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Yukio Yamada · Felix Scholkmann ·
Joseph C. LaManna *Editors*

Oxygen Transport to Tissue XLV

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Oxygen Transport to Tissue XLV

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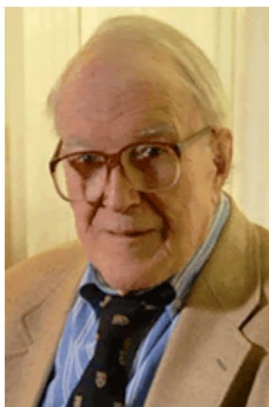
Commemorative Symposium Speakers



In Memory of Our Deceased



Prof. Melvin Knisely



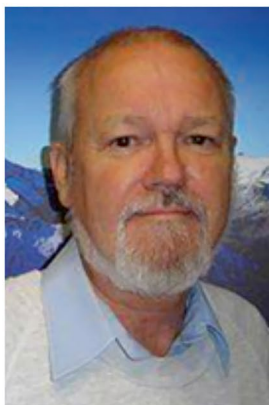
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Awards

The Melvin H. Knisely Award

It was established in 1983 to honor Dr. Knisely's accomplishments in the field of the transport of oxygen and other metabolites and anabolites in the human body. Over the years, he has inspired many young investigators, and this award is to honor his enthusiasm for assisting and encouraging young scientists and engineers in various disciplines. The award is to acknowledge outstanding young investigators. Award recipients:

1983: Antal G. Hudetz (Hungary)
1984: Andras Eke (Hungary)
1985: Nathan A. Bush (USA)
1986: Karlfried Groebe (Germany)
1987: Isumi Shibuya (Japan)
1988: Kyung A. Kang (Korea/USA)
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1999: Huiping Wu (USA)
2000: Valentina Quaresima (Italy)
2001: Fahmeed Hyder (Bangladesh)
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2011: Mei Zhang (USA)

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The Dietrich W. Lübbers Award

It was established in honor of Professor Lübbers's long-standing commitment, interest, and contributions to the problems of oxygen transport to tissue and to the society. Award recipients:

1994: Michael Dubina (Russia)
1995: Philip E. James (UK/USA)
1996: Resit Demit (Germany)
1997: Juan Carlos Chavez (Peru)
1998: Nathan A. Davis (UK)
1999: Paola Pichiule (USA)
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2001: Theresa M. Busch (USA)
2002: Link K. Korah (USA)
2003: James J. Lee (USA)
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2005: Charlotte Ives (UK)
2006: Bin Hong (China/USA)
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2017: Ben Jones (UK)
2018: Hamoon Zohdi (Switzerland)
2019: Kazaki Hotta (Japan)
2021: Masamichi Moriya (Japan)
2022: Frédéric Lange (UK)
2023: Jingjing Jiang (Switzerland)

The Britton Chance Award

It was established in honor of Professor Chance's long-standing commitment, interest, and contributions to the science and engineering aspects of oxygen transport to tissue and to the society. Award recipients:

2004: Derek Brown (Switzerland)
2005: James Lee (USA)
2006: Hanzhu Jin (China/USA)
2007: Eric Mellon (USA)
2008: Jianting Wang (USA)
2009: Jessica Spires (USA)
2010: Ivo Trajkovic (Switzerland)
2011: Alexander Caicedo Dorado (Belgium)
2012: Felix Scholkmann (Switzerland)
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2021: Maheen Siddiqui (UK)
2022: Samaneh Davoudi (Belgium)
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The Duane F. Bruley Travel Awards

They were established in 2003. They were created to provide travel funds for student researchers in all aspects of areas of oxygen transport to tissue. It is hoped that receiving the Duane F. Bruley Travel Award will inspire students to excel in their research and will assist in securing future leadership for ISOTT.

2004: Helga Blocks (Belgium), Jennifer Caddick (UK), Charlotte Ives (UK), Nicholas Lintell (Australia), Leonardo Mottola (Italy), Samin Rezanian (USA/Iran), Ilias Tachtsidis (UK), Liang Tang (USA/China), Iyichi Sonoro (Japan), Antonio Franco (Italy)
2005: Robert Bradley (UK), Harald Oey (Australia), Kathy Hsieh (Australia), Jan Shah (Australia)
2006: Ben Gooch (UK), Ulf Jensen (Germany), Smruta Koppaka (USA), Daya Singh (UK), Martin Tisdall (UK), Bin Wong (USA), Kui Xu (USA)
2007: Dominique De Smet (Belgium), Thomas Ingram (UK), Nicola Lai (USA), Andrew Pinder (UK), Joke Vanderhaegen (Belgium)
2008: Sebastiano Chicco (Italy)
2009: Lei Gao (UK), Jianting Wang (USA), Obinna Ndubizu (USA), Joke Vanderhaegen (Belgium)

- 2010: Zareen Bashir (UK), Tracy Moroz (UK), Mark Muthalib (Australia), Catalina Meßmer (USA), Takashi Eriguchi (Japan), Yoshihiro Murata (Japan), Jack Honeysett (UK), Martin Biallas (Switzerland)
- 2011: Catherine Hesford (UK), Luke S. Holdsworth (UK), Andreas Metz (Switzerland), Maria D. Papademetriou (UK), Patrik Persson (Sweden), Felix Scholkmann (Switzerland), Kouichi Yoshihara (Japan)
- 2012: Allann Al-Armaghany (UK), Malou Friederich-Persson (Sweden), Tharindi Hapuarachchi (UK), Benjamin Jones (UK), Rebecca Re (Italy), Yuta Sekiguchi (Japan), Ebba Sivertsson (Sweden), André Steimers (Germany)
- 2013: Allann Al-Armaghany (UK), Gemma Bale (UK), Alexander Caicedo-Dorado (Belgium), Luke Dunne (UK)
- 2014: Geraldine De Preter (Belgium), Benjamin Jones (UK), Stefan Kleiser (Switzerland), Nassimsadat Nasser (Switzerland), Marie-Aline Neveu (Belgium), Shinsuke Nirengi (Japan), Takuya Osawa (Japan)
- 2015: Gemma Bale (UK), Chenyang Gao (China), Chae Jeong Lim (Korea), Phong Phan (UK), Guennadi Saiko (Canada), Kuangyu Shi (Germany), Nannan Sun (China)
- 2016: Isabel De Roever (UK), Yuya Enokida (Japan), Jingjing Jiang (Switzerland), Frédéric Lange (UK), Andreas J Metz (Switzerland), Shun Takagi (Japan)
- 2017: Luca Giannoni (UK), Jingjing Jiang (Switzerland), Alexander Kalyanov (Switzerland), Zuzana Kovacsova (UK), Nassim Nasser (Switzerland), Maheen Faisal Siddiqui (UK), Shun Takagi (Japan), Yusuke Tsuruno (Japan), Lina Xu (Germany), Hamoon Zohdi (Switzerland)
- 2018: Andrei Belten (Canada), Aldo Di Costanzo Mata (Switzerland), Yuki Komuro (Japan), Joshua Russell-Buckland (UK), Dries Hendrikx (Belgium), Shun Takagi (Japan)
- 2019: Gary Angles (USA), Min Feng (USA), Thea Hüsing (Germany), Bhabuk Koirala (USA), Sho Kojima (Japan), Jonathan Nguyen (USA), Labiblais Rahman (Japan), Mandy Rauschner (Germany), Aarti Sethuraman (USA), Eileen Thiessen (USA)
- 2020: (meeting was omitted due to COVID-19 pandemic) 2021: (online meeting)
- 2022: Xianyin Hu (Japan), Djazia Yacheur (Switzerland), Victor Ochoa-Gutierrez (UK, Mexico), Tarcisi Cantieni (Switzerland), Tong Li (Switzerland)
- 2023: Hikari Otsuka (Japan)

Preface

This proceeding is from the landmark symposium celebrating the 50th anniversary of the International Society on Oxygen Transport to Tissue (ISOTT). Held in Tokyo, this event gathered global researchers, with a strong Asian contingent from Japan, Taiwan, Korea, and China. The conference showcased over 60 peer-reviewed papers, highlighting ongoing advancements in oxygen transport to tissue.

The 50th Anniversary Symposium was a key event, featuring lectures from influential members like Professors Duane Bruley, Peter Vaupel, Joseph C. LaManna, Edwin M. Nemoto, Harold Swartz, and Ann Barry Flood, plus contributions from Prof. Ursula Wolf, Prof. Sally Pias, and Prof. Eiji Takahashi. Their insights shaped ISOTT's history and future research directions.

A poignant moment was the 50th Anniversary Ceremony, opened by a video message from Shinji Suematsu, Member of the National Parliament of Japan and Former Minister of Education and Science Technology, who extended his congratulations and support. The ceremony honored deceased members like Prof. Melvin Knisely, Prof. Britton Chance, Prof. Dietrich Lübbers, Prof. Wilhelm Erdmann, Prof. David K. Harrison, Prof. John W. Severinghaus, and Prof. Mamoru Tamura with a moment of silence, acknowledging their lasting impact.

Reflecting on the past 50 years, we appreciate the visionaries who propelled ISOTT from its inception to its status as a premier scientific body in oxygen transport research. We anticipate further evolution and collaboration in scientific discovery.

These proceedings present findings from ISOTT 2023, advancing our understanding of oxygen transport mechanisms and their applications. We hope this stimulates further research and deepens understanding of the biological systems we study.

Thank you to all who contributed to making ISOTT 2023 a memorable and successful event. We trust these proceedings will be a valuable research resource and exemplify our society's vibrant, collaborative spirit.

ISOTT2023
Kashiwa, Chiba, Japan
Chofu, Tokyo, Japan

Co-Presidents
Kaoru Sakatani
Kazuto Masamoto

Acknowledgments

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As co-presidents of the 2023 Conference of the International Society on Oxygen Transport to Tissue, we, Kaoru Sakatani and Kazuto Masamoto, would like to gratefully acknowledge the support of the following sponsors:

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- The Uehara Memorial Foundation

Assistant

We, Kaoru Sakatani and Kazuto Masamoto, would like to express our gratitude to Harumi Igarashi, our secretary, for helping us prepare for ISOTT 2023 and this proceeding book.

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The manuscripts were reviewed by the following experts:

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Part I

50th Anniversary

Celebration of the 50th Anniversary of ISOTT (September 27, 2023 Tokyo, Japan)

ISOTT—A Meaningful Idea That Is Successful

Duane F. Bruley

Abstract

In the early 1960s, I was working as a traditional chemical engineer studying inanimate objects without the slightest clue of the biological world. At that time, I met Dr Melvin H. Knisely and he encouraged me to use my engineering skills to improve on the Krogh capillary tissue cylinder. I derived a detailed mathematical model and performed a complex computer simulation to achieve that goal. We attended professional meetings on oxygen transport to tissue all over the world, but mainly in Europe, presenting case studies. It became my goal to honour Dr Knisely with a meeting on oxygen transport to tissue at Clemson University in South Carolina, USA. Melvin's wife, Verona, convinced me to also have the meeting at the Medical University of South Carolina located in Charleston, since it was meant to honour Dr Knisely's work with his quartz rod crystal for illumination. He is credited as the first human being to observe the particulate matter in blood flowing in the microcirculation. He was nominated for the Nobel prize four times as a result of his discoveries. When I decided to have part of the

meeting at the medical school, I invited Dr Haim Bicher to work with me from there and I focused on Clemson University and the combined meeting structure. As the meeting evolved, we decided it would be a good idea to establish an international society and call it the "International Society on Oxygen Transport to Tissue" (ISOTT). I wrote a paper on the pillars of our young society, "ISOTT from the Beginning: A Tribute to Our Deceased Members (Icons)," and another that shares more detail about its beginnings, "The Founding of ISOTT: The Shamattawa of Engineering Science and Medical Science". The roots of ISOTT are all the members, new and old, who continue to make valuable contributions to an exceedingly important component of human health. I hope that the society lasts for a long time, continuing to make important contributions to the medical world. It is a society that has been instrumental in bringing together brilliant scientists from the medical, engineering, and natural science fields to work together. It has contributed to the evolution of "bioengineering" as we know it today.

Keywords

Founding · History · Success · Future · Quality · Legacy

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It's a minor miracle that a small society founded in 1973 can still be in existence 50 years later. When I graduated from the University of Tennessee with a traditional PhD in chemical engineering and zero knowledge of living systems, it was my good fortune that my colleague at Clemson, Dr William Barlage, invited me to go with him to Charleston, to the Medical University of South Carolina to see if we could become involved in any way with medical research. I accepted, and we travelled by car the 250 miles from Clemson University in Clemson, South Carolina, to Charleston, South Carolina. While there for two-and-a-half days, I talked to many researchers, but on our last day, we stopped by the anatomy department, where Dr Melvin H. Knisely was the Chairman. When he heard that we were engineers from Clemson, he invited us to go to lunch with him. His typical lunch was a McDonald's hamburger, and we accepted and discussed potential research over the lunch hour. Dr Knisely described how he often wondered if engineering expertise could improve the calculations existing on the Krogh Capillary Tissue Cylinder and asked if we could make contributions to the improvement. I had just completed my dissertation for the PhD in Engineering [1] at the University of Tennessee in Knoxville, TN, which dealt with the frequency response analysis of a wetted wall column including an experimental phase and a mathematical model and computer simulation of the column. Since the computer model was in cylindrical coordinates, it allowed me to expand the research and apply it to the Krogh cylinder with a much more sophisticated approach. At the time, I told Dr Knisely that I might be interested in the project, but I would have to learn much more about the fundamentals of the living system. He gave me two German papers, one of which was by Opitz and Schneider [2] and another by Dr Gerhard Thews [3]. Fortunately, I had taken technical German as a PhD requirement, and with the help of Dr Elsie Tabor and Dr Isabel Lockard, we successfully completed the translation and learned enough to confirm with Knisely that I could definitely improve upon the model with computer simulations. The Bruley Model included a multicompo-

nent system with nonlinearities and time-dependence. The purely analytical solution of a strictly linear system such as the Krogh Capillary Tissue Cylinder could not examine many fundamentals of oxygen transport that the Bruley Model could. The Bruley Model was developed from basic principles of flow in the microcirculation using the shell balance strategy. The Bruley Model and its solutions were presented at many national and international meetings and received many awards during the 1960s [4, 5].

My interest in working with the medical field became very pronounced and I involved myself in the development of a new engineering discipline: bioengineering. At that point in time, traditional engineers working on living systems were frowned upon by colleagues. But my interest peaked and following Dr Dietrich Lübbers' meeting in Dortmund, Germany, I decided to have a meeting on oxygen transport at Clemson University in Clemson, South Carolina, and honour Dr Knisely and his work related to the microcirculation. Among many things, he developed the quartz rod crystal illumination technology that he then used to examine the microcirculation in vivo. He could look into the human eye and actually see particulate matter flowing in real time. These studies led to the analysis of many disease states, including malaria, etc. He also showed that alcohol consumption does cause clumping of the flowing cells. For this, he was honoured around the world and nominated for the Nobel Prize four times. He was a gentleman and scholarly person who had the patience to deal with novice engineers like me. I greatly appreciated his help and interest and feel that he was my mentor into the medical world.

As a result, I began to put together the plans for the meeting. I got the approval of Dr Edwards, the President of Clemson University. While I was organising, I talked to Verona Knisely on the telephone from Clemson to Charleston and told her about my plans to honour Melvin and his work. At that time, she suggested that if the meeting was to honour Melvin, the meeting should take place at the medical school. I thought about it, but didn't want to take the engineering part out of the

meeting, and still wanted to have the meeting at Clemson. I finally made the decision to have the meeting at both universities, and with Verona's help, got the approval of President William Mellon McCord from the medical school to do so. To organise the meeting, I invited my colleague, Dr Haim Bicher, to join me and organise the medical school portion while I took care of the Clemson side of the meeting as well as the coordination of both sections. As we went ahead with the process, we found there was tremendous interest around the globe. So we decided that there should be an international society and we made the decision to found one, which we named the "International Society on Oxygen Transport to Tissue" (ISOTT). After making the decision, and naming the society, Dr Bicher and I developed the constitution and by-laws along with a format for the annual meetings that would ensure engineering and medical researchers to share their approach to the solution of medical problems. We decided that all sessions of the annual meeting should be in one room, with all participants (medical and engineering) taking part in every session. This way we could accomplish a transfer of engineering and medical knowledge to all the participants. We decided it was going to be a high-level research society and we were not going to worry about size. It was to meet once a year, alternating between the United States and Europe (later expanded to include Asia). If there were only two people in the world that had that interest, they could have a symposium and meet somewhere to discuss the principles of oxygen transport to tissue.

In the early 1980s, while I was head of biomedical engineering at Louisiana Tech University in Ruston, LA, USA, Dr Rene Borgaine, an ISOTT member from Belgium, was visiting my lab and our son Randall developed a blood clot in his leg. Dr Borgaine suggested that we check if Randall was protein C-deficient. It turned out that there were only three places in the world to assay for protein C, which is the pivotal anticoagulant in human blood. A deficiency of protein C in the blood leads to clumping of the cells and formation of blood clots, preventing oxygen transport to the tissues. We sent a sample of Randy's blood

to Dr Richard Marlar at the Milwaukee Blood Center in Wisconsin and the result came back that Randy was deficient in protein C. When I learned about this, I had our entire family tested for protein C deficiency and found that about half of our family members were deficient and would be prone to forming blood clots. At this point in time, I focused my research on trying to develop a zymogen protein C product. To get support, I contacted Dr Bill Drohan at the American Red Cross research centre in Gaithersburg, MD, USA. He was in charge of the derivatives division and became interested in considering the production of protein C. I was working on a process using immunoaffinity chromatography to separate zymogen protein C from fraction IV of blood plasma. We decided to work together and he supported the research with a number of people from his program too. We did the basic process development to produce some of the first protein C in the world and then the red cross began interaction with a small derivatives company from Austria. The company had a research facility in California where the process was actually put together and zymogen protein C is being produced today at that facility.

When I had to have my left hip replaced, I started working with a researcher and clinician, Dr Michael Streiff, at Johns Hopkins Hospital in Baltimore, MD, USA. He was, and still is today, my haematologist. While doing the surgery on me, he and the surgeon were very worried about blood clots because of the protein C deficiency. I suggested to them that we try using zymogen protein C perioperatively to make sure that I was protected against clotting. They thought it was a good idea and so I was proudly one of the first human beings in the world to use zymogen protein C to prevent clots without bleeding and provide safe invasive surgery [6]. The operation was completely successful with no clotting or bleeding. We tested the procedure multiple times, each case being successful with no clotting and no bleeding [7, 8]. We were extremely happy with the results, however, using immunoaffinity chromatography to separate the zymogen was so expensive that it has been impractical to use it as a general medical treatment. I believe there are

many medical issues where zymogen protein C could prevent people from blood clots (hereditary or acquired protein C deficiency) without causing bleeding. That includes women during pregnancy, where protein C levels often drop tremendously. I have focused my research on trying to find a technology that will be cheaper for production. We switched to metal affinity chromatography that would reduce the cost significantly and had some success, but by that time, I had become elderly with many medical problems and I had to stop my research. I still feel that zymogen protein C could be a very valuable solution to many oxygen transport problems involving blood clots.

Eli Lilly put their money into developing activated protein C using a bioreactor. It was costly in the range of \$500 million for the research itself, but then it was found to cause bleeding when administered as a bolus to try to cure sepsis, so the project was cancelled and they discontinued their production of activated protein C. This same thing would not happen with zymogen protein C because it circulates in the blood in an inactive form and is only activated at the site where it is needed. It has its own protective characteristics that prevent unwanted clotting without causing bleeds. I think nature has designed zymogen protein C as an incredibly safe anticoagulant for human blood, and probably all mammalian blood. I hope that some research company will develop a cost-effective process for the production of zymogen and people will continue finding applications for its use in serious and deadly medical situations.

To this day, the important thing for ISOTT is to attract high-level research via its membership and not be concerned about the growth of the society. Dr Bicher and I founded the annual society volume via Plenum Press in 1973, which still exists, but is being published through Springer Publishing now. It was a serendipitous process, but looking back, has been very successful, as some of the great minds of the world have been part of our group and members of ISOTT. My hope is that this format will continue for the annual meeting and that ISOTT can attract top talent to further the research goals on an incredibly important part of human health.

I'm not going to go into detail about the founding as I have already written and published several papers about this subject in ISOTT volumes. I'm going to reference them so that you can read and get a better picture of the details having to do with our society. One of these papers is "The Founding of ISOTT: The Shamattawa of Engineering Science and Medical Science" [9]. It explains the process and how and why the pieces were put together to found a society.

I also wrote a paper (ISOTT from the Beginning: A Tribute to Our Deceased Members (Icons)) on many of our original members who are deceased, but I wanted to pay a tribute to them because of their brilliant work and contributions to the intellectual growth of ISOTT [10]. I will not be participating much longer in the society and it makes me unhappy because some of my best experiences in life have been attending the meetings and being with the membership through the technical discussions and the evening dinners and beer jams, as well as the ultimate annual banquet with the various young investigators getting awards for their contributions. The future of the society does depend on maintaining the interest and getting fresh young blood as well as well-established researchers to join. But ISOTT is a very focused, high-level research organisation without concern about a large membership. I thank everyone who has participated in our society for the contributions they have made and continue making. My biggest wish is that everyone who is involved has a successful and healthy career as a scientist, engineer, clinician, or now, a "bioengineer". Our society has been a leader in putting into practice that which is necessary to get medical researchers and engineering researchers to share their expertise in solving high-level medical problems.

As a final note, it is my desire that ISOTT remains a high-end research-focused society working with medical scientists and engineering scientists to push back the boundaries on solutions to our major health problems. It is important for the work contributed by ISOTT to meet high standards in research. It has been a pleasure to be a major part of this journey. I can justifiably say that the annual ISOTT meetings have been the best intellectual and social gatherings that I have attended in my professional life.

It is an honour to be a part of the 50th anniversary celebration and share our success. Thanks for being a part of this success story due to your participation and contributions.

God bless.

References

1. Bruley DF, Prados JW (1964) The frequency response analysis of a wetted wall adiabatic humidifier. *AIChE J* 11:612
2. Opitz E, Schneider M (1950) The oxygen supply of the brain and the mechanism of deficiency effects. In: *Ergebnisse der Physiologie, Biologischen Chemie, und Experimentellen Pharmakologie* 46. Springer, pp 126–260
3. Thews G (1973) Oxygen diffusion in the brain. A contribution to the question of the oxygen supply of the organs. *Pflugers Archiv* 271:197–226
4. Reneau DD, Bruley DF, Knisely MH (1967) A mathematical simulation of oxygen release, diffusion and consumption in the capillaries and tissue of the human brain. *Chemical engineering in medicine and biology*, pp 135–241. Winner of a National ACS Award
5. Reneau DD, Bruley DF, Knisely MH (1969) A digital simulation of transient oxygen transport in capillary-tissue systems (cerebral grey matter). *AIChE J* 15(6):916–925
6. Bruley DF (2007) Zymogen protein C concentrate for safer heterozygote surgery, “i am a guinea pig”! *Adv Exp Med Biol* 645:115–121
7. Bruley DF, Mears SC, Streiff MB (2008) Safer surgery using zymogen protein C concentrate. *Adv Exp Med Biol* 662:439–445
8. Bruley DF, Shulick RD, Strieff MB (2012) Pancreaticoduodenectomy using perioperative zymogen protein C to help prevent blood clotting. A trilogy on increased patient safety. *Adv Exp Med Biol* 789:299–307
9. Bruley DF (2014) The founding of ISOTT: the Shamattawa of engineering science and medical science. In: *Oxygen transport to tissue XXXVI. Advances in experimental medicine and biology*, vol 812. Springer, New York, pp 59–63
10. Bruley DF, Thiessen E (2016) ISOTT from the beginning: a tribute to our deceased members (Icons). In: *Oxygen transport to tissue XXXVIII. Advances in experimental medicine and biology*, vol 923. Springer, Cham, p 1

Biophysical Modelling for Insight into Oxygen Diffusion, Distribution, and Measurement

Sally C. Pias

Abstract

Building on an extensive history of physiological and systems-oriented modelling, my group and others have recently used molecular simulation studies to understand oxygen (O_2) transport and localisation. Molecular simulations enable biophysical insight into processes difficult to study with experiments alone and are sometimes described as a “computational microscope.” Our work has emphasised lipid membrane contributions to oxygen diffusion and uptake, suggesting that lipid-based pathways along membranes and lipid deposits are likely to accelerate diffusive transport through cells and tissues. Moreover, the lipid and fluid fractions of the tissue are expected to be primary determinants of the local oxygen partial pressure (pO_2) as well as the oxygen permeability. Measurements using molecular probes can be influenced by the local molecular environment, due to differential solubility of both the probe and the oxygen molecules in various components of the cell’s complex solvent system. The biomolecular simulation work complements experimental studies, which enable evaluation of the models’ accuracy and their

applicability to real biological systems. Further work is needed to assess fully the possible influence of nanoscale crowders and obstacles (especially protein molecules) on tissue-level diffusive transport of oxygen. Likewise, water-rich carbohydrate layers, such as the glycocalyx, should be evaluated as potential barriers to oxygen transport. Insights gained through biophysical modelling studies could be broadly relevant to clinical phenomena affected by tissue oxygenation, such as tumour radiotherapy, ischaemia, neuropathy, and wound healing.

Keywords

Molecular dynamics simulation · TEMPO-choline · Lipid conjugate · Interfacial resistance · Computational microscope

1 Historical Context

Modelling work has contributed significantly to the theoretical framework for oxygen transport to tissue. Oxygen transport modelling has an extensive history, including work by Metzger [1], Bruley [2, 3], Thews [4], Grunewald, Lübbers, Hutten, Vaupel, and others [5, 6]. A 1994 review article by Bruley recounts a good deal of early oxygen transport modelling work [7]. The work has employed primarily physiological approaches

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(mathematical models informed by physiological parameters) as well as systems-oriented approaches (“integrative” models incorporating many interacting factors). Collaborations among clinical and biological researchers as well as engineers and physical scientists have yielded sophisticated theoretical models that meaningfully represent complex physiological systems.

2 Molecular Biophysical Modelling

Recent theoretical work on oxygen diffusive transport by my research group and others has employed a molecular biophysical approach, focused mainly on oxygen diffusive transport at the level of cell membranes. Such biophysical modelling is often likened to a “computational microscope” because it enables visualisation of structures and processes too small to image through experimental microscopy. The most common strategy handles atoms or clusters of atoms using a Newtonian physics-based model that accounts for the positions and movements of atoms but does not allow for chemical reactions (changes in bonding arrangements). Associated “molecular dynamics simulations” calculate atomic motion over time using a potential energy equation, which includes terms to represent the dynamics of atoms associated with bonds, angles, and torsion angles, as well as electrostatic and dispersion-type attractions and repulsions. The equation is coupled with well-optimised “force field” parameters, which can represent biomolecules (especially proteins) with a high degree of accuracy.

The molecular biophysical approach has the advantage of being “model-free” from a physiological perspective, enabling naive investigation of biomolecular processes without imposition of physiological assumptions. Even so, integrating the molecular biophysical data with physiological models could be of value—and presents an opportunity for further work. In addition to being model-free, biophysical simulations enable careful isolation of molecular structural variables, as

well as valuable estimation of thermodynamic quantities.

Small size and spatial scales limit the scope of the technique, but major advances in recent years have expanded those boundaries significantly. In particular, advances in computing hardware driven by computer gaming have led to routine accessibility of simulation timescales on the order of a microsecond [8]. A dedicated special-purpose computing machine for molecular dynamics simulations, with optimised hardware and software configurations, has rendered millisecond timescales accessible, as well [9]. Still, many biological processes of interest occur on timescales of seconds to tens of seconds or longer. These include macromolecular interactions (such as protein-protein and protein-DNA) and diffusional processes over longer spatial ranges. For relatively short spatial scales (~10 nm), oxygen diffusion can be modelled quite effectively using all-atom or “atomistic” molecular dynamics simulations. With this technique, physical properties such as free energy, permeability, partition coefficient, and diffusion coefficient can be estimated reproducibly and compared with experiments.

3 Radial Diffusion and Oxygen “Buffering”

A. Ghysels and colleagues (notably Richard Pastor and Andreas Krämer) have carried out several oxygen diffusion modelling studies using molecular dynamics simulations. These studies have led to several key insights. Foremost, innovative Bayesian analysis was used to determine that “radial” diffusion along the midplane between the two lipid bilayer leaflets is favoured relative to transbilayer diffusion [10]. As such, oxygen molecules can diffuse radially across distances up to about ten times the bilayer thickness, before leaving the nonpolar interior of the lipid bilayer [11]. Further, layered membranes, such as those that occur in myelin sheaths, could “buffer” cellular oxygen levels, by providing a local oxygen sink and associated oxygen enrichment [12]. On the other hand, raft-like liquid-ordered lipid

domains exhibit low oxygen permeability, but facilitate radial oxygen diffusion along the bilayer midplane [13].

Ghysels and Davoudi have applied a “coarse-grained” biophysical modelling approach to enable investigation of membrane structures at larger spatial scales. Coarse-grained modelling treats molecules as consisting of “beads,” or atom clusters, rather than individual atoms. The work supports biophysical study of caveolae—balloon-shaped lipid-rafts implicated in energy metabolism and oxygen sensing [14–16].

4 Aqueous Barriers and Lipid-Based Pathways

My own research group has also conducted extensive oxygen diffusion modelling, primarily based on all-atom molecular dynamics simulations. Our work has focused on lipid compositional variations, especially membrane cholesterol and breast tumour lipid changes, as well as on membrane protein abundance and crowding. We have also pursued detailed comparison with biophysical experimental measurements acquired using electron paramagnetic resonance (EPR) spin-label probes. Our work has generated insights focused on the pathway of oxygen diffusion, the differential solubility of oxygen in lipids versus aqueous fluids, and the interpretation of molecular-probe-based oxygen measurements.

Our studies indicate that lipid-based pathways within cells and tissues may accelerate oxygen diffusive transport [17]. This finding is consistent with Longmuir’s suggestion in 1981 that oxygen travels diffusively via “channels of high solubility” comprised of lipids [18]. Our work also indicates that the lipid and fluid fractions of tissues may influence oxygenation, in terms of the amount of oxygen (pO_2) available within tissue at a given time and the responsiveness of the tissue to acute O_2 depletion (the kinetics of replenishment) [19]. The primary reason for this sensitivity to fluid and lipid fractions is the substantially greater solubility of oxygen in lipids, compared with aqueous (water-based) fluids. The lipid/water solubility

ratio (partition coefficient) is at least 5 to 1 towards the lipid phase, or perhaps up to 25 to 1, according to Longmuir’s measurements [17, 18].

5 Ambiguity of Measurements Using Molecular Probes

Simulation studies by our group have led to the insight that molecular-probe-based measurements can be influenced by phase partitioning behaviour. Specifically, phase partitioning influences the interpretation of measurements obtained with electron paramagnetic resonance (EPR) probes meant to measure oxygen in the headgroup region of lipid bilayer membranes. Lipid bilayers are well-known to present a complex barrier that varies in character as solutes, such as oxygen, progress through the polar headgroups on one side, across the nonpolar lipid tail region, and again through a polar headgroup region on the other side. This complexity is captured in the widely accepted inhomogeneous solubility-diffusion model [20, 21]. For oxygen (O_2), the headgroup regions present interfacial free-energy barriers, while the lipid tails provide a local, high-solubility sink [22, 23].

Efforts to measure the magnitude of the interfacial barriers have been confounded by the physical and chemical character of the TEMPO-choline phospholipid-conjugated EPR probes targeted to the headgroup regions. These probes feature bulky ring structures with significant hydrophobic character. Even though the TEMPO-choline probe moiety replaces the lipid headgroup and is physically long enough to visit the headgroup region, these probes have been found through molecular dynamics simulation to be largely excluded from the headgroup region and to localise primarily to a more oxygen-rich sub-interfacial region beneath the headgroups and towards the nonpolar bilayer interior (Fig. 1 and ref. [24]). Knowledge of the probe’s partitioning behaviour is critical to accurate assessment of lipid bilayer permeability, as the headgroups present the rate-limiting barrier to oxygen permeation [24, 25]. Our work suggests that misconstrual of the TEMPO-choline probe’s localisation

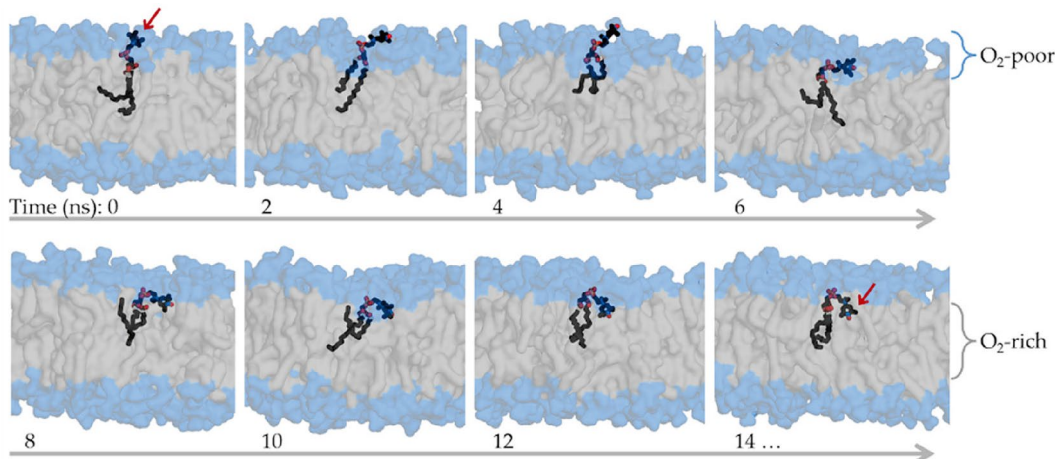


Fig. 1 Molecular dynamics simulation shows the lipid-conjugated TEMPO-choline EPR probe leaving the O₂-poor polar headgroup region and partitioning preferentially into the O₂-rich lipid tail region. Panels show consecutive snapshots of an all-atom molecular dynamics simulation, spaced 2 nanoseconds (ns) apart. Within 14 ns, the TEMPO probe moiety (red arrows) settles into the lipid tail region, where it remains for 75 ns more (data not shown). The simulation system consists of 127 molecules of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) phospholipid, along with one molecule of 1-palmitoyl-2-oleoyl-glycero-3-TEMPO-choline (T-PC), and

6400 water molecules (not shown). The T-PC molecule is highlighted (C atoms in black, H atoms hidden for clarity). POPC bilayer shown with headgroups in blue and tails in grey. *Simulation conditions:* isobaric-isothermal ensemble at 298 K at 1 atm with periodic boundary conditions, non-bonded interaction cut-off distance 10 Å, 2 femtosecond timestep, SHAKE algorithm active [26], TIP3P water [27], Lipid14 force field for POPC [28], and Lipid14 atom types for T-PC (charge-fitted with RESP [29], according to the capped headgroup charge-fitting approach of Lipid11 [30]). Image generated using PyMOL software [31]

leads to overestimation of the oxygen permeability of lipid bilayers by several fold, due to underestimation of the rate-limiting free-energy barrier at the lipid-water interface [24].

Partitioning effects are likely to influence most or all oxygen measurements utilising molecular probes, although they depend on the specific physical and chemical characteristics of the probes used. EPR probes are especially likely to exhibit partitioning towards hydrophobic (lipid-rich) phases because they typically include bulky hydrophobic functional groups to stabilise an unpaired electron and shield it from reaction.

Researchers using molecular probes should take into account the probes' lipid-water partition coefficients and consequent localisation, or at least should account for the fact that they will partition preferentially into one phase or another [32], usually lipid. Probe localisation non-uniformity can combine with non-uniformity in the oxygen distribution to produce substantial ambiguity in interpreting the measurements. In

the complex, multiphase environment of cells, it is often difficult to know what phases are represented in the data and to infer accurate whole-tissue partial pressures.

6 Open Questions

The chemical and physical complexity of tissues presents an ongoing challenge for understanding oxygen diffusive transport and for interpreting oxygen measurements. In particular, the influence of water-rich phases and interfaces on oxygen partial pressures and diffusional kinetics has not been fully evaluated. Since carbohydrates are especially water-rich, the glycocalyx (cell-surface carbohydrate layer) and other carbohydrate-coated interfaces should be investigated as potential barriers to oxygen permeation. Macromolecular crowding may, likewise, influence oxygen diffusion and solubility, affecting the kinetics and preferential pathways of diffusion.

7 Conclusion

Molecular biophysical modelling provides a powerful technique for studying oxygen transport. This “computational microscope” can enable visualisation and mechanistic understanding of molecular structural dynamics and diffusive processes. It also enables exacting isolation of variables and estimation of physical parameters such as permeability and free energy. Combining these parameters with physiological models may prove valuable for bridging the gap between subcellular (small nanoscale) mechanisms and tissue-level observations.

The molecular biophysical modelling approach provides detailed mechanistic insight into physical processes underlying biological function, in a physiologically agnostic, or “model free,” context. Though typically quite accurate from a biophysical perspective (or having *known* inaccuracies), these models are necessarily highly simplified and focus on one or a small number of variables. Cells and organisms, on the other hand, are profoundly multivariate and structurally complex as well as inhomogeneous. These complexities extend all the way from the molecular scale to the whole organism. Identifying critical variables that determine biological outcomes can be highly challenging and is a limitation of the molecular biophysical approach, when considered alone. The approach requires validation and crosstalk with experimental work, in order to evaluate and improve its applicability to real living systems. Through collaborative efforts with clinical and biological researchers, its insight into oxygen diffusive transport may benefit various clinical areas, such as tumour radiotherapy, ischaemia, neuropathy, and wound healing. However, closing the spatial as well as conceptual gap between the molecular and tissue scales remains a significant challenge.

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References

1. Metzger H (1973) Geometric considerations in modeling oxygen transport processes in tissue. In: Bruley DF, Bicher HI (eds) Oxygen transport to tissue: pharmacology, mathematical studies, and neonatology. Springer US, New York, pp 761–772
2. Bruley DF (1973) Mathematical considerations for oxygen transport to tissue (introductory paper). In: Bruley DF, Bicher HI (eds) Oxygen transport to tissue: pharmacology, mathematical studies, and neonatology. Springer US, New York, pp 749–759
3. Kang KA, Bruley DF, Bicher H (1988) A computer simulation of simultaneous heat and oxygen transport during heterogeneous three dimensional tumor hyperthermia. In: Mochizuki M, Honig CR, Koyama T et al (eds) Oxygen transport to tissue X. Springer US, New York, pp 747–756
4. Groebe K, Thews G (1989) Effects of red cell spacing and red cell movement upon oxygen release under conditions of maximally working skeletal muscle. In: Rakusan K, Biro GP, Goldstick TK, Turek Z (eds) Oxygen transport to tissue XI. Springer US, Boston, pp 175–185
5. Hoofd L, Turek Z (1996) Realistic modelling of capillary spacing in dog gracilis muscle greatly influences the heterogeneity of calculated tissue oxygen pressures. In: Ince C, Kesecioglu J, Telci L, Akpir K (eds) Oxygen transport to tissue XVII. Springer US, Boston, pp 333–340
6. Secomb TW, Hsu R, Braun RD et al (1998) Theoretical simulation of oxygen transport to tumors by three-dimensional networks of microvessels. In: Hudetz AG, Bruley DF (eds) Oxygen transport to tissue XX. Springer US, Boston, pp 629–634
7. Bruley DF (1994) Modeling oxygen transport: development of methods and current state. In: Vaupel P, Zander R, Bruley DF (eds) Oxygen transport to tissue XV. Springer US, Boston, pp 33–42
8. Salomon-Ferrer R, Götz AW, Poole D et al (2013) Routine microsecond molecular dynamics simulations with AMBER on GPUs. 2. Explicit solvent particle mesh Ewald. J Chem Theory Comput 9:3878–3888. <https://doi.org/10.1021/ct400314y>
9. Shaw DE, Adams PJ, Azaria A et al (2021) Anton 3: twenty microseconds of molecular dynamics simulation before lunch. In: Proceedings of the international conference for high performance computing, networking, storage and analysis. Association for Computing Machinery, New York, pp 1–11
10. Ghysels A, Venable RM, Pastor RW, Hummer G (2017) Position-dependent diffusion tensors in anisotropic media from simulation: oxygen transport in and through membranes. J Chem Theory Comput 13:2962–2976. <https://doi.org/10.1021/acs.jctc.7b00039>
11. De Vos O, Venable RM, Van Hecke T et al (2018) Membrane permeability: characteristic times and

- lengths for oxygen and a simulation-based test of the inhomogeneous solubility-diffusion model. *J Chem Theory Comput* 14:3811–3824. <https://doi.org/10.1021/acs.jctc.8b00115>
12. Ghysels A, Vervust W (2022) Oxygen storage in stacked phospholipid membranes under an oxygen gradient as a model for myelin sheaths. In: *Oxygen transport to tissue XLIII. Advances in experimental medicine and biology*, vol 1395. Springer, Cham
 13. Ghysels A, Krämer A, Venable RM et al (2019) Permeability of membranes in the liquid ordered and liquid disordered phases. *Nat Commun* 10:5616. <https://doi.org/10.1038/s41467-019-13432-7>
 14. Davoudi S, Wang Q, Patel HH et al (2023) Understanding the role of caveolae in oxygen buffering: the effect of membrane curvature. *Adv Exp Med Biol* 1438:87–91
 15. Davoudi S, Ghysels A (2024) Understanding oxygen “buffering” by caveolae using coarse-grained molecular dynamics simulations. *Adv Exp Med Biol* xxxx:xx–xx
 16. Zuniga-Hertz JP, Patel HH (2019) The evolution of cholesterol-rich membrane in oxygen adaption: the respiratory system as a model. *Front Physiol* 10:1–18. <https://doi.org/10.3389/fphys.2019.01340>
 17. Pias SC (2021) How does oxygen diffuse from capillaries to tissue mitochondria? Barriers and pathways. *J Physiol* 599:1769–1782. <https://doi.org/10.1113/JP278815>
 18. Longmuir IS (1981) Channels of oxygen transport from blood to mitochondria. *Adv Physiol Sci* 25:19–22. <https://doi.org/10.1016/B978-0-08-027346-4.50007-3>
 19. Pias SC (2023) Do vascular and extracellular measurements consistently reflect intracellular pO₂? *Adv Exp Med Biol* 1438:185–190
 20. Marrink S-J, Berendsen HJC (1994) Simulation of water transport through a lipid membrane. *J Phys Chem* 98:4155–4168. <https://doi.org/10.1021/j100066a040>
 21. Marrink SJ, Berendsen HJC (1996) Permeation process of small molecules across lipid membranes studied by molecular dynamics simulations. *J Phys Chem* 100:16729–16738. <https://doi.org/10.1021/jp952956f>
 22. Dotson RJ, Smith CR, Bueche K et al (2017) Influence of cholesterol on the oxygen permeability of membranes: insight from atomistic simulations. *Biophys J* 112:2336–2347. <https://doi.org/10.1016/j.bpj.2017.04.046>. PMID: PMC5474842
 23. Dotson RJ, McClenahan E, Pias SC (2021) Updated evaluation of cholesterol’s influence on membrane oxygen permeability. *Adv Exp Med Biol* 1269:23–30. https://doi.org/10.1007/978-3-030-48238-1_4
 24. Angles G, Hail A, Dotson RJ, Pias SC (2021) Atomistic simulations modify interpretation of spin-label oximetry data. Part 1: intensified water–lipid interfacial resistances. *Appl Magn Reson* 52:1261–1289. <https://doi.org/10.1007/s00723-021-01398-z>. PMID: PMC10249954
 25. Diamond JM, Katz Y (1974) Interpretation of non-electrolyte partition coefficients between dimyristoyl lecithin and water. *J Membr Biol* 17:121–154. <https://doi.org/10.1007/BF01870176>
 26. Ryckaert J-P, Ciccotti G, Berendsen HJC (1977) Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of *n*-alkanes. *J Comput Phys* 23:327–341. [https://doi.org/10.1016/0021-9991\(77\)90098-5](https://doi.org/10.1016/0021-9991(77)90098-5)
 27. Jorgensen WL, Chandrasekhar J, Madura JD et al (1983) Comparison of simple potential functions for simulating liquid water. *J Chem Phys* 79:926. <https://doi.org/10.1063/1.445869>
 28. Dickson CJ, Madej BD, Skjevik ÅA et al (2014) Lipid14: the Amber lipid force field. *J Chem Theory Comput* 10:865–879. <https://doi.org/10.1021/ct4010307>
 29. Bayly C, Cieplak P, Cornell W, Kollman P (1993) A well-behaved electrostatic potential based method using charge restraints for deriving atomic *J Phys Chem*:10269–10280
 30. Skjevik AA, Madej BD, Walker RC, Teigen K (2012) LIPID11: a modular framework for lipid simulations using Amber. *J Phys Chem B* 116:11124–11136. <https://doi.org/10.1021/jp3059992>
 31. The PyMOL molecular graphics system, Version 1.7.6.5 Schrödinger, LLC
 32. Aliaga C, Bravo-Moraga F, Gonzalez-Nilo D et al (2016) Location of TEMPO derivatives in micelles: subtle effect of the probe orientation. *Food Chem* 192:395–401. <https://doi.org/10.1016/j.foodchem.2015.07.036>

Master Role of Hypoxia in Cancer Progression: Major Insights During ISOTT's Half-Century

Peter Vaupel  and Gregg L. Semenza 

Abstract

On the occasion of ISOTT's half-century, in this chapter, tumour hypoxia (i.e. critically reduced oxygen levels on macro- and microscopic scales), recently classified as an additional hallmark of cancer, its various aetiology-related classifications (diffusion- or perfusion-limitations, hypoxaemic), time-frames of exposure (acute, chronic, cyclic), and different levels (moderate, mild, severe) within and across tumours, and its Janus-face-like role ("dichotomy") in tumour regression (e.g. apoptosis, necrosis) *versus* "adaptive" tumour progression have been updated and summarised. This latter knowledge is, to a

great extent, based on (a) direct, reliable assessments and mapping of the heterogeneous tumour oxygenation status using minimally invasive polarographic pO_2 microsenors in clinical settings since the late 1980s, and (b) the discovery of the hypoxia-inducible factors (HIFs) in the early 1990s. These data have clarified the role of hypoxia in stimulating a variety of biologic responses that mediate cancer progression through changes (a) in the genome (associated with clonal selection and expansion), (b) in the transcriptome, and (c) in the proteome, as well as its role as a barrier to the effectiveness of anti-cancer therapies.

Keywords

Tumour hypoxia · Tumour oxygenation status · pO_2 microsenors · Hypoxia detection · Hypoxia levels · Hypoxia classifications · Hypoxia-inducible factors · HIFs · Malignant progression · Treatment barrier · Patient prognosis

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1 Introduction

Cancer cells exposed to critically reduced O_2 supplies, mostly as a consequence of long-term restrictions in microvascular perfusion, of limitations in oxygen diffusion from the chaotic microvasculature to distant cells, and of a reduced

transport capacity of the blood that cannot meet the cellular O_2 demand, can reduce their overall protein synthesis, followed by restrained proliferation, apoptosis, or necrotic cell death, i.e. for these cells hypoxia acts as an *anti-proliferative signal*. In contrast, populations of hypoxic cancer cells can respond with reversible changes in the transcriptome and proteome ($pO_2 < 10$ mmHg) or with irreversible alterations in the genome ($pO_2 < 1$ mmHg) with subsequent expansion through clonal selection (*pro-proliferative effects*). The latter cell population drives the development of aggressive phenotypes, tumour progression, and contributes to therapeutic resistance, thus resulting in poor clinical outcome and patient prognosis. Colloquially, these opposing reactions are often referred to as “Janus face” or dichotomy of tumour hypoxia [1].

Hypoxia-induced changes in gene expression at tumour pO_2 values < 10 mmHg ($< 1.33\%$ O_2) are predominantly coordinated by hypoxia-inducible transcription factors HIF-1 (mainly in acute hypoxia) and HIF-2 (preferentially during prolonged hypoxia). Currently, the total number of RNAs that have been shown to be induced by hypoxia in a HIF-dependent manner in a cell type is > 8000 (unpublished data, G.L. Semenza). As an example, exposure of the SUM159 human triple-negative breast cancer cell line to 1% O_2 for 24 h significantly induced the expression of 1307 RNAs and repressed the expression of 817 RNAs at least 1.5-fold [2]. Targeted genes by the “co-opted master regulators” mediate tumour angiogenesis, metabolic reprogramming, regulation/maintenance of intracellular/extracellular pH-gradients, modulation of epithelial-mesenchymal transition (EMT), extracellular matrix remodelling, increased cell motility, invasion and metastasis, cancer stem cell specification and maintenance, suppression of anti-tumour immune responses (i.e. promotion of immune evasion), and resistances to chemo- and radiotherapies (for a recent review see [3]). Of note, the expression of HIF-1 can also be achieved in a hypoxia-independent (i.e. non-canonical) manner, e.g. by reactive oxygen species (ROS), by gain-of-function of oncogenes (e.g. cMyc, mTORC1, Akt, K-ras), or loss-of-function of tumour suppressors (e.g. mutant-p53, mutant-PTEN), and by activation of growth factors and

significantly induced the expression of receptor tyrosine kinases, and subsequently orchestrated by several signalling pathways [4]. In addition, altered expression of several microRNAs and of some sirtuins with tumour suppressor functions can activate HIF [5].

Independent of HIF, cancer cells can respond to severe hypoxic stress with an accumulation of unfolded/misfolded proteins in the endoplasmic reticulum which triggers the adaptive Unfolded Protein Response (UPR) pathway upon upregulation of the mammalian target of rapamycin-(mTOR-) mediated protein synthesis [6]. UPR promotes the survival of cancer cells and mediates resistance to anti-cancer therapies and, thus, can also trigger the development of more aggressive phenotypes.

Considering the seminal impact of hypoxia (and HIF) on the development of aggressive cancer phenotypes, this chapter will focus primarily on reliable descriptions of the “hostile” oxygenation status of experimental solid tumours and of most human malignancies, on the detailed pathogenesis of tumour hypoxia and its detection, and on the role of hypoxia in malignant progression, already described in excerpts in 85 communications, published earlier in the “Oxygen Transport to Tissue” series (Vols. I – XLIV) during ISOTT’s half-century.

2 Methods

This chapter aims to summarise “cornerstone” investigations exploring and core-data describing the oxygenation status of cancers, the detailed pathogenesis and detection of tumour hypoxia, and its implications for the oncologic setting, starting in the mid-1920s and extending into ISOTT’s lifetime 1973–2023. Particular emphasis will be placed on research data published by members (and former presidents) of ISOTT who have significantly contributed to the field.

3 Results

Table 1 presents information for the time period starting in 1909 up to 1973, the founding year of ISOTT. Table 2 contains references to relevant

Table 1 The history of research on tumour hypoxia (between 1909 and 1973, the founding year of ISOTT)

Timeline	Information	Refs.
1909	First description of the “protective” effect of (ischaemic) hypoxia upon X-irradiation.	[7]
1910	First mention of improved irradiation effects upon increased blood flow.	[8]
1923, 1956	O. H. Warburg’s historical postulates: (a) defective mitochondria are responsible for enhanced glycolysis in cancers, and (b) irreversible respiratory injury is a universal cause for carcinogenesis.	[9, 10]
Since mid-1950s	Interpretation of the data was questioned by B. Chance et al.	[11–14]
1962	Warburg conceded his original concept.	[15]
2013, 2021	Re-interpretation: Warburg effect is a component of metabolic reprogramming induced by genetic alterations in cancer cells.	[5, 16]
Since early 1940s	Membrane-covered Clark-type electrodes for direct measurements of local O ₂ pressures (pO ₂ values) in living tissues (cancers included).	[17]
1953	Existence of hypoxic radioresistance.	[18]
1955	Estimation of O ₂ diffusion distances in human lung cancer. ^a	[19]
Since late 1950s	pO ₂ measurements in human tumours in situ.	[20, 21]
Since late 1960s	pO ₂ measurements in cervical cancers of patients using polarographic O ₂ sensors.	[22, 23]
Since 1967/70	In situ-measurements of blood flow and O ₂ - consumption rates of “tissue-isolated” tumour isografts in rodents.	[24, 25]
Since 1972/73	pO ₂ measurements in isograft tumours reveal tumour hypoxia as a function of tumour vascularisation and blood flow, resp.	[26, 27]

^aSingular “typical” diffusion distances cannot exist in malignant tumours. Instead, a broad distribution (i.e. a continuum) of O₂ diffusion distances is usually observed [28]

Table 2 The history of research on tumour hypoxia (1974—1992)

Timeline	Information	Refs.
1974	Mathematical analyses of the critical O ₂ supply conditions in solid tumours based on experimental in vivo data. Evaluations of the pathogeneses of tumour hypoxia.	[29]
1977	Measurements of O ₂ diffusion coefficients in tumour tissues. ^a	[30]
1979	Description of acute hypoxia as a result of short-term flow interruptions.	[32, 33]
Early 1980s	Use of a cryo-spectrophotometric <i>ex-vivo</i> micro-method to describe the oxygenation status of primary cancers of the oral cavity and of the rectum of patients.	[34, 35]
1981	Technique for marking hypoxic tumour micro-areas with radiolabelled misonidazole reported.	[36]
1984	“Oxygen-regulated proteins” (ORPs) described.	[37]
1988	Computed O ₂ diffusion distances for human breast cancer tissue cords during diffusion-limitations, hypoxaemia, and compromised microcirculation.	[38]
1988	Observation of a causal relationship between moderate hypoxia (mean pO ₂ ≤ 8 mmHg) in lymph node metastases of head and neck cancers and response to radiotherapy.	[39]
1989	¹⁹ F-MRI for in vivo measurements of intravascular O ₂ -tensions in experimental tumours.	[40]
1991	Systematic clinical studies on the oxygenation status of primary cancers of the breast and uterine cervix using the Eppendorf pO ₂ histography system. Experimental proof of the existence of (a) irregularly distributed hypoxic/anoxic tissue areas, (b) steep pO ₂ gradients on a sub-millimetre scale, and (c) extreme heterogeneity within and across tumours.	[41, 42]
1992	¹⁸ F-FMISO, thereafter widely used as PET- imaging agent for tumour hypoxia in the clinical setting (“indirect oxygen measurement”).	[43]

^aMost cancers are hyperhydrated compared to their normal tissues of origin and, consequently, have distinctly higher diffusion coefficients [31]

Table 3 The history of research on tumour hypoxia (1992–2021)

Timeline	Information	Refs.
1992/95	Identification, purification, and cDNA isolation of the transcription factor HIF-1.	[44, 45]
1993	Characterisation of HIF-1. General involvement of HIF-1 in the transcriptional response to hypoxia.	[46, 47]
1993/96	Tumour hypoxia (pO ₂ <10 mmHg) predicts malignant progression and poor prognosis of patients with advanced cancers of the uterine cervix.	[48, 49]
	Prognostic significance of pre-therapeutic tumour hypoxia was validated for cancers of the uterine cervix, head and neck cancers, and soft tissue sarcomas (review).	[50]
1993/94	Direct assessment of tumour hypoxia using electron paramagnetic resonance (EPR).	[51, 52]
1996	Hypoxia selects for clones exhibiting mutant-p53.	[53]
1998/99	Most local tumour recurrences and metastases have a higher hypoxic fraction than the respective primary tumours. Hypoxic cancers of the uterine cervix with a low apoptotic index are highly aggressive.	[54, 55]
2001	Identification of the HIF prolyl hydroxylases (PHDs), enzymes leading to HIF inactivation in well-oxygenated cells	[56]
2007	Hypoxia in cancer: Significance and impact on clinical outcome (review).	[57]
	Computerised polarographic O ₂ microelectrode most accurate and direct system available for accessible tumours.	[58]
2021	Pivotal clinical trial leading to FDA approval of HIF-2 inhibitor Belzutifan for treatment of tumours in patients with the von Hippel-Lindau syndrome.	[59]
2021	Detailed oxygenation status of 17 different human tumour types vs. normal tissues (review).	[60]

data published between 1974 and 1990. In Table 3, substantial research information is reported for the years thereafter, the latter time period with groundbreaking, seminal gain in knowledge of compromised oxygen transports to cancers.

4 Conclusions

Reliable pO₂ measurements have unequivocally shown that hypoxia is a chief hallmark of most solid malignancies in adults. Clinical investigations have detected hypoxic sub-volumes which are heterogeneous in (a) extensions, (b) spatial distributions within a tumour, (c) over time, (d) extent (severity), and (e) aetiologies. Tumour hypoxia (pO₂ < 10 mmHg) has been identified as a strong independent and adverse prognostic factor of a series of solid tumours due to changes in the genome, transcriptome, and proteome (e.g. upon HIF-1 α expression). In addition, hypoxia is known to induce resistance to therapy.

References

1. Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 26:225–239. <https://doi.org/10.1007/s10555-007-9055-1>
2. Wang Y, Lyu Y, Tu K et al (2021) Histone citrullination by PADFI4 is required for HIF-dependent transcriptional responses to hypoxia and tumor vascularization. *Sci Adv* 7(35). <https://doi.org/10.1126/sciadv.abe3771>
3. Wicks EE, Semenza GL (2022) Hypoxia-inducible factors: cancer progression and clinical translation. *J Clin Invest* 132:e159839. <https://doi.org/10.1172/JCI159839>
4. Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3:721–732. <https://doi.org/10.1038/nrc1187>
5. Vaupel P, Multhoff G (2021) Revisiting the Warburg effect: historical dogma versus current understanding. *J Physiol (Lond.)* 599:1745–1757. <https://doi.org/10.1113/JP278810>
6. Feldman DE, Chauhan V, Koong AC (2005) The unfolded protein response: a novel component of the hypoxic stress response in tumors. *Mol Cancer Res* 3:597–605. <https://doi.org/10.1158/1541-7786.MCR-05-605>
7. Schwarz G (1909) Über Desensibilisierung gegen Röntgen- und Radiumstrahlen. *Münchener Med Wsch* 24:1–2

8. Müller C (1910) Eine neue Behandlungsmethode bösartiger Geschwülste. *Münchener Med Wschr* 28:1490–1493
9. Warburg O (1923) Versuche an überlebendem Carcinomgewebe. *Biochem Zschr* 152:319–344
10. Warburg O (1956) On the origin of cancer cells. *Science* 123:309–314
11. Chance B, Castor LN (1952) Some patterns of the respiratory pigments of ascites tumors in mice. *Science* 116(3008):200–202. <https://doi.org/10.1126/science.116.3008.200>
12. Chance B (1953) Dynamics of respiratory pigments of ascites tumor cells. *Trans NY Acad Sci* 16:74–75
13. Chance B, Hess B (1956) On the control of metabolism in ascites tumor cell suspension. *Ann NY Acad Sci* 63:1008–1016
14. Chance B (2005) Was Warburg right? Or was it that simple? *Cancer Biol Ther* 4:132–133. <https://doi.org/10.4161/cbt.4.1.1462>
15. Warburg OH (1962) Über die Klassifizierung der Krebszellen auf der Basis der Anaerobiose in vivo. In: *New methods of cell physiology*. Interscience Publisher, New York, pp 631–632
16. Semenza GL (2009) Regulation of cancer cell metabolism by hypoxia-inducible factor 1. *Semin Cancer Biol* 19:51–56. <https://doi.org/10.1016/j.semcancer.2008.11.009>
17. Davies PW, Brink F (1942) Micro-electrodes for measuring local oxygen tension in animal tissues. *Rev Sci Instrum* 13:524. <https://doi.org/10.1063/1.1769961>
18. Gray LH, Conger AD, Ebert M et al (1953) The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol* 312:638–648. <https://doi.org/10.1259/0007-1285-26-312-638>
19. Thomlinson RH, Gray LH (1955) The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer* 4:539–549. <https://doi.org/10.1038/bjc.1955.55>
20. Urbach F (1956) Pathophysiology of malignancy. I. Tissue oxygen tension of benign and malignant tumors of the skin. *Proc Soc exp Biol Med (N.Y.)* 92:644–649
21. Cater DB, Silver IA (1958) The measurement of oxygen tension in tumour. *Brit J Radiol* 31:340
22. Bergsjø P, Evans JC (1968) Oxygen tension of cervical carcinoma during the early phase of external irradiation. I. Measurements with a Clark micro electrode. *Scand J clin Lab Invest, Suppl* 106:159–166
23. Kolstad P (1968) Intercapillary distance, oxygen tension and local recurrence in cervix cancer. *Scand J clin Lab Invest, Suppl* 106:145–157
24. Gullino PM, Grantham FH, Courtney AH (1967) Utilization of oxygen by transplanted tumors in vivo. *Cancer Res* 27:1020–1030
25. Vaupel P et al (1971) Respiratory gas exchange and glucose metabolism of tumors (DS-carcinoma) in vivo. *Z exp Med* 156:283–294
26. Vaupel P et al (1972) Dreidimensionale Registrierung stationärer pO₂ – Werte im Tumorgewebe unter in vivo- Bedingungen bei Verwendung von Multi- Goldmikroelektroden. *Verhandl. Dtsch. Ges. Innere Medizin* 78:133–136. https://doi.org/10.1007/978-3-642-85448-4_22
27. Vaupel P et al (1973) Critical O₂ and glucose supply and microcirculation in tumor tissue. *Bibl Anat* 12:527–533
28. Vaupel P, Mayer A (2017) Tumor oxygenation status: facts and fallacies. *Adv Exp Med Biol* 977:91–99. https://doi.org/10.1007/978-3-319-55231-6_13
29. Vaupel P, Thews G (1974) pO₂ distribution in tumor tissue of DS-Carcinoma. *Oncology* 30:475–484. <https://doi.org/10.1159/000224991>
30. Grote J, Süsskind R, Vaupel P (1977) Oxygen diffusion constants D and K of tumor tissue (DS-Carcinoma) and their temperature dependence. *Adv Exp Med Biol*. https://doi.org/10.1007/978-1-4684-8890-6_47
31. Vaupel P, Piazena H (2023) Hyperhydration of cancers: A characteristic biophysical trait strongly increasing O₂, CO₂, glucose and lactate diffusivities, and improving thermophysical properties of solid malignancies. *Adv Exp Med Biol*. https://doi.org/10.1007/978-3-031-42003-0_22
32. Brown JM (1979) Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. *Br J Radiol* 620:650–656. <https://doi.org/10.1259/0007-1285-52-620-650>
33. Yamaura H, Matsuzawa (1979) Tumour regrowth after irradiation. *Int J Radiat Biol Rel Stud Phys Chem Med* 35:201–219
34. Mueller-Klieser W, Vaupel P, Manz R, Schmidseider R (1981) Intracapillary oxyhemoglobin saturation of malignant tumors in humans. *Int J Radiat Oncol Biol Phys* 10:1397–1404. [https://doi.org/10.1019/0360-3016\(81\)90036-5](https://doi.org/10.1019/0360-3016(81)90036-5)
35. Wendling P, Manz R, Thews G, Vaupel P (1984) Heterogeneous oxygenation of rectal carcinomas in humans: a critical parameter for preoperative irradiation? *Adv Exp Med Biol* 180:293–300. https://doi.org/10.1007/978-1-4684-4895-5_28
36. Chapman JD, Franko AJ, Sharpin (1981) A marker for hypoxic cells in tumours with potential clinical applicability. *Br J Cancer* 43:546–550. <https://doi.org/10.1038/bjc1981.79>
37. Sciandra JJ, Subjeck JR, Hughes CS (1984) Induction of glucose-regulated proteins during anaerobic exposure and of heat-shock proteins after reoxygenation. *Proc Natl Acad Sci USA* 15:4843–4847. <https://doi.org/10.1073/pnas.81.15.4843>
38. Groebe K, Vaupel P (1988) Evaluation of oxygen diffusion distances in human breast cancer xenografts using tumor-specific in vivo data: role of various mechanisms in the development of tumor hypoxia. *Int J Radiat Oncol Biol Phys* 15:691–697. [https://doi.org/10.1016/0360/3016\(88\)90313-6](https://doi.org/10.1016/0360/3016(88)90313-6)
39. Gatenby RA, Kessler HB, Rosenblum et al (1988) Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. *Int J Radiat Oncol Biol Phys* 14:831–838. [https://doi.org/10.1016/0360-3016\(88\)90002-8](https://doi.org/10.1016/0360-3016(88)90002-8)

40. Fishman JE, Joseph PM, Carvlin M et al (1989) In vivo measurements of vascular oxygen tension in tumors using MRI of a fluorinated blood substitute. *Invest Radiol* 24:65–71. <https://doi.org/10.1097/00004424-198901000-00014>
41. Vaupel P, Schlenger K, Knoop C, Höckel M (1991) Oxygenation of human tumors: Evaluation of tissue oxygen distributions in breast cancers by computerized O₂ tension measurements. *Cancer Res* 51:3316–3322
42. Höckel M, Schlenger K, Knoop C, Vaupel P (1991) Oxygenation of carcinomas of the uterine cervix: evaluation by computerized O₂ tension measurements. *Cancer Res* 51:6098–6102
43. Koh WJ, Rasey JS, Evans ML et al (1992) Imaging of hypoxia in human tumors with [F-18]-fluoromisonidazole. *Int J Radiat Oncol Biol Phys* 22:199–212. [https://doi.org/10.1016/3060-3016\(92\)91001-4](https://doi.org/10.1016/3060-3016(92)91001-4)
44. Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer as a site required for transcriptional activation. *Mol Cell Biol* 12:5447–5454. <https://doi.org/10.1128/mcb.12.12.5447-5454.1992>
45. Wang GL, Jiang B-H, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci USA* 92:5510–5514
46. Wang GL, Semenza GL (1993) General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Acad Sci USA* 90:4304–4308. <https://doi.org/10.1073/pnas.90.9.4304>
47. Wang GL, Semenza GL (1993) Characterization of hypoxia-inducible factor 1 and regulation of DNA-binding activity by hypoxia. *J Biol Chem* 268(29):21513–21518
48. Höckel M, Knoop C, Schlenger K et al (1993) Intratumoral pO₂ predicts survival in advanced cancer in the uterine cervix. *Radiother. Oncol.* 26:45–50. [https://doi.org/10.1016/0167-8140\(93\)90025-4](https://doi.org/10.1016/0167-8140(93)90025-4)
49. Höckel M, Schlenger K, Aral B et al (1996) Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res* 56:4509–4515
50. Vaupel P, Höckel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO₂ histography. *Antioxid Redox Signal* 9:1221–1235. <https://doi.org/10.1089/ars.2007.1628>
51. Swartz HM, Bacic G, Friedman et al (1994) Measurements of pO₂ in vivo, including human subjects, by electron paramagnetic resonance. *Adv Exp Med Biol* 361:119–128. https://doi.org/10.1007/978-1-4615-1875-4_16
52. Halpern HJ, Yu C, Peric M et al (1994) Oxymetry deep in tissues with low-frequency electron paramagnetic resonance. *Proc Natl Acad Sci USA* 13047–13051. <https://doi.org/10.1073/pnas91.26.13047>
53. Giaccia AJ (1996) Hypoxic stress proteins: survival of the fittest. *Semin Radiat Oncol* 6:46–58. [https://doi.org/10.1016/S1053-4296\(96\)80035-X](https://doi.org/10.1016/S1053-4296(96)80035-X)
54. Höckel M, Schlenger K, Höckel S et al (1998) Tumor hypoxia in pelvic recurrences of cervical cancer. *Int J Cancer* 79:365–369. [https://doi.org/10.1002/\(sci\)1097-0215\(19980821\)79:4<365:aid-ijc10>3.0.co;2-4](https://doi.org/10.1002/(sci)1097-0215(19980821)79:4<365:aid-ijc10>3.0.co;2-4)
55. Höckel M, Schlenger K, Höckel S, Vaupel P (1999) Hypoxic cervical cancers with low apoptotic index are highly aggressive. *Cancer Res* 59(18):4525–4528 328
56. Epstein AC, Gleadle JM, McNeill LA et al (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF prolyl hydroxylation. *Cell* 107(1):43–54. [https://doi.org/10.1016/S0092-8674\(01\)00507-4](https://doi.org/10.1016/S0092-8674(01)00507-4)
57. Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev*:225–239. <https://doi.org/10.1007/s10555-007-9055-1>
58. Moeller BJ, Richardson RA, Dewhirst MW (2007) Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev* 26:241–248. <https://doi.org/10.1007/s10555-007-9056-0>
59. Jonasch E, Donskov F, Iliopoulos O et al (2021) Belzutifan for renal cell carcinoma in von Hippel-Lindau disease. *N Engl J Med* 385:2036–2046. <https://doi.org/10.1056/NEJMoa2103425>
60. Vaupel P, Flood AB, Swartz HM (2021) Oxygenation status of malignant tumors vs. normal tissues: Critical evaluation and updated data source based on direct measurements with pO₂ microsenors. *Appl Magnet Reson* 52:1451–1479. <https://doi.org/10.1007/s00723-021-0138-6>

Microvascular Shunts, Intracranial Pressure, and the Impact of Drag-Reducing Polymers

Edwin M. Nemoto, Denis E. Bragin,
and Howard Yonas

Abstract

In the 50 years of my membership in ISOTT, I, Edwin M Nemoto, have enjoyed the application of many of the technologies developed in our society including microelectrodes for pH, PO₂, and near-infrared spectroscopy (NIRS) in the measurement of tissue oxygenation and metabolism. The greatest joy has been the number of great scientists I have had the pleasure of knowing and exchanging scientific ideas with across the United States, Europe, and Asia. This will be the enduring legacy of ISOTT for me personally as we continue beyond our half-century existence.

Every organ in our body, including the tegmentum, is endowed with microvascular shunts (MVS), which may be involved in physiological regulation, i.e. temperature regulation or pathophysiological responses to tis-

sue injury and oedema. MVS that open in response to increased capillary resistance and tissue oedema in the brain, heart, kidneys, liver, and muscles conduct neither nutrient nor gas exchange with tissue promoting tissue oedema in a vicious cycle. Pharmacologic arteriolar vasodilation cannot correct the MVS flow as may occur after a stroke or traumatic brain injury because pan arteriolar vasodilation would shunt flow to the normal tissue and away from the injured brain in a “reverse” steal or a “Robin Hood” phenomenon. A high molecular weight (4000 kDa) drag-reducing polymer (DRP) of polyethylene oxide or Lamiflo™ enhances blood flow by altering the physical dynamics of red blood cells (RBC) and blood flow, increasing the shear rate in the microvasculature and capillaries where shear rate is highest as it is inversely proportional to the 3rd power of blood vessel diameter. The shear rate sensed on the endothelium through the glycocalyx exerts precise control of endothelial function, including endothelial water permeability, nitric oxide synthase activity, lymphocyte adhesion to and transport across the endothelium, and microglial activation, all in response to low endothelial shear rate. DRP has proven effective in reversing MVS flow and increasing capillary flow in haemorrhagic shock, myocardial ischaemia, stroke, renal ischaemia, traumatic brain injury, stroke, sepsis, and Alzheimer’s Disease. Our aim is to

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establish the universality of MVS in the pathogenesis of vascular disease and in taking DRP to clinical treatment of vascular diseases.

Keywords

Brain perfusion · Cerebral perfusion pressure · Autoregulation · Traumatic brain injury · Lamiflo™

1 Introduction

How did we get to our description of microvascular shunts (MVS) in the brain? We first asked the question whether cerebral venous pressure is always higher than intracranial pressure (ICP) if brain perfusion is to persist. In three miniature swine, we placed catheters into a cortical cerebral vein, the cisterna magna (connected to a reservoir to increase ICP), in the sagittal sinus, and in the subarachnoid space and found that cortical cerebral venous pressure was always higher than ICP as ICP was increased. Based on these results, we hypothesised that ICP compression of cerebral veins increases venous back pressure into cerebral capillaries leading to tissue oedema and a viscous feedback loop of increasing ICP, increased cerebral venous pressure, and tissue oedema (Fig. 1) [1].

Histological sections of the human cerebrum also described “Thoroughfare Channels, TFC” [2, 3]. MVS were also suggested when neurosurgeons observed “red veins” upon opening the brain of traumatic brain injury (TBI) patients. Reports of “shunt peaks” in radioactive inert gas clearance methods of CBF measurements also suggested MVS [4]. Evidence of MVS in the brain was based upon the observation that when cerebral perfusion pressure (CPP) was decreased by increasing ICP instead of decreasing mean arterial pressure (MAP) as was conventionally done in describing the cerebral blood flow (CBF) autoregulation curve, the critical CPP fell from 50 mmHg to 30 mmHg [5, 6]. This was unexplained but we surmised that it was due to MVS maintaining a falsely elevated CBF at lower CPP [7]. Hudetz et al. [8] described two types of capil-

lary (CAP) flow in the brain with decreasing CPP: 95% with flow autoregulation and 5% without autoregulation which we surmised was indicative of MVS. What really piqued my interest in MVS was the Xenon-CT cerebral blood flow (CBF) image of a young 7-year-old girl whose left hemisphere was completely infarcted and who was in barbiturate coma for ICP control (Fig. 2). Upon seeing this tragic image, I was motivated to prove the existence of MVS in the brain using two-photon laser scanning microscopy (2PLSM) with my colleagues Dr Bragin, with his expertise in 2PLSM, and Dr Yonas, Chair of the Department of Neurosurgery, who invited me as the Director of Neurosurgery Research at the University of New Mexico, Albuquerque, NM.

2 Methods

2.1 Two-Photon Laser Scanning Microscopy Studies

Briefly, laboratory-acclimated, male, Sprague-Dawley rats weighing 250 to 300 grammes were studied by 2PLSM under 2% isoflurane/70%nitrous oxide/28% oxygen anaesthesia via a craniotomy over the left parietal cortex. Fluorescent serum (tetramethyl rhodamine isothiocyanate (TAMRA) dextran, 155 kDa in physiological saline, 5% wt./vol) was visualised using an Olympus BX 51WI upright microscope and water-immersion LUMPlan FL/IR 20X/0.50 W objective. Excitation was provided by a Prairieview Ultima multiphoton microscopy laser scan unit powered by a Millennia VI 6 W diode laser source pumping a Tsunami Ti: sapphire laser (Spectra-Physics, Mountain View, CA) tuned to 750-nm centre wavelength. Band-pass-filtered epifluorescence (560–660 nm for TAMRA and 425–475 nm for NADH) was collected by the photomultiplier tubes of the Prairie View Ultima system. Images (512 × 512 pixels, 0.15 µm/pixel in the x- and y-axes) or line scans were acquired using Prairie View software. Red blood cell flow velocity was measured in microvessels ranging from 3 to 25 µm diameter up

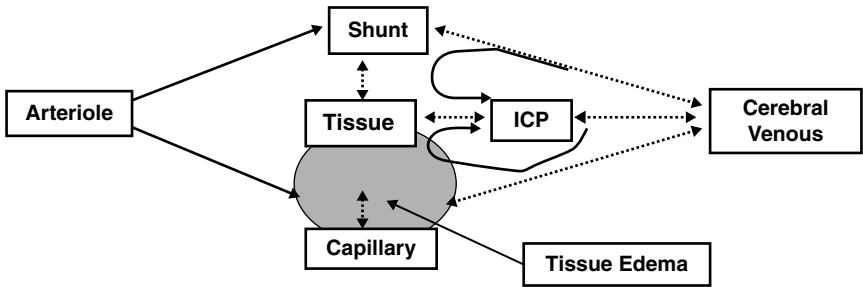


Fig. 1 Interaction of cerebrovascular and intracranial compartments resulting in the opening of thoroughfare channels (TFC) due to brain oedema, increased tissue pressure and capillary resistance secondary to increased intracranial pressure (ICP), and cerebral venous and capillary pressures. Dotted lines and double-ended arrows indi-

cate reciprocal interaction. Circular arrows indicate hydrostatic pressure recoil of ICP through the cerebral venous system back to the capillaries contributing to tissue oedema, raised ICP, and a further increase in cerebral venous and capillary pressures in a positive feedback loop [1]

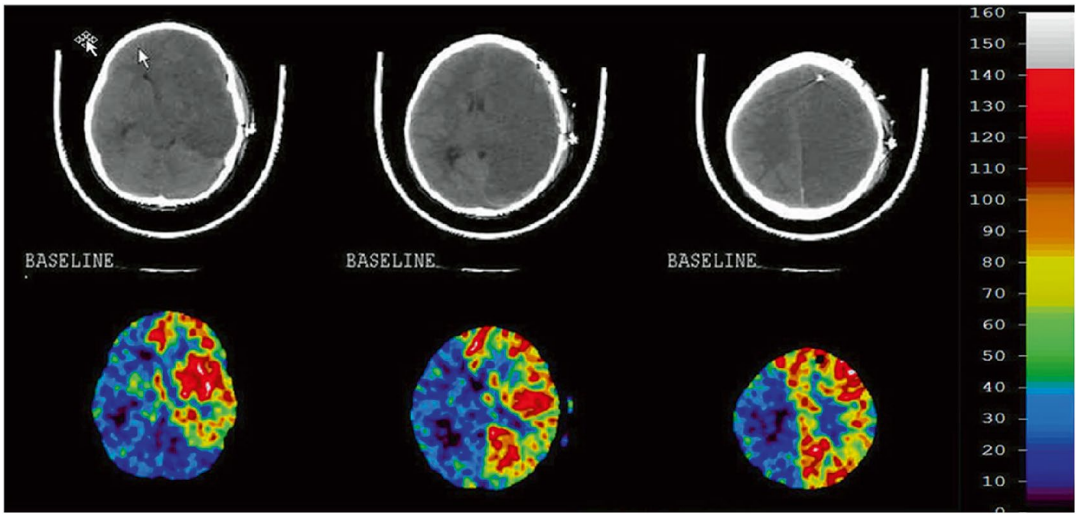


Fig. 2 Stable Xe-CT CBF images of a 7-year-old girl whose left hemisphere was completely infarcted on barbiturate coma to manage intracranial pressure. She eventually recovered but with severe neurologic deficits

to 500 μm below the surface of the parietal cortex as previously described [7, 8].

Tissue hypoxia was measured by NADH autofluorescence. Using NIH ImageJ software, three-dimensional anatomy of the vasculature in regions of interest was reconstructed from two-dimensional (planar) scans of the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack) were monitored intermittently. The rats were maintained at CPP of 70 mmHg with arterial blood gases and pH controlled within normal limits throughout the five hours of the study with measurements of

cerebral variables by 2PLSM and systemic physiological variables for 30 min at 30 min intervals.

3 Results

3.1 Development of Microvascular Shunts at High Intracranial Pressure

We studied the effect of high ICP on CAP and MVS flow and showed that an ICP of 30 mmHg increased the ratio of MVS/CAP flow which was

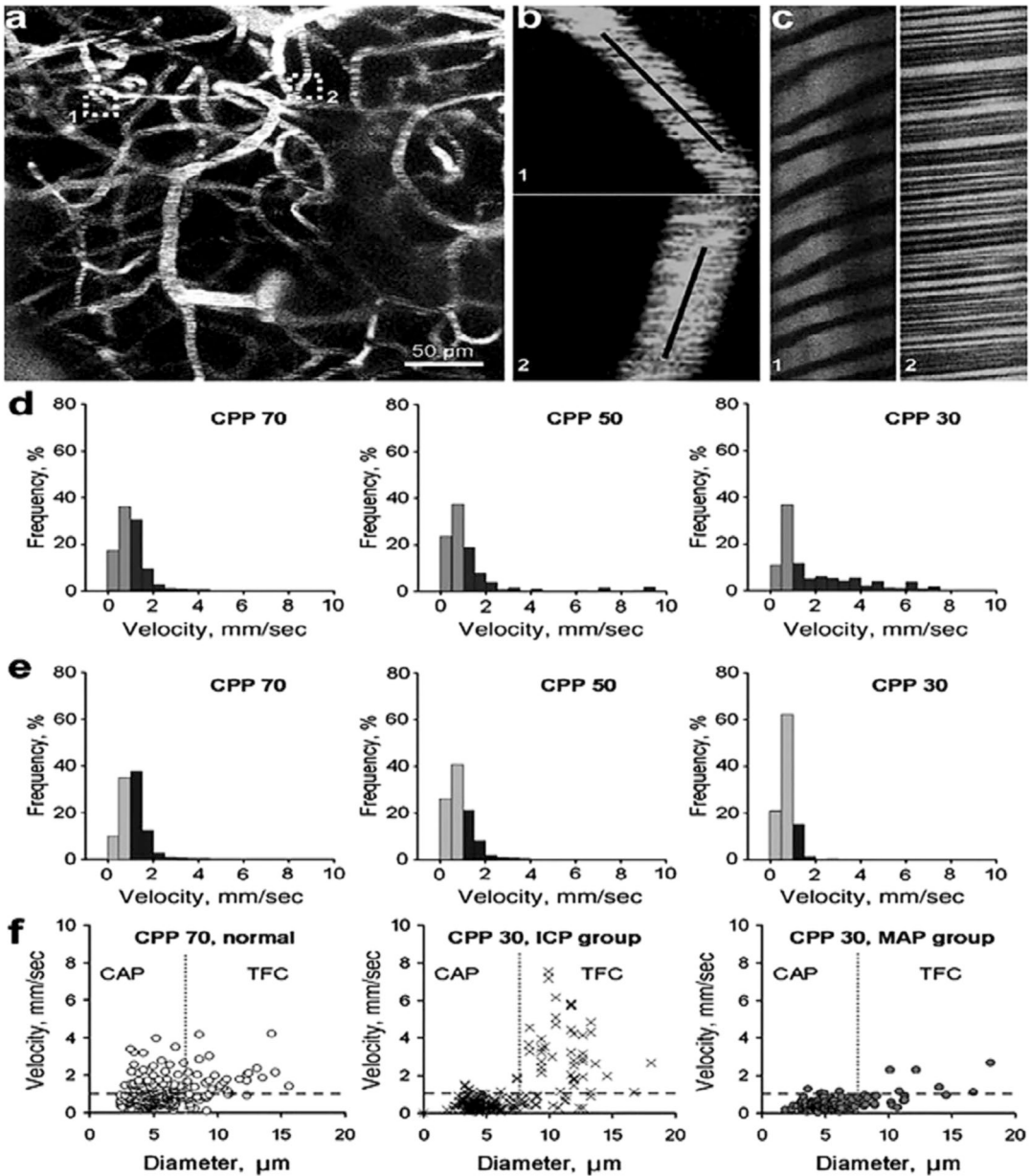


Fig. 3 (a) Z projection of region where microflow was recorded. (b) RBC flow in microvessels. (c) Line scan of blood flow velocities. (d) Normalised frequency histograms showing shift of flow velocities from below to above 1 mm/s by decreased CPP by increasing ICP ($n = 10$). (e) Normalised frequency histograms of the effect of decreasing CPP by decreasing

MAP reduced flow velocity without an increase in higher flow velocities. (f) Scatterplots of flow velocity at normal CPP of 70 mmHg and at CPP of 30 mmHg by increasing ICP and decreasing MAP showing the effect of increasing ICP on thoroughfare channels (TFC) in vessels greater than 8 μm which did not occur in the MAP group

reduced by increasing CPP, whereas in the MAP group, MVS flow did not occur (Fig. 3) [7]. MVS flow does not show flow autoregulation and does

not conduct gas or nutrient exchange with tissue and the volume of MVS appears to be directly correlated with ICP. CAP flux, NADH fluores-

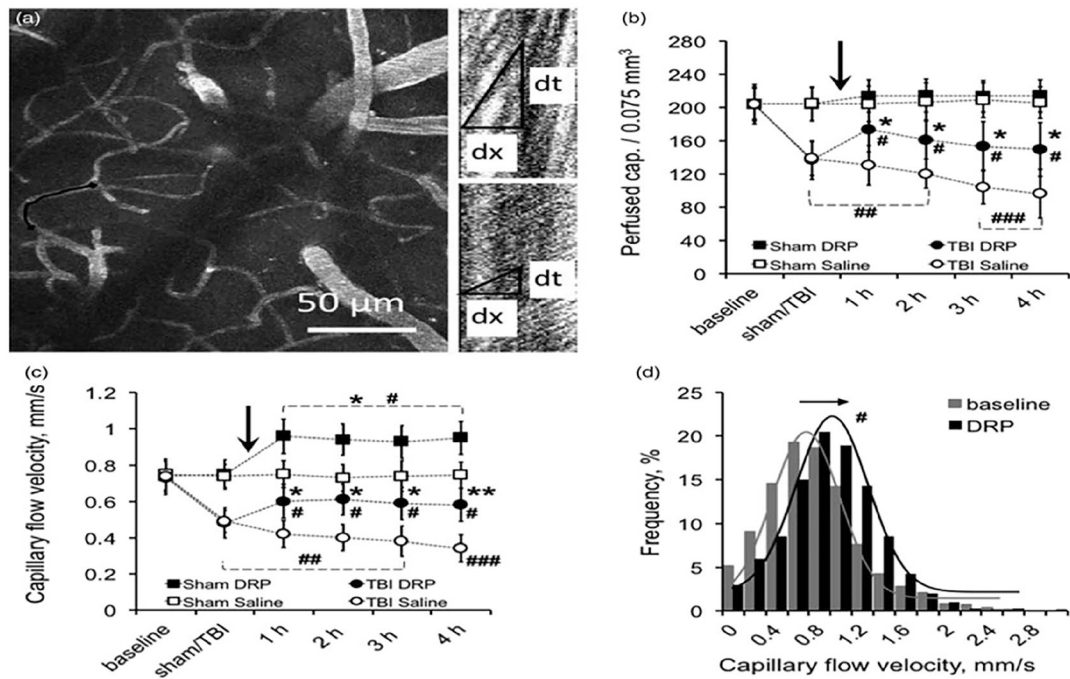


Fig. 4 (a) 2PLSM micrograph of region from which microvascular flow was recorded. (b) Graph showing a significant reduction in perfused capillaries after TBI and the improvement with DRP. $n = 10$ rats/group. $*P < 0.05$ from saline, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ from baseline. Arrow points to the point of saline or

DRP injection. (c) Time series plots showing reduction of capillary flow velocities with time after TBI and the effect of DRP. $*P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ from baseline. $n = 10$ rats/group. (d) Frequency histogram of capillary flow velocity distribution with DRP from baseline in one rat

cence, and water content were higher in the ICP group (Fig. 4) [8].

3.2 Microvascular Shunts and High ICP After Traumatic Brain Injury and Effects of a Drag-Reducing Polymer (Lamiflo™)

Our aim in this study on induced high ICP was to determine whether DRP, prepared from high molecular weight (4000 kDa) polyethylene oxide (PEO), would attenuate the transition from CAP to MVS flow at high ICP. We used a rat fluid percussion model of TBI induced by a gas-driven device; we developed TBI injury models of varying severity inducing a sustained increase in ICP of 20 mmHg (mild TBI), 30 mmHg (moderate TBI), and 40 mmHg (severe TBI) for up to 6 h

after injury. Using the moderate TBI model resulting in ICP of 30 mmHg for up to six hours after injury, our aim was to determine whether DRP at doses resulting in plasma levels of 1–2 ppm would improve perfusion, decrease MVS volume, and decrease ICP. DRP reduced MVS after TBI as reflected by the reduction in the MVS/CAP ratio, increased the number of perfused capillaries, increased tissue oxygenation, and showed a decrease in ICP and was effective in TBI complicated by haemorrhagic shock [9–11].

4 Conclusions

Low blood flow sensed by the glycocalyx provides precise control of endothelial function including activation of the inflammatory-immune, coagulopathic systems that can be

treated by DRP, selectively increasing endothelial shear rate without affecting normal flow. We will develop these concepts related to MVS and its resolution by DRP and other therapeutic interventions clinically.

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References

1. Nemoto EM (2006) Dynamics of cerebral venous and intracranial pressures. *Acta Neurochir Suppl* 96:435–437
2. Hasegawa T, Ravens JR, Toole JF (1967) Precapillary arteriovenous anastomoses. “Thoroughfare channels” in the brain. *Arch Neurol* 16:217–224
3. Ravens JR (1974) Anastomoses in the vascular bed of the human cerebrum. In: Cervos-Navaro J (ed) *Pathology of cerebral microcirculation*. Walter de Gruyter, Berlin, pp 16–38
4. Lassen NA (1959) Cerebral blood flow and oxygen consumption in man. *Physiol Rev* 39:138–238
5. Miller JD, Stanek A, Langfitt TW (1972) Concepts of cerebral perfusion pressure and vascular compression during intracranial hypertension. *Prog Brain Res* 35:411–432
6. Grubb RL Jr, Raichle ME, Phelps ME, Ratcheson RA (1975) Effects of increased intracranial pressure on cerebral blood volume, blood flow, and oxygen utilization in monkeys. *J Neurosurg* 43:385–398
7. Bragin DE, Bush RC, Müller WS, Nemoto EM (2011) High intracranial pressure effects on cerebral cortical microvascular flow in rats. *J Neurotrauma* 28:775–785
8. Bragin DE, Bush RC, Nemoto EM (2013) Effect of cerebral perfusion pressure on cerebral cortical microvascular shunting at high intracranial pressure in rats. *Stroke* 44:177–181
9. Bragin DE, Kameneva MV, Bragina OA, Thomson S, Statom GL, Lara DA, Yang Y, Nemoto E (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. *J Cereb Blood Flow Metab* 37(3):762–775
10. Bragin DE, Lara DA, Bragina OA, Kameneva MV, Nemoto EM (2018) Resuscitation fluid with drag reducing polymer enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock. *Adv Exp Med Biol* 1072:39–43
11. Bragin DE, Bragina OA, Trofimov A, Berliba L, Kameneva MV, Nemoto EM (2021) Improved cerebral perfusion pressure and microcirculation by drag reducing polymer-enforced resuscitation fluid after traumatic brain injury and hemorrhagic shock. *Acta Neurochir Suppl* 131:289–293

Optical Methods for the Study of Brain Metabolism In Situ

Joseph C. LaManna

Abstract

From its inception, the International Society of Oxygen Transport to Tissue (ISOTT) included several core members who were intrigued by the possibility of using light to probe the metabolic state of the cerebral cortex. This 50th anniversary review will focus on optical methods (UV, Visible, NIR) used to study brain oxygen metabolism in the intact brain. Chance, Lübbers, and Jöbsis did fundamental work in the 1970s and 1980s. These investigators spearheaded the use of fluorescence of pyridine nucleotides (e.g., NADH) and flavoprotein, and visible and near-infrared spectroscopy of haemoglobin amount and saturation, as well as the mitochondrial cytochrome chain. Two of our society awards are named for these pioneers and founders. By the new millennium, their many scientific descendants (although still too few for the task at hand) have been productive and moved the field forward. Technical advances in materials, detectors, devices, and computer power have made great advances possible. Physiological theory and understanding of cell and molecular mechanisms still lag, pro-

viding fertile territory for future goals. There are at least two major directions currently that will certainly continue to illuminate brain metabolism (obvious—and well worn—pun intended). These are the burgeoning NIR instrumentation variations, and second, the tremendous potential of two-photon phosphorescence lifetime microscopy. These advances have been introduced through the years and continue to be advanced at our annual ISOTT symposia.

Keywords

Jöbsis · Lübbers · Chance · NADH fluorescence · Visible spectrophotometry · Near-infrared spectroscopy · NIRS

1 Lübbers and Visible Wavelength Reflection Spectrophotometry

In 1959, Dietrich Werner Lübbers (1917–2005) [1] introduced what would become the “*rapid-spectroscope*” [2]. This instrument was used to record reflection spectra in the visible wavelength range. The photomultiplier circuit design and construction was apparently borrowed from Gerhard Thews, the Society’s first elected president (personal communications from Oliver Thews and Peter Vaupel). Lübbers applied the

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method to intact brain preparations in situ in animals with haemoglobin removed by saline perfusion to avoid the complications of spectral interference from haemoglobin absorption spectrum. He was a member of the International Committee for the very first ISOTT meeting in 1973 and was one of the first presenters at the meeting. It was presented in the very first ISOTT session “Instrumentation and Methods”, where he was one of the session chairs along with William Whalen and Leland Clarke, reminding us that instrumentation and methods were a primary interest and origin motivation of the Society. The paper (he had five at the meeting) was a study of the haemoglobin spectrum from human skin [3]. Lübbers was the president of ISOTT in 1982, hosting the meeting that year in Dortmund. The Lübbers award was established in 1994. David Harrison published an extended biography of Lübbers, presented at the Louisville meeting in 2006 [1].

Lübbers research and presentations at ISOTT meetings in the 1970s and 1980s helped to inform and inspire my own research at the time. My first ISOTT paper, presented at the Anaheim meeting in 1976, was a collaboration with Laz Mandel while we were at Duke and described a “rapid scanning spectrophotometer” for in vivo application [4].

2 Chance and In Situ Brain Mitochondrial Fluorescence

Britton Chance (1913–2010) had a remarkable career and is a significant contributor to and influence on the society. There is a detailed summary of his life and career, presented at the Georgetown meeting in 2011 [5]. Out of all his accomplishments and contributions, this review will discuss the small subset that includes pyridine nucleotide and flavoprotein fluorescence. The initial observations were reported in *Science* in 1962 [6]. NADH was still known as DPNH at that time. (Notably, one of the authors of this

chapter was Frans Jöbsis). Most of the early brain studies were done in collaboration with Avram Mayevsky. Indeed, Mayevsky was a participant of the 1973 meeting and authored two papers [7, 8]. These two papers laid out the theory of in vivo NADH fluorometry and described a method using implanted cannulae that included quartz fibre optics. He has provided an extensive review of his work in book form [9].

A major advance in metabolic fluorometry was the idea to measure fluorescence from both NAD and flavoprotein [10]. The advantage of the Fp/PN ratio method is that the signal intensity due to the redox based fluorescence of Fp is opposite to that of NADH (e.g., the reduced form of NADH fluoresces, but the oxidised form of flavoprotein fluoresces. This has the property of cancelling the non-specific optical interference. Another useful innovation was also used in that study, that of the freeze-trapping instrument of Quistorff reported at the first Cambridge meeting in 1978 [11]. Lin Z. Li (ISOTT President in 2015) and his colleagues are currently continuing the metabolic monitoring efforts at the Johnson Foundation at the University of Pennsylvania.

Jöbsis continued to study NADH fluorescence from intact brain after moving to Duke University to establish his lab, primarily by his collaboration with Mike Rosenthal [12]. Rosenthal’s significant contributions were to pursue the technique of using the signal from the reflected excitation light as a calibrated reference signal and to show that the amplitude of the NADH oxidation response to electrical stimulation was correlated with stimulus intensity and extracellular potassium ion concentration [13, 14]. Another important observation was that the metabolic response to brief stimulation persisted for minutes rather than seconds, a finding that was corroborated when BOLD fMRI became available, e.g., [15].

The other major group working with NADH fluorescence in situ was in Budapest, Hungary, where Arisztid Kovach (ISOTT President in 1980) had established a productive lab which included E. Dora, L. Gyulai, A. Hudetz (ISOTT

President in 1997, Knisely 1983), and A. Eke (ISOTT President in 1998, Knisely 1984) [16]. Kovach was an early contributor to the ISOTT meetings, serving on the International Committee for the 1975 meeting in Mainz.

3 Jöbsis and NIRS

Jöbsis played an important role in the early years of the society, in part due to his connection with the American Physiological Society, where he was on the programme committee for the annual FASEB meetings. So he was able to programme the 1974 meeting of ISOTT as a session of the Federation meeting that year in Atlantic City. Proceedings of that meeting can be found in *Microvascular Research* vol. 8, pp 275–376. Two years later, Jöbsis organised the five-day Colloquium on Oxygen and Physiological Function, which also included the ISOTT session for that year in Anaheim. These proceedings were published in book of the same name [17].

In the mid-1970s, Jöbsis turned his attention to the near-infrared optical spectrum. The main advantage of NIR techniques for in vivo monitoring is the much deeper penetrating power compared to light in the visible spectrum. A second advantage is that the relative absorption of haemoglobin to cytochrome is much more favourable. The paper that established the field of NIR spectroscopy appeared in *Science* in 1977 [18]. The history of this significant advance has been well-documented in an issue of the *Journal of Biomedical Optics* in 2007 [19].

Jöbsis announced his progress in NIRS at the Nijmegen ISOTT meeting in 1984 with a series of seven presentations (Fig. 1). It should be noted that the presentation following these seven was by Marco Ferrari [20] who made the important contribution of showing that tissue oxygen saturation could be quantitatively estimated from the

NIRS method. This was the first paper that revealed the promise of the power of NIRS, that it could provide quantitative results.

It was David Delpy (University College London and Co-chair of ISOTT 1996) who fully established the NIRS technique. He was at the 1986 meeting in Cambridge [21], and the following year in Sapporo, Japan, presented with M. Cope the key paper for NIRS quantitation, measuring or estimating pathlength [22]. The group of Mamoru Tamura in Japan also presented their NIRS methodology at the Cambridge meeting [23]. The work is being carried on by Clare Elwell (ISOTT President in 2014, Knisely 1995) [24], Chris Cooper (Knisely 1997) [25], Gemma Bale (Lübberts 2016), and Ilias Tachtsidis (ISOTT President elect 2025, Knisely 2007) [26].

Current ISOTT members who are leading the efforts in NIRS include Ursula Wolf (ISOTT President 2020–2022), Felix Scholkmann (Knisely 2017, Lübberts 2014, Chance 2012), and Martin Wolf (ISOTT President 2010, Knisely 1998) in Switzerland. But this is a topic that will be covered in greater detail in a separate paper in this volume by Ursula Wolf.

4 Closing Thoughts

This review has attempted to discuss the early research in optical techniques used for brain oxygen metabolism studies and to show how the scientists developing these methods were integral in the founding of ISOTT. The progress of these studies benefitted strongly from being presented for critical discussion at the annual meetings as documented in the published proceedings of the society. The review also includes many examples of how the scientists involved in brain optical methods were leaders of the society; many served as the president, many were society award winners.

Fig. 1 This is a page from the table of contents of Oxygen Transport to Tissue VII, edited by Kreuzer, Cain, Turek and Goldstick, Adv. Exp. Med. Biol. Vol.191, p.xix, 1985. This was the proceedings of the 1984 ISOTT meeting in Nijmegen, The Netherlands

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References

1. Harrison D (2008) Dietrich W. Lubbers: celebration of a life dedicated to research into oxygen transport to tissue. Adv Exp Med Biol 614:9–17
2. Lubbers D, Niesel W (1959) [The short-term spectral analysator; a high-speed spectral photometer for continuous measurement of absorption or extinction spectra]. Pflugers Arch Gesamte Physiol Menschen Tiere 268:286–295.
3. Lubbers D (1973) Spectrophotometric examination of tissue oxygenation. Adv Exp Med Biol 37a:45–54
4. Mandel L, Riddle T, LaManna J (1977) A rapid scanning spectrophotometer and fluorometer for in vivo monitoring of steady-state and kinetic optical properties of respiratory enzymes. In: Jöbsis F (ed) Oxygen & physiological function. Professional Information Library, Dallas, pp 72–78

5. Li L, Nioka S, Kang K (2013) Dedication: Britton Chance, Md, PhD, Dsc. In: Welch W, Plum F, Bruley D, Harrison D (eds) *Oxygen transport to tissue Xxxiv*. Springer, New York. 765: p V–Xiv
6. Chance B, Cohen P, Jobsis F, Schoener B (1962) Intracellular oxidation-reduction states in vivo. *Science* 137:499–508
7. Chance B, Oshino N, Sugano T, Mayevsky A (1973) Basic principles of tissue oxygen determination from mitochondrial signals. *Adv Exp Med Biol* 37a:277–292
8. Mayevsky A, Chance B (1973) A new long-term method for the measurement of nadh fluorescence in intact rat brain with chronically implanted cannula. *Adv Exp Med Biol* 37a:239–244
9. Mayevsky A (2015) *Mitochondrial function in vivop evaluated by nadh fluorescence*. Springer International Publishing, Cham. Imprint: Springer: 1 Online Resource (Xiv, 276 Pages 234 Illustrations, 263 Illustrations In Color
10. Chance B, Schoener B, Oshino R et al (1979) Oxidation-reduction ratio studies of mitochondria in freeze-trapped samples. Nadh and flavoprotein fluorescence signals. *J Biol Chem* 254:4764–4771
11. Chance B, Quistorff B (1978) Study of tissue oxygen gradients by single and multiple indicators. *Adv Exp Med Biol* 94:331–338
12. Rosenthal M, Jobsis F (1971) Intracellular redox changes in functioning cerebral cortex. Ii. Effects of direct cortical stimulation. *J Neurophysiol* 34:750–762
13. Rosenthal M, Somjen G (1973) Spreading depression, sustained potential shifts, and metabolic activity of cerebral cortex of cats. *J Neurophysiol* 36:739–749
14. Lothman E, Lamanna J, Cordingley G et al (1975) Responses of electrical potential, potassium levels, and oxidative metabolic activity of the cerebral neo-cortex of cats. *Brain Res* 88:15–36
15. Chen Z, Calhoun V (2012) Volumetric bold fmri simulation: from neurovascular coupling to multivoxel imaging. *BMC Med Imaging* 12:8
16. Kovach A, Eke A, Dora E, Gyulai L (1976) Correlation between the redox state, electrical activity and blood flow in cat brain cortex during hemorrhagic shock. *Adv Exp Med Biol* 75:289–297
17. Jobsis F (1977) *Oxygen & physiological function*. Dallas, Professional Information Library
18. Jobsis F (1977) Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 198:1264–1267
19. Delpy D, Ferrari M, Piantadosi C, Tamura M (2007) *Pioneers in biomedical optics: special section honoring Professor Frans F. Jobsis of Duke University*. *J Biomed Opt* 12:062101
20. Ferrari M, Giannini I, Sideri G, Zanette E (1985) Continuous non invasive monitoring of human brain by near infrared spectroscopy. *Adv Exp Med Biol* 191:873–882
21. Van Der Zee P, Delpy D (1987) Simulation of the point spread function for light in tissue by a Monte Carlo method. *Adv Exp Med Biol* 215:179–191
22. Cope M, Delpy D, Reynolds E et al (1988) Methods of quantitating cerebral near infrared spectroscopy data. *Adv Exp Med Biol* 222:183–189
23. Hazeki O, Seiyama A, Tamura M (1987) Near-infrared spectrophotometric monitoring of haemoglobin and cytochrome A, A3 in situ. *Adv Exp Med Biol* 215:283–289
24. Elwell C, Cope M, Edwards A et al (1992) Measurement of cerebral blood flow in adult humans using near infrared spectroscopy--methodology and possible errors. *Adv Exp Med Biol* 317:235–245
25. Cooper C, Sharpe M, Elwell C et al (1997) The cytochrome oxidase redox state in vivo. *Adv Exp Med Biol* 428:449–456
26. Bale G, Elwell C, Tachtsidis I (2016) From jobsis to the present day: a review of clinical near-infrared spectroscopy measurements of cerebral cytochrome-C-oxidase. *J Biomed Opt* 21:091307

Part II

Brain

Brain Tissue Oxygen Dynamics Under Localised Hypoxia in the Awake State and the Physical Neuroprotective Effects of General Anaesthesia

E. D. Doubovikov and D. P. Aksenov

Abstract

Brain health directly depends on maintaining a level of tissue oxygen that is high enough to avoid global hypoxia and local brain ischaemia. It is well documented that general anaesthesia has an anti-hypoxic neuroprotective effect. Previous studies of this effect primarily assessed the biochemical actions of anaesthetics. Physical actions were not well studied because the quantification of oxygen dynamics has only recently been described. Based on known oxygen, blood, and neuronal measurements, under various anaesthesia protocols and in the awake state, we mathematically analysed physical anaesthesia effects on oxygen distribution for localised hypoxia. From this, we built a universal equation of oxygen dynamics which can be applied to

both animal and human subjects in awake and anaesthetised states, under normoxia, hyperoxia, and hypoxia. Using this equation, we determined that a proper anaesthesia protocol can protect up to 167 mm³ of local hypoxic cortical brain tissue via oxygen diffusion from healthy neighbouring areas.

Keywords

Neuroprotection · Cerebral ischemia · Stroke · Oxygen delivery · Sevoflurane · Isoflurane

1 Introduction

The dynamics of oxygen in brain tissue comprise one of the most important fundamental problems in neuroscience and medicine. The brain is a metabolically demanding organ and its health directly depends on maintaining a sustainable level of tissue oxygen. When this level decreases, the surrounding brain tissue goes into a hypoxic state with a high risk of neuronal death leading to an area of infarction. Here, we analyse and compare brain tissue oxygen dynamics in the healthy awake state, during global and local hypoxia, and under general anaesthesia, which is suggested to have neuroprotective and anti-hypoxic effects. Our study is focused mainly on the cerebral cortex, which represents 80% of human brain mass.

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The neuroprotective effects of general anaesthesia have been reported for decades. The initial finding [1] showed that after global hypoxia, the survival time was significantly increased in groups of mice exposed to isoflurane. Other studies have since confirmed the neuroprotective effects of general anaesthesia under various protocols and pathological states, including stroke [2], neonatal hypoxia-ischaemia [3], and even hypoxic models on a cell culture [4]. Biochemical mechanisms for these effects have been suggested. For example, treatment with isoflurane revealed an activated S1P/PI3K/Akt signalling pathway [3], which has been shown to participate in preventing apoptosis due to hypoxia [5]. In addition, these neuroprotective effects can also have physical mechanisms of action.

Based on oxygen and neuronal measurements under various anaesthesia protocols and in the awake state, we analysed the effects of anaesthesia on the oxygen distribution for localised hypoxia. We hypothesised that the dynamics of brain tissue oxygen are substantially different under awake and anaesthetised states, and that the anaesthetised state indeed has an immediate and profound neuroprotective effect which can be explained in terms of physical dynamics and the diffusion of oxygen.

2 Methods

2.1 Finite Difference Method

The equation describing the oxygen dynamics in tissue, which is derived from an established model of oxygen transport and re-uptake in extracellular space [6], is given by

$$\frac{d\phi_T}{dt} = D \frac{d^2\phi_T}{dx^2} - R(\phi_T - \phi_V) - \Psi \quad (1)$$

Where ϕ_T , ϕ_V denote oxygen concentration in brain tissue and venules, respectively, D is the diffusivity coefficient (m^2/s), R is a rate constant ($1/\text{s}$) representing passive diffusion into neighbouring venules, and Ψ is the rate of neuronal consumption (mmHg/s). Note that implicit in Eq. (1) is the requirement that $\phi_T(x, t) > \phi_V(x, t)$, since

venules cannot effuse oxygen into tissue. Thus, the location of capillaries must be sufficiently close enough to ensure an adequate supply of oxygen. Both R and Ψ depend on the density of capillaries and neurons in the brain tissue, respectively. The firing rate also effects the neuronal consumption rate Ψ . For this study, we set $D = 1000 \mu\text{m}^2/\text{s}$ [7]. Ψ is calculated as amount of oxygen consumption per spike (mmHg/spike) multiplied by the firing rate (spike/s) and scaled by the volume density of neurons within a cubic volume (a.u.), which serves as a proxy of CMRO_2 . The amount of oxygen consumption per spike is set to $0.26 \text{ mmHg}/\text{spike}$ in accordance with Ozuger et al. [8]. R is set to a flat rate constant of $1 (1/\text{s})$ which is scaled by the capillary density (a.u.) [6]. We note that R is set to zero when $\phi_T - \phi_V < 0$ as otherwise this would imply that venules diffuse oxygen to tissue (which is physiologically inconsistent). The equation is numerically iterated using a 3-dimensional Alternating-Direction Implicit (ADI) scheme until a steady state is reached, implemented in CUDA in accordance with the methodology described by Chopp [9]. Capillaries were distributed over a 3-dimensional grid using a Monte-Carlo method.

We assume that the sensory-motor cortex has an average capillary density of 385 capillaries per mm^2 [10], a neuron density of 200,000 neurons per mm^3 [11], and an average baseline firing rate of 3 Hz [12]. We compare the effects of two inhalation anaesthetics, sevoflurane and isoflurane. Sevoflurane was chosen because it is one of the most common anaesthetics in the USA [13], while isoflurane is frequently employed in research as well as in clinical practice in developing countries [14]. Moreover, we consider the anaesthetics, sevoflurane and isoflurane, due to their different respective physiological effects on the neurovascular unit. Specifically, 1 minimum alveolar concentration (MAC) of sevoflurane causes an increase in the cerebral blood flow by 7% [15, 16] of the baseline level, a decrease in the respiratory rate by 36%, and a decrease in the firing rate to 11% of its baseline [17]. Meanwhile, 1 MAC of isoflurane results in increases in the cerebral blood flow by 35% above the baseline

[15, 16], decreases the respiratory rate by 30%, and decreases the firing rate to 25% of the baseline frequency [17]. Along with anaesthesia, we also consider the delivery of supplemental oxygen. The treatment of a patient with 60% and 100% supplemental oxygen leads to an average increase of 110% and 260% of the baseline oxygen level, respectively [18]. Assuming an average baseline of 30 mmHg [19] oxygen pressure in the capillaries, supplemental oxygen at 100% would cause an increase in the capillary oxygen pressure to $(30 \cdot (1 + 0.07 - 0.36 + 2.6)) = 99.3$ mmHg when used with sevoflurane and increase to $(30 \cdot (1 + 0.35 - 0.3 + 2.6)) = 109.5$ mmHg when used with isoflurane.

3 Results

3.1 Oxygen Delivery Under Local Ischaemia

Using our finite difference method, and using a grid size of $128 \times 128 \times 128$, we looked at the steady state oxygen distribution under normal resting state conditions and under stimulation. Figure 1 shows the steady state oxygen level (at $z = 64$) in the cerebral cortex when local brain area in a diameter of 1 or 1.5 mm loses the blood supply (Fig. 1a, c). Figure 1b shows the oxygen concentration during sustained stimulation (at $z = 64$) for the areas of infarction (1 mm in diameter). Under an infarction radius of 1 mm, stimulation causes oxygen to deplete at the centre of the infarction.

3.2 Treatment of Localised Hypoxia with Anaesthesia and Supplemental Oxygen

Applying the mathematical model in one dimension, we calculated the maximum distance that oxygen can be provided from an oxygen source under increasing rates of neuronal activity for a neuronal population of 100,000 per mm^3 and 500,000 per mm^3 (Fig. 2a). Using properties of the cerebral cortex, we simulated the maximum

provided oxygen in the centre of infarction (i.e. the lowest level of oxygen concentration in the tissue) under increasing diameters of vascular damage, after separately applying sevoflurane and isoflurane, along with adding 100% supplemental oxygen (Fig. 2b). Sevoflurane can provide sufficient oxygen up to an ischaemic diameter of around 6000 microns, while isoflurane provides oxygen up to a diameter of about 5000 microns.

4 Discussion and Conclusion

We used a combination of established and novel techniques to build a detailed model of brain tissue oxygen dynamics in the healthy, awake state as well as under local and global hypoxia and effects of various protocols of general anaesthesia. We uncovered the spatial limits for when oxygen diffusion from healthy brain regions can no longer compensate for localised hypoxia and for a physical mechanism of how anaesthesia provides immediate neuroprotective effects during hypoxia. Previous mathematical models of oxygen diffusion that used a rate constant to account for effusion of oxygen into venules did not include the differential between oxygen concentration in the capillaries versus the venules. In the areas of ischaemia, any effusion of oxygen into venules is not expected, and thus our model accounts for the fact that the primary mechanism of oxygen depletion is via direct consumption with neurons. A limitation of our approach is that we did not explicitly include venules in our 3D grid and accounted for this by scaling our rate constant in accordance with the capillary density to account for passive effusion.

Brain tissue oxygen dynamics are very different under anaesthesia due to several factors, including: decreased neuronal consumption, the level of vasodilation, frequency of oxygen oscillations, the rate of spontaneous respiration or volume of mechanical ventilation, and the concentration of supplemental oxygen. Under 100% oxygen and the administration of sevoflurane, almost 167 mm^3 of cerebral tissue will get a sufficient oxygen supply from neighbouring areas. In this scenario, sevoflurane has higher

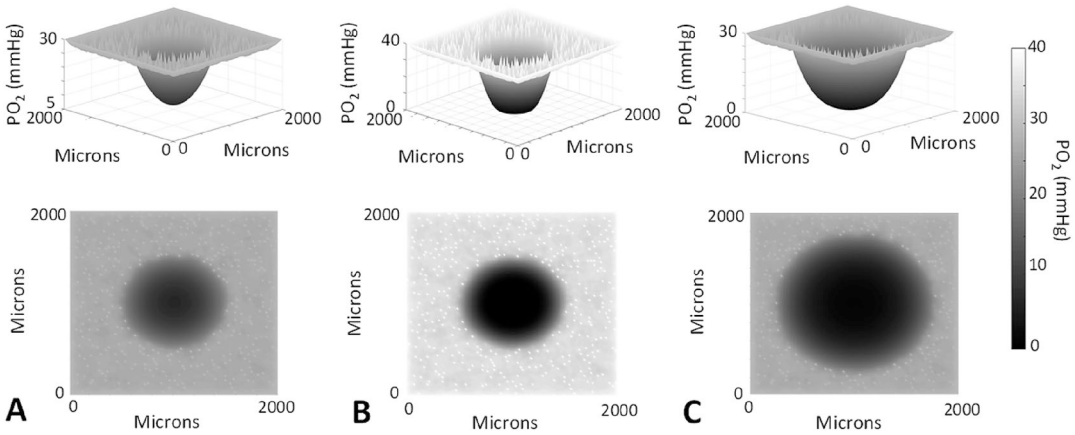


Fig. 1 The relationship between area of ischaemia and neuronal firing rate at low frequencies. Brain tissue oxygen in the cerebral cortex does not go below 5 mmHg if 1 mm area cannot be supplied by oxygen through capillaries (area of potential ischaemia (API)) and neuronal firing rate is 3 Hz (a). The oxygen diffusion from neighbouring tissue can provide sufficient oxygen in the centre of API as indicated by 3D upper panel and 2D lower panel. Note

that a 3 Hz baseline firing rate is typical for somatosensory cortex in awake state. However, during sensory stimulation, when firing rate increases up to 8 Hz and functional hyperaemia delivers more oxygen (40 mmHg), brain tissue oxygen goes down to 0 mmHg in the centre of API (b). If API increases up to 1.5 mm in diameter, hypoxia is observed in the API under resting state (c)

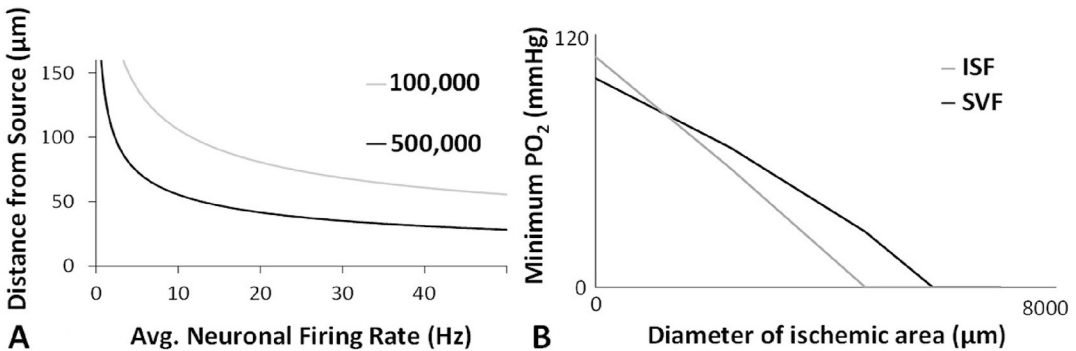


Fig. 2 Relationship between neuronal firing rate and oxygen delivery and the effects of IMAC sevoflurane and isoflurane anaesthesia with supplemental oxygen on ischaemic areas in the brain. The distance of delivery oxygen from a single source decreases non-linearly with respect to an increase in the firing rate, with a lower neuronal den-

sity (100,000 neurons per cubic mm) able to provide higher oxygen at the same firing rate relative to a higher neuronal density (500,000 neurons per cubic mm) (a). For the cortex, sevoflurane along with 100% oxygen can cover up to 6 mm diameter of ischaemic area, while isoflurane only allows for up to 5 mm brain tissue to be saved (b)

physical anti-hypoxic properties than isoflurane due to the greater suppression of neuronal activity [17]. Overall, our results suggest that in the case of single or multiple small areas of interrupted cerebral blood flow, any type of environmental stimulation or behavioural task should be avoided to protect the largest volume of brain tissue from hypoxic conditions.

Currently, there is limited clinical evidence to guide anaesthetic choice under acute ischaemic stroke, which is likely due to the methodological shortcomings of preclinical studies [20]. Our results indicate that the previous confusion regarding the general neuroprotective effects of anaesthesia can be related to the tangled interpretation of the physical neuroprotective effect in

humans and animal models. This effect seems to be extremely useful only in case of localised strokes up to 6 mm in diameter. We also argue that the scope and application of the results described above should be applicable to both single and disseminated ischaemic strokes.

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References

1. Newberg LA, Michenfelder JD (1983) Cerebral protection by isoflurane during hypoxemia or ischemia. *Anesthesiology* 59:29–35
2. Chen F, Long Z, Yin J, Zuo Z, Li H (2015) Isoflurane post-treatment improves outcome after an embolic stroke in rabbits. *PLoS One* 10:e0143931
3. Zhou Y, Lekic T, Fathali N, Ostrowski RP, Martin RD, Tang J, Zhang JH (2010) Isoflurane posttreatment reduces neonatal hypoxic-ischemic brain injury in rats by the sphingosine-1-phosphate/phosphatidylinositol-3-kinase/Akt pathway. *Stroke* 41:1521–1527
4. Wise-Faberowski L, Raizada MK, Summers C (2001) Oxygen and glucose deprivation-induced neuronal apoptosis is attenuated by halothane and isoflurane. *Anesth Analg* 93:1281–1287
5. Hasegawa Y, Suzuki H, Sozen T, Rolland W, Zhang JH (2010) Activation of sphingosine 1-phosphate receptor-1 by FTY720 is neuroprotective after ischemic stroke in rats. *Stroke* 41:368–374
6. Nicholson C (2001) Diffusion and related transport mechanisms in brain tissue. *Rep Prog Phys* 64(7):815
7. Suwa K (1992) Analysis of oxygen transport to the brain when two or more parameters are affected simultaneously. *J Anesth* 6(3):297–304
8. Ozugur S, Kunz L, Straka H (2020) Relationship between oxygen consumption and neuronal activity in a defined neural circuit. *BMC Biol* 18:76
9. Chopp D (2019) Introduction to high performance scientific computing. SIAM
10. Borowsky IW, Collins RC (1989) Metabolic anatomy of brain: a comparison of regional capillary density, glucose metabolism, and enzyme activities. *J Comp Neurol* 288:401–413
11. Diene LD, Costa-Ferro ZSM, Barbosa S, Milanese BB, Lazzari GZ, Neves LT, Paz LV, Neves PFR, Battisti V, Martins LA, Gehlen G, Mestriner RG, Da Costa JC, Xavier LL (2019) Selective brain neuronal and glial losses without changes in GFAP immunoreactivity: young versus mature adult Wistar rats. *Mech Ageing Dev* 182:111128
12. Aksenov DP, Li L, Miller MJ, Iordanescu G, Wyrwicz AM (2015) Effects of anesthesia on BOLD signal and neuronal activity in the somatosensory cortex. *J Cereb Blood Flow Metab* 35:1819–1826
13. Barash P, Cullen B, Stoelting R, Cahalan M, Stock C (2011) Clinical anesthesia. Lippincott Williams & Wilkins
14. Tobias JD, Kim Y, Davis J (2002) Anesthetic care in developing countries: equipment and techniques. *South Med J* 95:239–247
15. Li CX, Patel S, Wang DJ, Zhang X (2014) Effect of high dose isoflurane on cerebral blood flow in macaque monkeys. *Magn Reson Imaging* 32:956–960
16. Matta BF, Heath KJ, Tipping K, Summors AC (1999) Direct cerebral vasodilatory effects of sevoflurane and isoflurane. *Anesthesiology* 91:677–680
17. Aksenov DP, Miller MJ, Dixon CJ, Wyrwicz AM (2019) The effect of sevoflurane and isoflurane anesthesia on single unit and local field potentials. *Exp Brain Res* 237:1521–1529
18. Menzel M, Doppenberg EM, Zauner A, Soukup J, Reinert MM, Bullock R (1999) Increased inspired oxygen concentration as a factor in improved brain tissue oxygenation and tissue lactate levels after severe human head injury. *J Neurosurg* 91:1–10
19. Parpaleix A, Goulam Houssen Y, Charpak S (2013) Imaging local neuronal activity by monitoring PO(2) transients in capillaries. *Nat Med* 19:241–246
20. Archer DP, Walker AM, McCann SK, Moser JJ, Appireddy RM (2017) Anesthetic neuroprotection in experimental stroke in rodents: a systematic review and meta-analysis. *Anesthesiology* 126:653–665

Cerebral Oximetry in the Neonatal Intensive Care Unit: Discussion of Two Contradicting Study Results (SafeBoosC-II, SafeBoosC-III)

Martin Wolf, Marco Ferrari, and Felix Scholkmann

Abstract

Recently, the usefulness of near-infrared spectroscopy-based cerebral oximetry in extremely preterm infants to prevent death or severe brain injury was evaluated in a study called *SafeBoosC-III* ($N = 1601$). In this study, the incidence of both outcomes was similar between the cerebral oximetry and the control group. In a previous smaller study called *SafeBoosC-II* ($N = 166$), there was a borderline significant advantage for cerebral oximetry. There is therefore a highly significant ($p = 0.010$) difference between the two outcomes. What could be the reason for the difference? One major difference was that many centres were not familiar with cerebral oximeters in the new study compared with the old

study. This could be a reason for the discrepancy. We conclude that cerebral oximetry should not be disregarded as a method of neuromonitoring in neonatology.

Keywords

Cerebral oximetry · Near infrared spectroscopy · Preterm infants · Brain injury · Death

1 Introduction

Near-infrared spectroscopy-based cerebral oximetry is a non-invasive technique to determine cerebral oxygenation by measuring the proportion of haemoglobin carrying oxygen in the blood [1]. Recently, Hansen et al. [2] evaluated the usefulness of cerebral oximetry in extremely preterm infants in combination with a treatment guideline [3] to prevent death or severe brain injury (*SafeBoosC-III*, $N = 1601$). This randomised trial concluded that treatment guided by cerebral oximetry monitoring [4, 5] in the first 72 hours after birth did not lead to a lower incidence of death or severe brain injury at 36 weeks postmenstrual age than usual care. In a previous smaller study (*SafeBoosC-II*, $N = 166$) [6–8], there was a borderline significant advantage for cerebral oximetry (death: unadjusted $p = 0.049$, adjusted $p = 0.10$; brain injury: unadjusted $p = 0.053$, adjusted $p = 0.11$). Therefore, the fol-

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lowing questions arise: Is this difference between the two study results significant? What could be the reason for the difference?

To address these questions, the aim was to test whether the discrepancy is statistically significant and to assess the differences between the two studies that could potentially explain the discrepancy.

2 Methods

We analysed the difference of pooled relative risks by calculating the ratio of relative risk and performing a test of interaction (z -score) which then was converted to a p -value (two-tailed). Visualisation was performed in R (version 4.1.2) [9] using the ggplot2 [10] package.

3 Results and Discussion

The incidences of death and severe brain injury were similar between the two control groups in the two studies [2, 6], while in the cerebral oximetry group these incidences were higher (death: by 94.0%, severe brain damage: by 52.2%) in the new SafeBoosC-III study [2] compared to the previous SafeBoosC-II study [6] (Fig. 1). These differences are highly significant ($p = 0.010$) as we have determined by our statistical analysis.

3.1 Comparison of the Control Groups

The control groups received the usual care according to the guidelines of the respective participating centre, but differed in one respect between SafeBoosC-II and SafeBoosC-III: In SafeBoosC-II, infants had a cerebral oximetry sensor installed, which measured brain oxygenation, but the values of this measurement were concealed and not visible to the clinical staff. It was therefore impossible to react to changes in brain oxygenation. In SafeBoosC-III, in comparison, no cerebral oximetry equipment was used at all. Thus, the difference between the two control

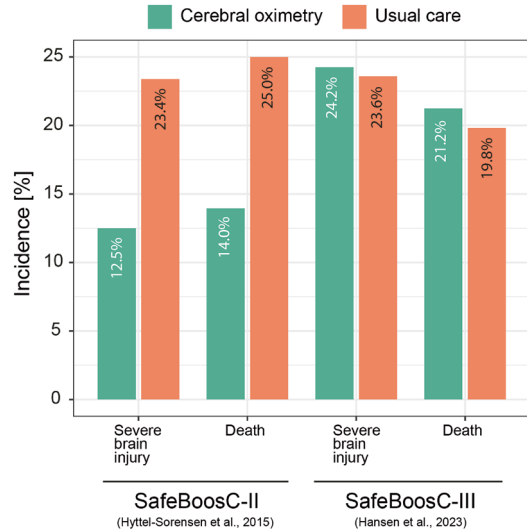


Fig. 1 Incidences (death and severe brain injury) in the two studies SafeBoosC-II (left) and SafeBoosC-III (right). The control group (usual care) is similar between the two studies, but the cerebral oximetry groups are significantly different

groups is the installation and operation of functioning cerebral oximetry sensors on the infant. This may give an indication of whether the process of installing cerebral oximetry equipment and the measuring of oxygenation might cause unwanted side effects that result in an unfavourable outcome for these vulnerable infants. It turned out that the incidence of severe brain injury was very similar between SafeBoosC-II (23.4% incidence of severe brain lesions) and SafeBoosC-III (23.6% incidence). This indicates that cerebral oximetry instrumentation has no effect on the incidence of severe brain injury. Looking at the incidence of death, there was a slightly lower incidence in SafeBoosC-III (19.8% mortality) versus SafeBoosC-II (25.0% mortality). This difference was not significant and it was small. What could be the reason? One possibility is that the additional handling of the infants during installation and operation of the cerebral oximetry instrument led to a higher incidence in SafeBoosC-II. Another possible explanation, which seems more relevant to us, is that SafeBoosC-III was conducted 8 years later than SafeBoosC-II and there was some progress in treating preterm infants in the meantime. This

progress could explain the slightly lower mortality in preterm infants. In general, it is known that the mortality decreases over the years [11, 12], while the incidence of brain injury remains more constant [13], since the additional survivors are likely to have more severe injuries. The data suggest that the cerebral oximetry equipment by itself does not have a significant impact on the outcome in preterm infants.

3.2 Comparison of the Treatment Groups

Why is there such a significant and huge discrepancy between the treatment groups? We looked at the differences between the two studies. One likely reason is that in the previous study, SafeBoosC-II, all the centres were experts in cerebral oximetry, but in the new study, SafeBoosC-III, they were not. We compared the two studies and found that in SafeBoosC-III, those who were not familiar with cerebral oximetry were instructed by a web-based training. There was also a training concerning the treatment guideline and, once completed, each training led to a certification. However, neither training nor certification was mandatory. The training included in our opinion insufficient information on how to place and attach the sensors to obtain accurate values, e.g. that the sensor must be tightly in contact with the tissue and how to deal with hair for the sensors to work properly [5]. This training was probably insufficient to properly understand cerebral oximetry and to draw conclusions from the cerebral oximetry values. In addition, cerebral oximetry instruments only provide correct values if they are applied correctly. This is a crucial point. For example, the sensor needs to be placed on the correct location of the head, and superficial inhomogeneities in particular, such as hair or moles, may lead to erroneous values if not handled properly. Furthermore, the treatment guideline did not prescribe a specific response to a low brain oxygenation value. It simply recommends that monitoring parameters are checked and appropriate action taken. It could be that staff who are

unfamiliar with the novel cerebral oximetry equipment are less likely to take the values seriously and therefore might be less likely to take appropriate action to resolve the hypoxia. There are a number of cerebral oximetry devices on the market and the sensitivity and accuracy of these devices vary [14–16]. In SafeBoosC-III, there was a larger variety of instruments admitted compared to SafeBoosC-II. All of these instruments had to undergo a phantom test to set comparable alarm limits. Still, the systematic variation between instruments could reduce the trust that clinical staff has towards cerebral oximetry [17, 18].

We think that these methodological differences therefore probably play an important role in explaining the different study results. This also means that the study results should not lead to the conclusion that the cerebral oximetry equipment is useless in preventing brain injury or death. We suggest that the cerebral oximetry equipment can reduce the incidence of death and serious brain injury when used properly by trained personnel.

Although web-based neonatal brain oximetry training programmes are always very useful for training NICU staff and verifying their competence, the complexity of cerebral oximetry data collection on the neonate's head also requires training in the presence of a nurse/neonatologist experienced in cerebral oximetry. For future studies, we suggest that staff be trained to use the cerebral oximetry devices professionally and to respond adequately to abnormal values of tissue oxygenation to ensure effective treatment.

4 Conclusion and Outlook

Although cerebral oximetry did not show any advantage in the newer larger study, we believe cerebral oximetry should not be disregarded for neuromonitoring in neonatology [19]. Our view is that more research is needed for evaluating the clinical effectiveness of cerebral oximetry in neonates. We are eager to see the results of the 1,276 infants (alive at 36 weeks' postmenstrual age) from the SafeBoosC-III trial who will participate in the recently announced two-year follow-up

study [4]. Data from routine follow-up programmes will be collected between the ages of 18 and 30 months.

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References

1. Wolf M, Ferrari M, Quaresima V (2007) Progress of near-infrared spectroscopy and topography for brain and muscle clinical applications. *J Biomed Opt* 12(6):062104
2. Hansen ML et al (2023) Cerebral oximetry monitoring in extremely preterm infants. *N Engl J Med* 388(16):1501–1511
3. Pellicer A et al (2013) The SafeBoosC phase II randomised clinical trial: a treatment guideline for targeted near-infrared-derived cerebral tissue oxygenation versus standard treatment in extremely preterm infants. *Neonatology* 104(3):171–178
4. Rasmussen MI et al (2023) Cerebral oximetry monitoring versus usual care for extremely preterm infants: a study protocol for the 2-year follow-up of the SafeBoosC-III randomised clinical trial. *Trials* 24(1)
5. SafeBoosC-III (2023) Available from: <https://www.rigshospitalet.dk/english/departments/juliane-marie-centre/departments-of-neonatology/research/SafeboosC-III/Sider/default.aspx>
6. Hyttel-Sorensen S et al (2015) Cerebral near infrared spectroscopy oximetry in extremely preterm infants: phase II randomised clinical trial. *BMJ* 350:g7635
7. Hyttel-Sorensen S et al (2013) A phase II randomized clinical trial on cerebral near-infrared spectroscopy plus a treatment guideline versus treatment as usual for extremely preterm infants during the first three days of life (SafeBoosC): study protocol for a randomized controlled trial. *Trials* 14:120
8. SafeBoosC-II (2023). Available from: <https://www.rigshospitalet.dk/english/departments/juliane-marie-centre/departments-of-neonatology/research/SafeboosC-II/Sider/default.aspx>
9. R-Core-Team (2018) R: a language and environment for statistical computing R foundation for statistical computing, Vienna, Austria
10. Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer, New York
11. Bell EF et al (2022) Mortality, in-hospital morbidity, care practices, and 2-year outcomes for extremely preterm infants in the US, 2013–2018. *JAMA* 327(3):248–263
12. van Beek PE et al (2021) Survival and causes of death in extremely preterm infants in the Netherlands. *Arch Dis Child Fetal Neonatal Ed* 106(3):251–257
13. Arnaud C et al (2021) Trends in prevalence and severity of pre/perinatal cerebral palsy among children born preterm from 2004 to 2010: a SCPE collaboration study. *Front Neurol* 12:624884
14. Gagnon MH et al (2020) Sensitivity of a next-generation nirs device to detect low mixed venous oxyhemoglobin saturations in the single ventricle population. *Anesth Analg* 131(3):e138–e141
15. Davie SN, Grocott HP (2012) Impact of extracranial contamination on regional cerebral oxygen saturation: a comparison of three cerebral oximetry technologies. *Anesthesiology* 116(4):834–840
16. Cho H et al (2000) Comparison of two commercially available near-infrared spectroscopy instruments for cerebral oximetry. Technical note. *J Neurosurg* 93(2):351–354
17. Evans KM, Rubarth LB (2017) Investigating the role of near-infrared spectroscopy in neonatal medicine. *Neonatal Netw* 36(4):189–195
18. Mintzer JP, Moore JE (2019) Regional tissue oxygenation monitoring in the neonatal intensive care unit: evidence for clinical strategies and future directions. *Pediatr Res* 86(3):296–304
19. El-Dib M et al (2023) Neuromonitoring in neonatal critical care part II: extremely premature infants and critically ill neonates. *Pediatr Res* 94(1):55–63

Cerebral Oxygen Dynamics in a Patient After Subdural Haematoma Removal Surgery: A Case Report

Naoyuki Hashimoto, Yu Takada, Masafumi Kubota, and Atsuhiko Tsubaki

Abstract

Near-infrared spectroscopy (NIRS) can be employed for the non-invasive real-time evaluation of cerebral oxygen dynamics. A portable wireless NIRS system has been developed to record measurements using a band worn around the head. In this case report, we describe the clinical findings and cerebral oxygen dynamics of a patient who underwent surgery to remove a left temporal subdural haematoma. The patient was a male in his 70s, who fell while riding a bicycle. Following the gradual onset of gait disturbance, the patient was admitted to our hospital for surgery. After surgical removal of the subdural haematoma, the cerebral oxygen dynamics of the left and right frontal cortical hemispheres were monitored using NIRS in the following sequence: supine, bed-up 70°, sitting, standing, and

supine, for 3 min each. Measurements were made on postoperative days (PODs) 2, 3, 7, and 10. Clinically, on POD2, the patient had a decreased level of consciousness, requiring assistance in maintaining each posture. On POD10, his level of consciousness improved and he was able to walk for short distances. The cerebral oxygen dynamics on the injured hemispheres showed the most remarkable changes on POD2 which decreased with the passage of time. NIRS may be useful in non-invasive assessment of the recovery of the cerebral circulation after neurosurgical intervention to inform the medical rehabilitation team on the progress in recovery.

Keywords

Near-infrared spectroscopy · Cerebral hemodynamics · Early mobilisation · Subdural haematoma · Autoregulatory dysfunction

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1 Background

Early mobilisation of patients with acute cerebrovascular disease has been recommended by several relevant guidelines [1–3], given that it is considered to prevent disuse syndrome and contribute to walking ability and functional prognosis. However, regarding the timing of early

mobilisation, very early mobilisation within 24 h reportedly decreases the modified Rankin Scale score after 3 months [4]. Importantly, acute mobilisation requires appropriate risk management according to the patient's condition. In acute cerebrovascular disease, cerebral blood flow autoregulation may be disrupted after onset [5], resulting in ischaemia or hyperaemia in regional cerebral circulation during bed rest. Accordingly, cerebral haemodynamics must be adequately assessed for risk management and condition assessment.

Near-infrared spectroscopy (NIRS) can non-invasively measure cerebral haemodynamics. The patient is less restrained during NIRS measurements than during magnetic resonance imaging and positron emission tomography. Hence, it can be used for bedside measurements during standing movements, which may have clinical applications in rehabilitation therapy. In the current case, we used portable NIRS to measure cerebral haemodynamics during bed rest after subdural haematoma surgery.

2 Case Presentation

The patient was a male in his 70s. Before admission, the patient was able to perform activities of daily life independently. On day X, he fell while riding a bicycle and injured the left side of his head. He was able to perform daily activities, but gradually developed speech impairment,

motor paralysis, and gait disturbances. On X+8, the patient was brought to our hospital, diagnosed with a subacute subdural haematoma, and a craniotomy was performed to remove the haematoma to prevent increased haematoma. Figure 1 shows the pre- and postoperative computed tomography (CT) images. Rehabilitation intervention was initiated on post-operative day (POD) 1. At the time of the intervention, the patient was able to perform isolated movements adequately, but had a speech impairment and required assistance to perform basic activities such as getting up, sitting, and holding a standing position. On the first day of measurement, the patient was verbally informed of the details of the procedure and written consent was obtained.

3 NIRS Measurement

NIRS (Hb-133; Astem Co., Japan) was used for the following measurements. The measurement sites included two channels in the left and right frontal regions (Fig. 2); the measurement waveform lengths were 770 ± 10 nm and 830 ± 10 nm, and the measured items included oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), total haemoglobin (THb), and tissue oxygen saturation (StO_2). The measurement protocol consisted of supine, bed-up at 70° , sitting, standing, and lying positions for 3 min each. Blood pressure was measured using a cuff manometer for each pos-

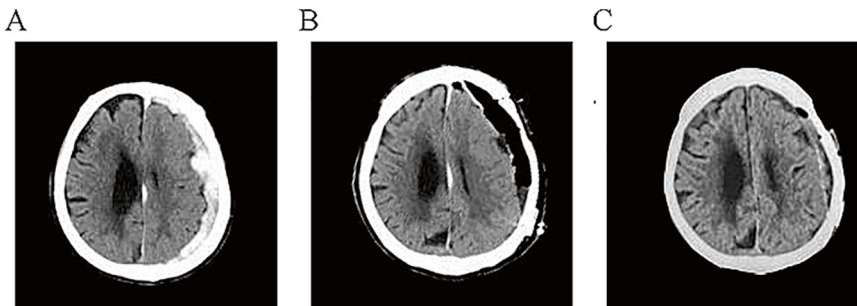


Fig. 1 CT image before surgery. (a) CT image obtained before the haematoma removal surgery (POD0). (b): CT image on POD2. (c) CT image on POD11. After haema-

toma removal surgery, an improvement of cerebrum compression was observed from **b** to **c**

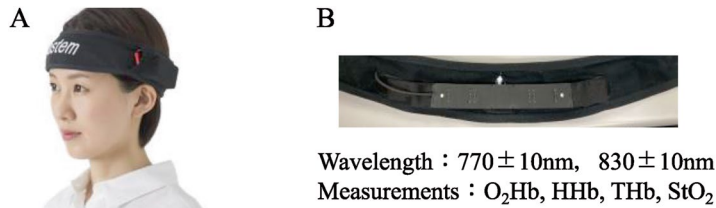


Fig. 2 NIRS device. (a) A cordless band is wrapped around the head for measurement with two channels for the left and right frontal lobe regions. (b) Two wave-

lengths are used to measure cerebral haemodynamics in the cerebral parenchyma

tural change. Measurements were performed before rehabilitation and the patients rested in the supine position prior to measurement.

4 Progress and NIRS Measurement

NIRS measurements were performed on PODs 2, 3, 7, and 10. The clinical findings were as follows: On POD2, the level of consciousness was eyes 3, verbal 3, and motor 6; total 12 on the Glasgow coma scale (GCS), and cognitive function was 10 on the mini mental state examination (MMSE). The patient required assistance in getting up from supine; bedside mobilisation was provided; on POD3, the consciousness level improved (GCS; eyes 3, verbal 4, motor 6; total 13) and he was able to walk with assistance on the parallel bars; on POD7, the patient's consciousness level improved further (GCS; eyes 3, verbal 6, motor 6; total 15) and he was able to walk independently on the parallel bars; and on POD10, his MMSE score was 24 and communication appeared effortless. The patient was able to walk short distances independently. Figure 3 shows the NIRS measured waveforms. Table 1 shows O_2Hb at each postural change and Table 2 StO_2 on each POD. In this report, the change in cerebral blood flow was judged by O_2Hb as the representative value of each index. On POD2, cerebral blood flow always showed a greater variability on the injured side than on the uninjured side; on POD2 and POD3, cerebral blood flow on the injured side increased with standing up; on

POD7 and POD10, cerebral blood flow on the injured side sometimes decreased with bed-up; and on POD10, cerebral blood flow on the injured side and uninjured side increased or decreased at the same time. Regarding the difference between the injured and uninjured side in StO_2 , the deviation observed on PODs 2 and 3 was no longer evident on POD10.

5 Discussion

We observed decreases in cerebral haemodynamic variability and left to right differences in a patient with postdural haematoma, correlated with clinical improvements. Although there have been reports on cerebral haemodynamics during orthostasis in patients with orthostatic hypotension [6], no reports have been reported on cerebral haemodynamics during bedside weaning in patients with cerebrovascular disorders or head trauma.

1. Cerebral Blood Flow Autoregulatory Capacity in Patients with Subdural Haematoma

Cerebral autoregulation can be impaired by any degree of traumatic brain injury (TBI), including mild TBI [7]. In this case, the patient underwent surgery 8 days after symptom onset, and neurological symptoms were observed during the measurement period, suggesting that he may have experienced deficits in cerebral blood flow autoregulation at the time of measurement.

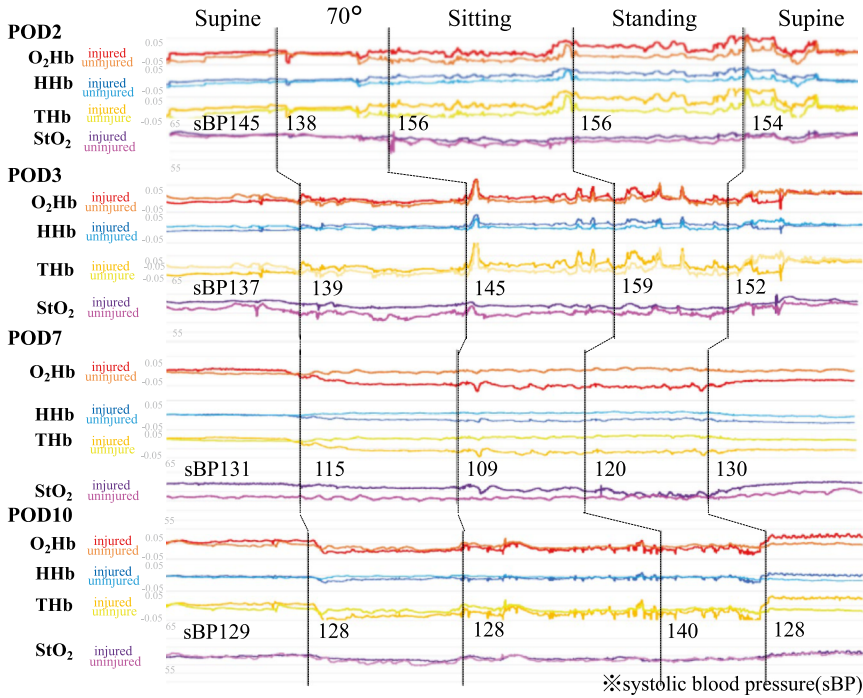


Fig. 3 NIRS measurements. Injured: Left prefrontal region. Uninjured: Right prefrontal region

Table 1 Changes in variables of O₂Hb from each posture

		O ₂ Hb values in each posture				Effect size on postural change			
		Supine	70°	Sitting	Standing	Supine	Supine → 70°	70° → Sitting	Sitting → Standing
POD2	Injured	-0.009 ± 0.009	0.007 ± 0.008	0.025 ± 0.008	0.036 ± 0.002	0.003 ± 0.011	1.88	2.25	1.89
	Uninjured	-0.012 ± 0.003	-0.026 ± 0.011	-0.012 ± 0.012	0.004 ± 0.012	0.000 ± 0.007	1.74	1.22	1.33
POD3	Injured	-0.009 ± 0.003	-0.003 ± 0.004	0.005 ± 0.003	0.008 ± 0.012	0.008 ± 0.018	1.70	2.26	0.34
	Uninjured	0.008 ± 0.005	-0.013 ± 0.005	-0.008 ± 0.004	0.004 ± 0.014	0.023 ± 0.004	4.20	1.10	1.17
POD7	Injured	-0.007 ± 0.010	-0.034 ± 0.008	-0.050 ± 0.008	-0.055 ± 0.002	-0.035 ± 0.002	2.98	2.00	0.86
	Uninjured	0.010 ± 0.002	-0.004 ± 0.004	0.001 ± 0.003	0.007 ± 0.003	-0.001 ± 0.002	4.43	1.41	2.00
POD10	Injured	0.002 ± 0.014	-0.026 ± 0.003	-0.014 ± 0.007	-0.017 ± 0.005	0.021 ± 0.006	2.77	2.23	0.49
	Uninjured	-0.004 ± 0.007	-0.013 ± 0.004	-0.003 ± 0.005	-0.008 ± 0.004	0.000 ± 0.003	1.58	2.43	1.10

mean ± standard deviation

Each value is for 1 minute after the posture change. There was a significant difference between each posture in the Bonferroni method after one-way ANOVA ($p < 0.01$). Cohen's d was calculated before and after the posture. Grey: Increase of O₂Hb; Blue: Decrease of O₂Hb

2. Changes in NIRS Indices

NIRS is a non-invasive device that can measure changes in cerebral oxygen metabolism and cerebral blood circulation; however, the measurements represent relative value changes from the initiation of the measurement. Hoshi et al. attributed the increase in O₂Hb levels to increased

blood flow or decreased metabolism in the brain cells [8]. However, in environments where oxygen saturation remains unaltered, an increase or decrease in O₂Hb levels reflects cerebral blood flow to a certain extent. During cerebral ischaemia, arterial blood inflow decreases, accompanied by a reduction in O₂Hb levels; however, HHb increases owing to the elevated cerebral oxygen

Table 2 Differences in StO₂ between injured and uninjured sides of POD

		StO ₂ values on each POD	Effect size
POD2	Injured	62.5 ± 0.4	0.98
	Uninjured	62.0 ± 0.6	
POD3	Injured	63.0 ± 0.7	1.74
	Uninjured	61.6 ± 0.9	
POD7	Injured	61.7 ± 0.7	3.33
	Uninjured	59.8 ± 0.4	
POD10	Injured	60.6 ± 0.5	0.36
	Uninjured	60.4 ± 0.6	

mean ± standard deviation
Significant difference between injured and uninjured sides (paired t-test, $p < 0.01$)
The effect size was calculated between the injured and non-injured side at Cohen’s d

uptake rate, and the sum of THb remains unchanged [9]. In the intensive care unit (ICU), StO₂ is monitored to observe decreased tissue hypoxia due to hypoperfusion. However, in the current case, an increase in StO₂ was observed on the injured side up to POD7. Severe brain injury decreases cerebral blood flow due to increased intracranial pressure; however, blood flow in the extracranial region via the external carotid artery is relatively increased. Therefore, NIRS monitoring over the scalp may increase local oxygen saturation [10]. The O₂Hb, HHb, and THb levels showed similar trends and may include factors other than overperfusion or hypoperfusion of the brain parenchyma. However, considering the clinical course, the left to right differences between the injured and uninjured sides and changes over time may provide clinically useful information.

5.1 Limitations

The patient was instructed to limit movement as much as possible and refrain from speech; however, the effects of body movement on arti-

facts and cortical activity could not be excluded. In addition, patients with TBI may be more susceptible to cutaneous blood flow on the injured side.

6 Conclusions

Measuring NIRS indices over time may allow the evaluation of the condition of patients with cerebrovascular disorders.

COI Disclosure The authors declare no conflicts of interest associated with this manuscript.

References

1. Winstein CJ et al (2017) Guidelines for adult stroke rehabilitation and recovery. *Stroke* 48:e369
2. Gittler M et al (2018) Guidelines for adult stroke rehabilitation and recovery. *JAMA* 319:820–821
3. The Japan Stroke Society (2015) Japanese guidelines for the management of stroke 2015 (in Japanese). *Kyowa kikaku*:277–280
4. AVERT Trial Collaboration group (2015) Efficacy and safety of very early mobilization within 24h of stroke onset (AVERT): a randomized controlled trial. *Lancet* 386:46–55
5. Lassen NA (1959) Cerebral blood flow and oxygen consumption in man. *Physiol Rev* 39:183–238
6. Klop M et al (2023) Capturing postural blood pressure dynamics with near-infrared spectroscopy-measured cerebral oxygenation. *Geroscience*. <https://doi.org/10.1007/s11357-023-00791>
7. Junger EC et al (1997) Cerebral autoregulation following minor head injury. *J Neurosurg* 86:425–432
8. Hoshi Y et al (2001) M: Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *J Appl Physiol* 90:1657–1662
9. Sakatani K, Katayama Y (eds) (2002) Near-infrared spectroscopy for clinicians (in Japanese). *Shinkohigaku*, Tokyo
10. Broud M et al (2021) Usefulness of cerebral oximetry in TBI by NIRS. *J Clin Med* 10:2938

A Case Report of Changes in Regional Cortical Blood Volume and Oxygenation During Induction of General Anaesthesia with Sevoflurane Versus Propofol in a Paediatric Moyamoya Disease, Evaluated by Near-Infrared Spectroscopy

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Abstract

A safe induction method of general anaesthesia for paediatric moyamoya disease patients has not been fully established. We had the opportunity to administer general anaesthesia twice to a two-year-old girl diagnosed with moyamoya disease. We used different induction methods for general anaesthesia at each session, i.e. slow induction with sevoflurane and rapid induction with propofol, and were able to evaluate changes in her left regional cortical blood volume (rCBV) and oxygen-

ation (rCBO) during both anaesthesia inductions using near-infrared spectroscopy (NIRS). The mean change value of total-Hb (rCBV) (mean $\pm$ SD; $\mu\text{mol/L}$) in the rapid induction was lower than that in the slow induction (-0.54 ± 1.43 vs. 1.82 ± 1.74). However, the TOI (rCBO) levels during both anaesthesia inductions were constantly higher than these respective baseline values (64% in the slow induction, 71% in the rapid induction), and these mean change values in each of the anaesthesia induction were about the same. The present results suggested that both the slow induction method with sevoflurane and the rapid induction method with propofol might be safe and effective for anaesthesia induction in paediatric patients with moyamoya disease.

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Keywords

Paediatric moyamoya disease · General anaesthesia · Anaesthesia induction · Cortical circulation · Near-infrared spectroscopy

1 Introduction

In the management of general anaesthesia for paediatric patients with moyamoya disease, maintenance of cerebral blood flow (CBF) is essential to avoid cerebral ischaemia, since hypocapnia, hypotension, and hypovolemia have all been identified as risk factors for ischaemic complications [1, 2]. However, a safe induction method of general anaesthesia for these patients has not been fully established. We recently had the opportunity to administer general anaesthesia twice to a two-year-old girl diagnosed with moyamoya disease. We used different induction methods for general anaesthesia at each session, i.e. slow induction with sevoflurane at the first anaesthesia and rapid induction with propofol at the second anaesthesia, and were able to evaluate changes in her regional cerebral blood volume (rCBV) and oxygenation (rCBO) during both anaesthesia inductions using near-infrared spectroscopy (NIRS).

2 Case Report

A 31-month-old girl (weight, 12 kg) presented with a history of few episodes of transient ischaemic attacks that were precipitated by crying. Evaluation using single photon emission computed tomography showed a decrease in cerebral blood flow in the left frontal region. Therefore, she received general anaesthesia twice: the first time for angiography and the second for revascularisation surgery (encephalo-duro-arterio-myosynangiosis; EDAMS).

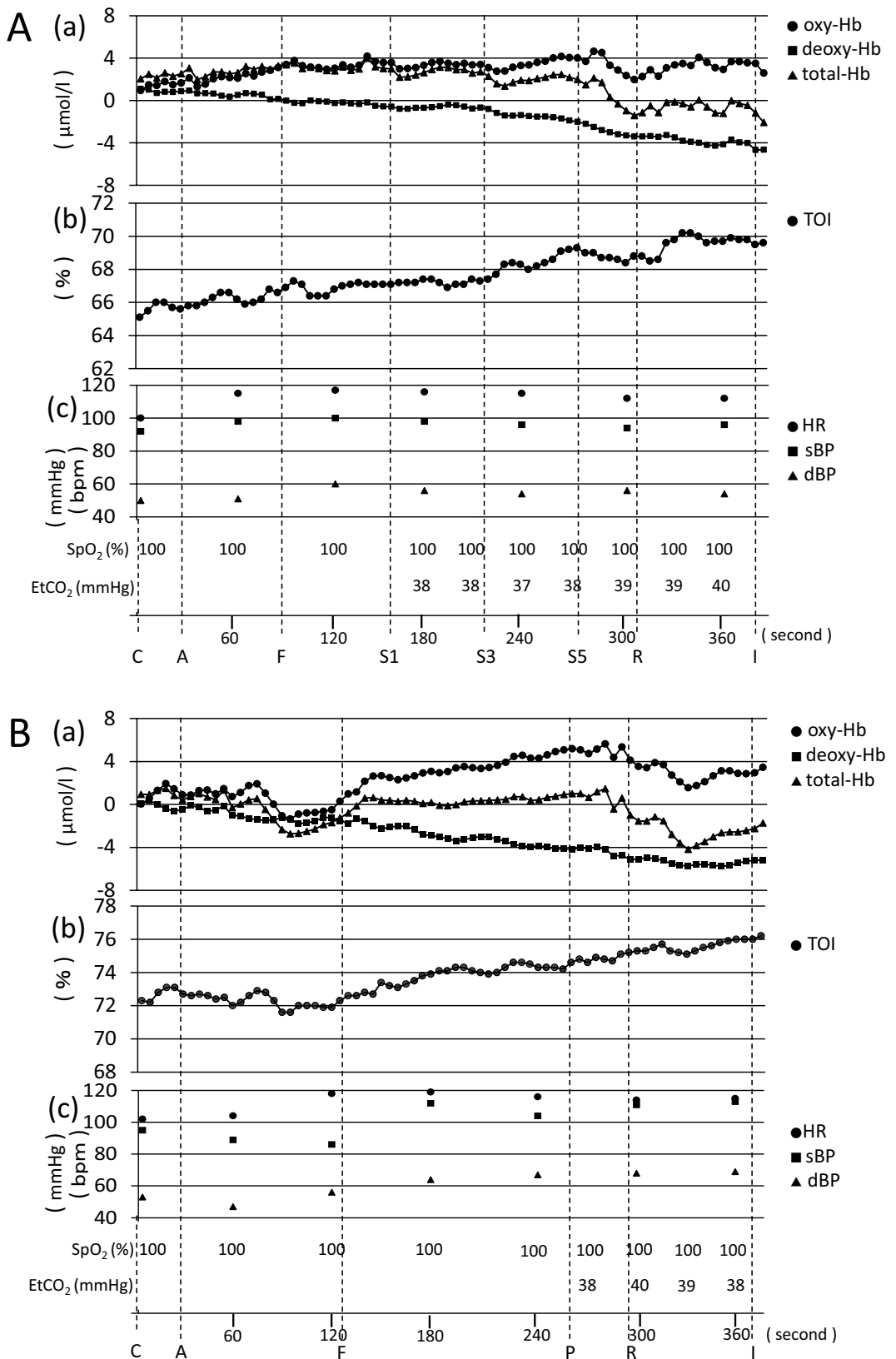
On each operation day, an intravenous line was inserted in the ward and the patient received 6 mg midazolam orally 1 hour before anaesthesia induction. Then, she was moderately sedated on

arrival to the operating room. We selected a slow induction method with sevoflurane in consideration of uncertainty of airway management in the first anaesthesia. After oxygenation with 6 L/min oxygen for 3 min, 0.12 mg atropine and 25 µg fentanyl were injected. Then, sevoflurane was administered, and the concentration slowly increased from 1% to 5%. Manual ventilation was gradually shifted from assisted ventilation to controlled ventilation via a fitted face mask, while maintaining an end-tidal carbon dioxide (EtCO₂) of 35–40 mmHg. After injection of 12 mg rocuronium, we performed orotracheal intubation. We chose a rapid induction method with propofol in the second anaesthesia, since we were able to manage manual artificial ventilation and tracheal intubation easily in the first anaesthesia. After oxygenation with 6 L/min for 3 min, 0.12 mg atropine, 25 µg fentanyl, and 24 mg propofol were injected. Then, orotracheal intubation was performed after injection of 12 mg rocuronium. Manual ventilation via a fitted face mask was performed with maintenance of an EtCO₂ of 35–40 mmHg.

In each of the anaesthesia inductions, the patient was monitored with automatic non-invasive blood pressure measurements on the right arm, pulse oximetry, and electrocardiography. In each case, the anaesthesia induction was commenced following attachment of an NIRS (Niro 200NXTM, Hamamatsu Photonics, Hamamatsu City, Japan) probe on the patient's left forehead. The NIR light from three laser diodes (775, 810, 850 nm) was directed at the forehead through a fibre optic bundle, and the reflected light was transmitted to a multi-segment photodiode detector array. The NIRS system measures the concentrations of oxy-haemoglobin (Hb), deoxy-Hb, total-Hb (total-Hb: oxy-Hb + deoxy-Hb), and the tissue oxygen index

Fig. 1 Changes in near-infrared spectroscopy (NIRS) measurements and cardiorespiratory parameters during induction of general anaesthesia with sevoflurane (graph A) versus propofol (graph B). Measurements: (a), oxy-haemoglobin (oxy-Hb), deoxy-Hb and total-Hb; (b), Tissue oxygen index (TOI); (c), blood pressure (sBP: systolic pressure, dBP: diastolic pressure), per-

cutaneous oxygen saturation (SpO₂), end-tidal carbon dioxide (EtCO₂). Time points: C complete oxygenation, A atropine injection, F fentanyl injection, S1 inhalation of 1% sevoflurane, S3 inhalation of 3% sevoflurane, S5 inhalation of 5% sevoflurane, P propofol injection, R rocuronium injection, I endotracheal intubation



(TOI). Measurements of the NIRS parameters, systolic blood pressure (sBP), diastolic blood pressure (dBP), heart rate (HR), EtCO₂, and SpO₂, were recorded every one min during both anaesthesia inductions.

The patient did not cry throughout anaesthesia induction on either occasion. The changes in NIRS measurements and cardiorespiratory parameters during induction of general anaesthesia with sevoflurane versus propofol are shown in the Fig. 1 (graph A and B). The changes in total-Hb (rCBV) during both anaesthesia inductions were maintained within the safe range, while the mean change value of total-Hb (mean $\pm$ SD; $\mu\text{mol/L}$) in the rapid induction was slightly lower than that in the slow induction (-0.54 ± 1.43 vs. 1.82 ± 1.74). The TOI (rCBO) levels during both anaesthesia inductions were constantly higher than these baseline values (64% in the slow induction, 71% in the rapid induction), and the mean change values in each of the anaesthesia induction were about the same. All cardiorespiratory parameters did not significantly change during both anaesthesia inductions. The infusion volumes during the first and the second anaesthesia induction were 17.8 mL and 19.2 mL, respectively.

3 Discussion

Reportedly, when maintaining general anaesthesia for patients with moyamoya disease, use of propofol might be preferred over sevoflurane for prevention of ischaemic complications [1, 3], although this remains a matter of debate since no previous reports have evaluated changes in CBF during anaesthesia induction in either adult or paediatric patients with moyamoya disease. Hence, it is unclear whether slow induction with sevoflurane or rapid induction with propofol should be the preferred anaesthesia induction method in these patients. This is the first report to define the changes in cerebral circulation evaluated by NIRS during both types of general anaesthesia induction, including slow induction with sevoflurane and rapid

induction with propofol, during two separate procedures in a paediatric patient with moyamoya disease.

In the NIRS device used in this case report, the measured values of total-Hb and TOI can be indices of tissue blood volume and oxygenation, respectively, when blood haematocrit is constant [4, 5]. Therefore, since the patient received a small amount of infusion during both anaesthesia inductions, the measured values of total-Hb and TOI were regarded as her rCBV and rCBO. In this case, total-Hb did not decrease markedly from baseline levels, and TOI increased slightly during both anaesthesia inductions. Based on the evaluation by NIRS, in which ischaemia should be suspected when TOI decreases by $>13\%$ from baseline, along with an increase in deoxy-Hb and decrease in oxy-Hb and total-Hb [6, 7], the patient's cerebral circulation might have been sufficiently maintained with both the induction methods. However, her total-Hb and oxy-Hb tended to decrease after inhalation of more than 5% sevoflurane and with propofol infusion, respectively, while deoxy-Hb did not change significantly and TOI was well maintained. These results suggest that her rCBV decreased slightly in both the above situations. Reportedly, hypotension and hypocapnia easily decrease CBF in patients with moyamoya disease [1, 2]. Although inhalation of sevoflurane increases CBF in patients without cerebrovascular disease, it causes the cerebral steal phenomenon when administered at a high concentration in patients with diseases such as moyamoya disease, resulting in a decrease in cortical blood flow [3]. Infusion of propofol, on the other hand, simultaneously decreases CBF secondary to cerebrovascular constriction and suppresses cerebral oxygen metabolism [8]. Considering that her cardiorespiratory measurements remained unchanged throughout both the anaesthesia inductions, the mild decrease in rCBV observed under the above conditions might reflect decreases in cortical blood flow due to cerebral steal induced by sevoflurane and suppression of cerebral oxy-

gen metabolism induced by propofol, respectively.

There were several reasons for successful maintenance of her cortical blood circulation during both anaesthesia inductions. First, optimal sedation during anaesthesia induction by midazolam premedication prevented crying, which is an important cause of ischaemic attacks in paediatric patients with moyamoya disease [1]. Second, it has been described that abrupt decrease in cardiac output (CO) decreases CBF [9], and that maintenance of HR is more important for maintenance of CO in children than in adults [10]. Therefore, injection of atropine before anaesthesia induction might have contributed to maintaining her CBF. Finally, the fact that we could easily and reliably assist and control her ventilation via a face mask was also beneficial, since it allowed adequate oxygenation and maintenance of adequate EtCO₂.

4 Conclusion

This case report suggests that both the slow induction method with sevoflurane and the rapid induction method with propofol might be safe and effective for anaesthesia induction in paediatric patients with moyamoya disease, if patients can receive optimal ventilation without crying.

References

1. Baykan N, Ozgen S, Ustalar ZS et al (2005) Moyamoya disease and anesthesia. *Paediatr Anaesth* 15:1111–1115
2. Sato K, Shirane R, Yoshimoto T (1997) Perioperative factors related to the development of ischemic complications in patients with moyamoya disease. *Childs Nerv Syst* 13:68–72
3. Sato K, Shirane R, Kato M et al (1999) Effect of inhalational anesthesia on cerebral circulation in moyamoya disease. *J Neurosurg Anesthesiol* 11:25–30
4. Sakatani K, Xie Y, Lichty W et al (1998) Language-activated cerebral blood oxygenation and hemodynamic changes of the left prefrontal cortex in poststroke aphasic patients: a near-infrared spectroscopy study. *Stroke* 29:1299–1304
5. Terborg C, Birkner T, Schack B et al (2003) Noninvasive monitoring of cerebral oxygenation during vasomotor reactivity tests by a new near-infrared spectroscopy device. *Cerebrovasc Dis* 16:36–41
6. Al-Rawi PG, Kirkpatrick PJ (2006) Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. *Stroke* 37:2720–2725
7. Kuroda S, Houkin K, Abe H et al (1996) Near-infrared monitoring of cerebral oxygenation state during carotid endarterectomy. *Surg Neurol* 45:450–458
8. Oshima T, Karasawa F, Satoh T (2002) Effects of propofol on cerebral blood flow and the metabolic rate of oxygen in human. *Acta Anaesthesiol Scand* 46:831–835
9. Neumann S, Burchell AE, Rodrigues JCL et al (2019) Cerebral blood flow response to simulated hypovolemia in essential hypertension: a magnetic resonance imaging study. *Hypertension* 74:1391–1398
10. McAuliffe G, Bissonnette B, Cavalle-Garrido T et al (1997) Heart rate and cardiac output after atropine in anaesthetised infants and children. *Can J Anaesth* 44:154–159

Comparison of Cerebral Saturation and Brain Net Water Uptake After Moderate Traumatic Brain Injury

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Abstract

The aim was to study the relationship between net water uptake (NWU) and cerebral oxygenation in patients with posttraumatic ischaemia (PTI) foci after moderate traumatic brain injury (moTBI).

Materials and Methods. Perfusion computed tomography (PCT) was performed for 72 patients with PTI foci after moTBI in 2013–2022. The mean age of the patients was 32.7 ± 12.5 years (from 18 to 65 years), 25 women and 47 men. Cerebral tissue oxygen saturation (SctO₂) was evaluated using Fore-

Sight 2030 (CAS Medical Systems Inc., USA) in the region of the frontal lobe pole (FLP). NWU was calculated from non-contrast CT. Data are shown as a median [interquartile range]. $P < 0.05$ was considered statistically significant.

Results. SctO₂ in FLP varied within the range from 61% to 88%. It was 62% [55.4;72.1] over the lesion frontal lobe with PTI and 64% [58.5;73.7] over the opposite FLP side. The average NWU in the FLP cortex on the PTI side was 4.98% [2.21;7.39]. In the case when there were no focal injuries in the frontal lobes, SctO₂ was significantly correlated with higher NWU ($R = -0.780$, $p < 0.00001$).

Conclusions. The cerebral oxygen tissue saturation correlates with net water uptake in patients with PTI after moTBI ($p < 0.005$).

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Keywords

Cerebral oxygenation · Net water uptake ·
Traumatic brain injury

1 Introduction

The most frequent consequences of the early stage of moderate traumatic brain injury (moTBI) are secondary insults, leading to cerebral posttraumatic ischaemia (PTI) and consequential cytotoxic

oedema [1]. Although the ischaemic stroke incidence in the acute period of TBI is about 3%, it increases in morbidity and mortality [2]. The contemporary tool for the brain oedema evolution assessment is based on the study of net water uptake (NWU) by comparing computed tomography (CT) brain density using Hounsfield unit (HU) in the ischaemic and non-ischaemic (contralateral) zones [3]. Recent systematic reviews have shown that NWU is a cerebral oedema development biomarker [4, 5]. The NWU perfusion computed tomography (PCT) assessment allows a more accurate evaluation of brain oedema. However, such studies are still rare, especially in moTBI [6].

Moreover, the relationship between cerebral oxygenation and brain hydration due to cerebral oedema remains underinvestigated.

The aim was to study the relationship between NWU and cerebral oxygenation in patients with PTI foci after moTBI.

2 Methods

In a protocol reviewed and approved by the Institutional Ethical Committee and conforming to the standards of the Declaration of Helsinki, a retrospective, observational, and non-randomised single-centre study was conducted as an analysis of a prospectively maintained database cohort (2013–2022). We analysed 72 patients with PTI foci after moTBI (women 25; men 47). The median age was 35.2 years [28.3; 43.2]. PTI was determined using PCT, and brain oedema was assessed using NWU on non-contrast CT images. Inclusion criteria were: moTBI <12 h after head injury; Glasgow Coma Score (GCS) ≥ 9 and ≤ 12 , Marshall II–III grade, PCT performed <5 days after injury; blast TBI, unilateral PTI foci in PCT. The exclusion criteria were as follows: age ≤ 18 and ≥ 60 years; GCS at admission <9 and >12 points; bilateral PTI foci, Injury Severity Scale ≥ 9 points.

All patients received multiphase PCT on days 1–5 after the moTBI (mean 3.3 ± 0.5 days) on a 160-slice scanner (Canon Aquilion Prime SP, Canon Medical Imaging, Japan). The PCT procedure was described earlier [7]. PCT data were processed using a Vitrea workstation (Vitrea FX, Vital Images, USA), where standard perfusion maps were built, including: cerebral blood volume (CBV), cerebral blood flow (CBF), and mean transit time (MTT). The region of interest (ROI) was established based on sub-cortical areas of the brain lesions and symmetrical zones of both frontal lobe poles (FLP). Quantitative perfusion indices, including cerebral blood flow, were calculated voxel-wise and used to generate colour-coded maps. The voxels with CBF >100 mL/100 g/min and/or CBV >8 mL/100 g were assumed to contain large vessels and removed from the perfusion map calculation of perfusion parameters. The ischaemic thresholds (core and penumbra) were: a decrease in CBV <2.0 mL/100 g or a CBF <145% compared with the contralateral hemisphere [8]. NWU was calculated using the following equation [9]:

$$\text{NWU} = (1 - D_{\text{ischaemic}} / D_{\text{normal}}) \times 100\%$$

where, $D_{\text{ischaemic}}$ – PTI “core” density, D_{normal} – the density of normal brain tissue in the symmetrical zone of the contralateral hemisphere (HU).

Cerebral near-infrared spectroscopy (CNIRS) was performed by a space-resolved cerebral tissue oximeter Fore-Sight 2030 (CAS Medical Systems Inc., USA) and included the detection of the SctO₂ values in the same FLP regions.

Data are shown as a median [interquartile range]. Statistical analysis was performed using the T-criterion Wilcoxon. The regression line and confidence limits for each are for illustrative purposes only. $P < 0.05$ was considered statistically significant.

3 Results

The saturation in the FLP tissue varied within the range from 61% to 88%. It was 62% [55.4;72.1] over the FLP lesion with PTI and 64% [58.5;73.7] over the opposite side (Table 1). The values below the ischaemic threshold ($SctO_2 < 60\%$) were detected in 10 patients.

The average NWU in the FLP cortex on the PTI side was 4.98% [2.21;7.39].

The average CBF in the FLP cortex on the PTI side was 20.9 ml/100g/min [11.9;22.6], and on the contralateral side was 21.4 ml/100g/min [18.7;36.7]. CBV were 1.8 ml/100g [0.8;2.2]

Table 1 Data on comparison of the analysed parameters

	PTI side	Non-PTI side	<i>p</i>
$SctO_2$ (%)	62 [55.4;72.1]	64% [58.5;73.7]	$p > 0.05$
CBF (ml/100g/min)	20.9 [11.9;22.6]	21.4 [18.7;36.7]	$p > 0.05$
CBV (ml/100g)	1.8 [0.8;2.2]	1.8 [1.3;2.5]	$p > 0.05$
MTT (s)	4.7 [3.6;5.0]	4.0 [3.4;4.5]	$p > 0.05$

PTI Posttraumatic ischaemia, *SctO₂* Cerebral tissue O₂ saturation, *CBF* Cerebral blood flow, *CBV* Cerebral blood volume, *MTT* Mean transit time

and 1.8 ml/100g [1.3;2.5], respectively. MTT were 4.7 s [3.6;5.0] and 4 s [3.4;4.5], respectively ($p < 0.05$).

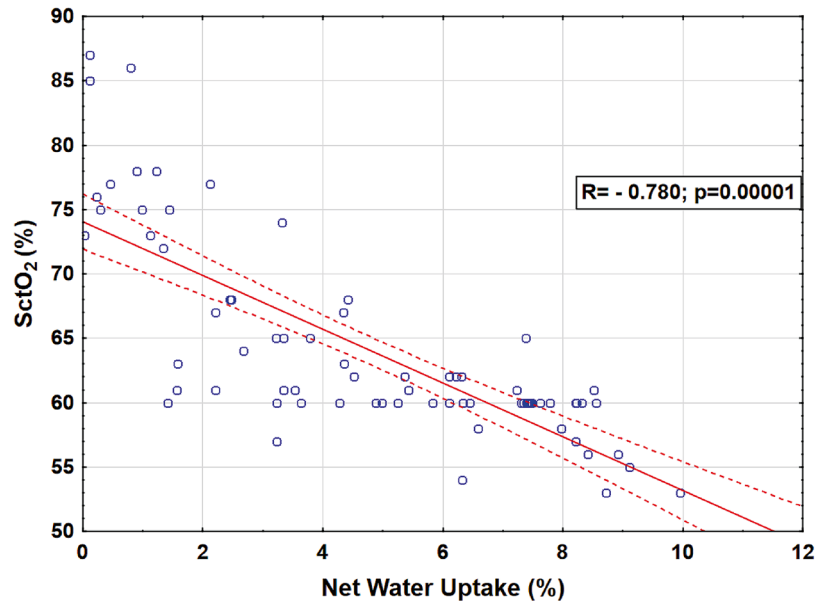
Cerebral NWU values and $SctO_2$, the brain saturation level in the FLP on the PTI side, were compared pairwise. In cases where there were no focal injuries in the frontal lobes, NWU was significantly and inversely correlated with cerebral oxygenation on the lesion foci side ($R = -0.780$, $p < 0.00001$) (Fig. 1).

4 Discussion

To our knowledge, this is one of the first studies reporting the relationship between NWU and the brain oxygen status. The main finding of this study is that NWU within the FLP on the PTI side was significantly correlated with cerebral oxygen saturation in moTBI patients.

In our opinion, there are several explanations for the identified phenomenon, but all of them are related to brain oedema phenotypes. Thus, PTI development is associated not only with hypoxia but also with an impairment of blood-brain barrier permeability that increases NWU [10]. It is assumed that disorders of cerebral metabolism during TBI alter intracellular kinase-phosphatase

Fig. 1 NWU was significantly correlated with $SctO_2$ on the PTI foci side: the linear regression results. Dashed red lines represent 95% confidence intervals for the regression (solid red line)



balance and aquaporin-4 phosphorylation state, leading to increased expression of water channels and intracellular swelling [11]. However, its effect on oxygen status remains poorly understood and requires further research.

As described earlier, a significant NWU increase in the ischaemic zone is made by the gradient between intravascular and extravascular (parenchymal) oncotic pressure [12]. At the same time, the question of what features this process has in PTI foci requires further study. Finally, it has been shown that reduced cerebral venous outflow also leads to a proportional NWU increase [13].

It should be noted that this study has several limitations. The main limitation was the single-centre and retrospective design and the lack of a control group. Another limitation was the technical impossibility of clinically assessing brain tissue hydration on CNIRS data distortions, which are currently being studied only in the experiments [14]. In addition, our study did not examine cerebral collateral circulation status, which can have a significant impact on PTI progression.

5 Conclusion

NWU was significantly and inversely correlated with cerebral oxygenation in PTI foci in mTBI patients.

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References

- Zipperle J, Schmitt FCF, Schöchl H (2023) Point-of-care, goal-directed management of bleeding in trauma patients. *Curr Opin Crit Care* 2023. <https://doi.org/10.1097/MCC.0000000000001107>
- Shen J, Zhu L, Shan Y et al (2023) Effects of remote ischemic preconditioning in severe traumatic brain injury: a single-center randomized controlled trial. *Medicine (Baltimore)* 102(38):e35190. <https://doi.org/10.1097/MD.00000000000035190>
- Broocks G, Meyer L, Elsayed S et al I-LAST Investigators (2023) Association between net water uptake and functional outcome in patients with low ASPECTS brain lesions: results from the I-LAST Study. *Neurology* 100(9):e954–e963. <https://doi.org/10.1212/WNL.000000000000201601>
- Xu T, Yang J, Han Q et al (2022) Net water uptake, a neuroimaging marker of early brain edema, as a predictor of symptomatic intracranial hemorrhage after acute ischemic stroke. *Front Neurol* 13:903263. <https://doi.org/10.3389/fneur.2022.903263>
- Schleicher RL, Vorasayan P, McCabe M et al, NN104 Investigators (2023) Analysis of brain edema in RHAPSODY. *Int J Stroke*, 17474930231187268. Advance online publication. <https://doi.org/10.1177/17474930231187268>
- Haupt W, Meyer L, Wagner M et al (2022) Assessment of irreversible tissue injury in extensive ischemic stroke-potential of quantitative cerebral perfusion. *Transl Stroke Res*. <https://doi.org/10.1007/s12975-022-01058-9>
- Trofimov AO, Agarkova DI, Trofimova KA et al (2023) Changes of arterial and venous cerebral blood flow correlation in moderate-to-severe traumatic brain injury: a CT perfusion study. *Adv Exp Med Biol* 1438:51–58. https://doi.org/10.1007/978-3-031-42003-0_9
- Lin L, Bivard A, Kleinig T, Spratt NJ et al (2018) Correction for delay and dispersion results in more accurate cerebral blood flow ischemic core measurement in acute stroke. *Stroke* 49:924–930. <https://doi.org/10.1161/STROKEAHA.117.019562>
- Lu SS, Wu R, Cao YZ et al (2022) ASPECTS-based net water uptake predicts poor reperfusion and poor clinical outcomes in patients with ischemic stroke. *Eur Radiol* 32(10):7026–7035. <https://doi.org/10.1007/s00330-022-09077-8>
- Boyko M, Gruenbaum BF, Frank D et al (2023) The integrity of the blood-brain barrier as a critical factor for regulating glutamate levels in traumatic brain injury. *Int J Mol Sci* 24(6):5897. <https://doi.org/10.3390/ijms24065897>
- Malaeb S, Izzetoglu M, McGowan J et al (2018) Noninvasive monitoring of brain edema after hypoxia in newborn piglets. *Pediatr Res* 83:484–490. <https://doi.org/10.1038/pr.2017.264>
- Ng FC, Yassi N, Sharma G et al ... HERMES Collaborators (2022) Correlation between computed tomography-based tissue net water uptake and volumetric measures of cerebral edema after reperfusion therapy. *Stroke* 53(8):2628–2636. <https://doi.org/10.1161/STROKEAHA.121.037073>
- Faizy TD, Kabiri R, Christensen S et al (2021) Venous outflow profiles are linked to cerebral edema formation at noncontrast head ct after treatment in acute ischemic stroke regardless of collateral vessel status at CT angiography. *Radiology* 299(3):682–690. <https://doi.org/10.1148/radiol.2021203651>
- Xie J et al (2011) Near-infrared spectroscopy technique to evaluate the effects of drugs in treating traumatic brain edema. *J Phys Conf Ser* 277:012041. <https://doi.org/10.1088/1742-6596/277/1/012041>

Comparison of Secondary Ischaemia Incidence, Intracranial Pressure, and Cerebrovascular Reactivity Dynamics During Intrahospital Transportation of Severe TBI Patients

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Abstract

The aims of the study were to evaluate post-traumatic cerebral ischaemia (PTCI) incidence in severe traumatic brain injury (TBI) patients and to assess the intracranial pressure (ICP) and cerebrovascular reactivity dynamics during intrahospital transportation (IT). *Materials:* A total of 153 severe TBI patients and 182 IT were included. The mean Glasgow

Coma Scale (GCS) score was 6.7 ± 2.1 . ICP and arterial pressure were invasively monitored, and an improved pressure reactivity index (iPRx) was calculated from the measured parameters. Statistical analysis was done using Student's t-criterion and Wilcoxon criterion where appropriate. *Results:* Perfusion computed tomography (PCT) revealed a neuroimaging PTCI pattern in all 153 severe TBI patients (100%). In 58 patients (37.9%), ischaemia extended to both hemispheres; in 95 patients (62.1%), it affected only one hemisphere. The mean ICP during IT was significantly higher (26.1 ± 13.5 mm Hg, $p < 0.001$) than before the IT (19.9 ± 5.3 mm Hg). All patients had increased ICP, especially during vertical movement in an elevator (maximum 75.2 mm Hg). *Conclusion:* PTCI was detected in all severe TBI patients in coma. The IT of comatose severe TBI patients leads to a significant increase in ICP and iPRx.

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Keywords

Cerebral ischaemia · Pressure reactivity index
· Intrahospital transport · Traumatic brain
injury

1 Introduction

Secondary posttraumatic cerebral ischaemia (PTCI) development in traumatic brain injury (TBI) patients is a dramatic event that significantly worsens outcomes and increases mortality [1].

Although the ischaemic stroke frequency at severe TBI is known and does not exceed 3–5%, secondary PTCI often occurs against the background of disorders of consciousness and pre-existing neurological deficit, which complicate its diagnosis and do not allow the actual frequency of its persistence to be established [2].

According to current guidelines for the diagnosis of ischaemic stroke, the intermediate modalities for its recognition are diffusion-weighted magnetic resonance imaging (DWI-MRI) and perfusion computed tomography [3]. However, such studies in TBI remain scarce to date.

Moreover, the utilisation of bedside, mobile computed tomography scanning is predominantly confined to prominent tertiary trauma centres, and the intrahospital transport (IT) of comatose patients suffering from severe TBI for both therapeutic and diagnostic purposes could subject these individuals to potential clinical hazards due to the compromised cerebrovascular reactivity and the risk of intracranial hypertension [4].

The aims of the study were (1) to establish posttraumatic PTCI incidence in severe TBI patients and (2) to assess the intracranial pressure and cerebrovascular reactivity dynamics during IT.

2 Methods

The protocol of this observational retrospective single-centre study was approved by the institutional Local Ethics Committee. Since all patients were in a coma, informed consent was given by the relatives. A total of 153 severe TBI patients and 182 IT were included. There were 93 men and 60 women, with a mean age of 34.3 ± 4.3 years (range 19–52 years). The mean Glasgow Coma

Table 1 The distribution of the patients according to the Marshall Classification

Marshall type	N	%
I (no visible intracranial pathology)	0	0
II (midline shift of 0–5 mm, basal cisterns remain visible, no high or mixed density lesions $>25 \text{ cm}^3$)	75	49
III (swelling)	41	26.8
IV (shift)	37	24.2
V (evacuated mass lesion)	0	0
VI (non-evacuated mass lesion)	0	0

Scale (GCS) score was 6.7 ± 2.1 . Inclusion criteria were as follows: severe TBI with a GCS score <8 within 6 h after a trauma; perfusion CT performance; Injury Severity Score ≤ 9 . The distribution of patients according to the Marshall scale is presented in Table 1.

The hospital's CT suite and intensive care department (ICD) were located on different floors (on the first and second floors, respectively); therefore, the whole IT included a horizontal part (in a gurney) and a vertical part (in an elevator—Thyssen Krupp Synergy mean vertical velocity—0.5 m/s) and required the use of an elevator twice. ITs from ICD to CT were carried out on the 2nd or 3rd days (according to the institutional protocol) or immediately in clinical deterioration cases. The IT team consisted of two nurses, an ICD physician, and a volunteer. IT was carried out lying on the back. The head end was elevated $15\text{--}20^\circ$. All sufferers were ventilated using the Puritan-Bennett 840 respirator (Medtronic-Covidien Co, USA) during IT. The mean hospital temperature was about 25°C , and the mean atmosphere pressure in Nizhny Novgorod was about 755 mmHg.

All patients were subjected to dynamic multislice CT using a 64-slice Philips Ingenuity CT scanner (Philips Medical Systems, Cleveland, USA). The perfusion CT procedure was described early [5]. Perfusion parameters of cerebral blood flow (CBF), mean transit time (MTT), and cerebral blood volume (CBV) were derived by the Bayesian method. The core of the ischaemia was calculated based on MTT and CBV (MTT $>145\%$ of the contralateral side values and CBV $<2.0 \text{ mL}/100 \text{ g}$) [6].

Table 2 Data on comparison of the analysed parameters

	Before IT	After IT	<i>p</i>
CPP (mm Hg)	95.9 ± 10.7	73.4 ± 18.4	<i>p</i> < 0.05
ICP (mm Hg)	19.9 ± 5.3	26.1 ± 13.5	<i>p</i> < 0.05
iPRx	0.23 ± 0.14	0.52 ± 0.04	<i>p</i> < 0.001

CPP cerebral perfusion pressure, ICP intracranial pressure, iPRx improved pressure reactivity index (autoregulation coefficient in supratentorial spaces)

In 91 patients, the main physiological parameters (parenchymal intracranial pressure (ICP), mean arterial pressure (MAP), cerebral perfusion pressure (CPP), heart rate, etc.) were recorded every 5 s using an intensive care monitor MP5 IntelliView (Philips, Germany) on the ICD, during IT and dynamic multislice PCT. Based on the improved pressure reactivity index (iPRx), the cerebrovascular reactivity state was calculated as the moving correlation coefficient between the thirty consecutive MAP and ICP values [7]. ICP, CPP, and iPRx values were measured 1 h before, during, and after a CT scan. Statistical analysis was done using Statistica software package 12 (TIBCO Software Inc., Palo Alto, USA). Data are presented as a mean ± standard deviation for continuous variables with normal and median [interquartile range] with abnormal distribution and validated for normality using the Shapiro-Wilk test. Statistical analysis was done using Student’s t-criterion and Wilcoxon criterion where appropriate. The significance level was set at *p* < 0.05. The logistic regression was performed to estimate the probability of PTCI development due to intracranial hypertension and cerebrovascular reactivity.

3 Results

Perfusion CT revealed a neuroimaging PTCI pattern in all 153 severe TBI patients (100%). In 58 patients (37.9%), ischaemia extended to both hemispheres; in other cases (95 patients – 62.1%), it affected only one hemisphere. The ICV at bihemispheric lesions was 152.38 cm³ [63.46; 188.44]. In the remaining patients, ICV was 32.57 cm³ [14.22; 58.21].

Before IT, the mean CPP and ICP were 95.9 ± 10.7 mmHg and 19.9 ± 5.3 mmHg, respectively.

Before CT scans, the mean CPP and ICP were 81.5 ± 12.5 mmHg and 22.6 ± 13.5 mmHg, respectively. However, mean CPP and ICP were already 73.4 ± 18.4 mmHg and 26.1 ± 13.5 mmHg at the end of the IT. Thus, the mean ICP at the end of the IT (26.1 ± 13.5 mmHg) was significantly higher (*p* < 0.001) than before IT (19.9 ± 5.3 mmHg). All patients had increased ICP during the vertical elevator movement (min 23.2 mmHg; max 75.2 mmHg), which remained increased during horizontal transportation on the gurney (*p* < 0.05).

The average duration of the IT (including perfusion CT) was 18.4 ± 5.2 min, and the average time of ICP disturbances reached 75.2 ± 27.5 min. The mean dynamic iPRx after and before IT was 0.52 ± 0.04 and 0.23 ± 0.14, respectively (*p* < 0.001) (Table 2).

There were no significant correlations between the ICV and outcomes according to the Glasgow Outcome Score (GOS), as well as between the ICV and ICP values (*p* > 0.05). However, there was a significant correlation between the GOS and iPRx values (*p* < 0.01). Logistic regression analysis showed that cerebrovascular reactivity disturbances (iPRx) were the most significant factor associated with PTCI development during IT of severe TBI patients (Table 3). The model showed its accuracy and statistical significance in assessing the probability of developing PTCI ($\chi^2 = 17.611$ *p* = 0.00015). The accuracy of the presented model was 75.71%.

4 Discussion

The first aim of the study was to determine post-traumatic PTCI incidence in severe TBI patients. For this, we used perfusion CT as the “gold standard” neuromonitoring method for recognised PTCI [8].

We found that all comatose patients with severe TBI had signs of PTCI foci. This is slightly higher than the frequency reported in previous studies, but the analysis of Westermarck maps and

Table 3 Cerebral autoregulation impairments were the most significant factor associated with PTCI development during IT of sTBI patients (logistic regression analysis results)

	iPRx	ICP
Estimate	14.242	0.081
Standard error	4.421	0.049
T	3.213	1.161
p-level	0.001	0.233
−95%CL	5.385	−0.049
+95%CL	23.123	0.222
Wald chi-square test	10.319	1.349
p-level	0.002	0.232
Odds ratio (unit ch)	1525353	1.074
−95%CL	21.711	0.949
+95%CL	1.043	1.220
Odds ratio (range)	126.545	5.149
−95%CL	6.241	0.309
+95%CL	256.554	86.558

iPRx improved pressure reactivity index (autoregulation coefficient in supratentorial spaces); *ICP* intracranial pressure; 95%CL – 95% centile

removal voxels from the large vessels makes our results more concise [9].

However, it requires cross-validation with another high-accuracy method of diffusion-weighted MRI [10].

The assumption that the incidence of PTCI was associated with transport issues can be refuted by the fact that no patients had episodes of brain desaturation at cerebral oximetry or a critical decrease in CPP at ICP-MAP monitoring. In addition, we did not find a significant correlation between the volume of PTCI and outcomes according to GOS, but this is perhaps due to the retrospective nature of the study and a selection shift towards the most severe patients.

Our results were consistent with previous studies [11, 12] that the causes of PTCI in severe TBI might be intracranial hypertension due to vasogenic oedema development, blood-brain barrier disruptions, and glymphatic system impediment [13]. However, further studies are required to confirm this.

The logistic analysis showed that both parameters (ICP and iPRx) significantly impacted PTCI development prediction during IT. The PTCI development probability reached 73% at the upper ICP (21 mmHg) and cerebrovascular reac-

tivity frontiers (iPRx 0.3). In addition, the PTCI probability was more than 50% even if ICP was normal (5 mmHg), but iPRx 0.3 and more.

At the same time, when solving the second aim to study ICP and cerebrovascular reactivity dynamics during IT, we found significant changes in these values during elevator movement. This might reflect a transient short-term “overlogging” in the blood supply of capacitance vessels at concomitant low values of intracranial compliance [14] and be similar to ICP plateau-waves formation [15].

Retrospective nature and small cohort size were the main limitations of this study. Multiphase PCT studies were repeated only in 23 out of 91 patients, and we were unable to measure PTCI foci dynamics.

The main implication of this study is the need for multimodal monitoring of severe TBI patients during IT and, broadly, during any transportation of critically ill patients. Moreover, the PTCI development risks in the IT of patients with severely impaired cerebrovascular reactivity and intracranial hypertension are probably underestimated. Further research is required on these issues.

5 Conclusion

According to PCT data, PTCI was detected in all severe TBIs in the coma. The IT of comatose severe TBI patients leads to a significant increase in ICP and iPRx.

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References

1. Rauchman SH, Zubair A, Jacob B et al (2023) Traumatic brain injury: mechanisms, manifestations, and visual sequelae. *Front Neurosci* 17:1090672. <https://doi.org/10.3389/fnins.2023.1090672>
2. Khellaf A, Khan DZ, Helmy A (2019) Recent advances in traumatic brain injury. *J Neurol* 266(11):2878–2889. <https://doi.org/10.1007/s00415-019-09541-4>

3. Carney N, Totten AM, O'Reilly C et al (2017) Guidelines for the management of severe traumatic brain injury, fourth edition. *Neurosurgery* 80(1):6–15. <https://doi.org/10.1227/NEU.0000000000001432>
4. Gomez A, Sekhon M, Griesdale D et al (2022) Cerebrovascular pressure reactivity and brain tissue oxygen monitoring provide complementary information regarding the lower and upper limits of cerebral blood flow control in traumatic brain injury: a Canadian High Resolution-TBI (CAHR-TBI) cohort study. *Intensive Care Med Exp* 10(1):54. <https://doi.org/10.1186/s40635-022-00482-3>
5. Trofimov A, Kopylov A, Dobrzeniecki M et al (2020) Comparative analysis of simultaneous transcranial doppler and perfusion computed tomography for cerebral perfusion evaluation in patients with traumatic brain injury. *Adv Exp Med Biol* 1232:55–62. https://doi.org/10.1007/978-3-030-34461-0_8
6. Lim NE, Chia B, Bulsara MK et al (2022) Automated CT perfusion detection of the acute infarct core in ischemic stroke: a systematic review and meta-analysis. *Cerebrovasc Dis Epub ahead of print* 3 June 2022:1–13. <https://doi.org/10.1159/000524916>
7. Bragin DE, Statom G, Nemoto EM (2016) Dynamic cerebrovascular and intracranial pressure reactivity assessment of impaired cerebrovascular autoregulation in intracranial hypertension. *Acta Neurochir* 122:255–260. https://doi.org/10.1007/978-3-319-22533-3_51
8. Yedavalli V, Kihira S, Shahrouki P et al (2023) CTP-based estimated ischemic core: a comparative multicenter study between Olea and RAPID software. *J stroke Cerebrovas Dis* 32(11):107297. <https://doi.org/10.1016/j.jstrokecerebrovasdis.2023.107297>
9. Shahrouki P, Kihira S, Tavakkol E et al (2023) Automated assessment of ischemic core on non-contrast computed tomography: a multicenter comparative analysis with CT perfusion. *J Neurointerv Surg*. <https://doi.org/10.1136/jnis-2023-020954>
10. Zhu C, Qin W, Xu J, Hu W (2023) Perfusion deficits in thrombolysis-treated acute ischemic stroke patients with negative or positive diffusion-weighted imaging. *BMC Neurol* 23(1):380. <https://doi.org/10.1186/s12883-023-03427-x>
11. Parmentier-Decrucq E, Poissy J, Favory R et al (2013) Adverse events during intrahospital transport of critically ill patients: incidence and risk factors. *Ann Intensive Care* 3(1):10. <https://doi.org/10.1186/2110-5820-3-10>
12. Marti M, Cook F, Lobo D et al (2017) Secondary insults and adverse events during intrahospital transport of severe traumatic brain-injured patients. *Neurocritical* 26(1):87–95. <https://doi.org/10.1007/s12028-016-0291-5>
13. Park J, Bae Y, Kim JS et al (2023) Glymphatic system evaluation using diffusion tensor imaging in patients with traumatic brain injury. *Neuroradiology* 65(3):551–557. <https://doi.org/10.1007/s00234-022-03073-x>
14. Link CD, Haese TM, Frigieri G et al (2023) Intracranial compliance and volumetry in patients with traumatic brain injury. *Surg Neurol Int* 14:246. https://doi.org/10.25259/SNI_314_2023
15. Pinto H, Dias C, Rocha AP (2022) Multiscale information decomposition of long memory processes: application to plateau waves of intracranial pressure. *Annual international conference of the IEEE Engineering in Medicine and Biology Society*, pp 1753–1756. <https://doi.org/10.1109/EMBC48229.2022.9870925>

Dependence of Cerebral Oxygenation and Task Performance on Coloured Light Exposure and Chronotype: Blue and Red Do Not Have the Same Effects on the Prefrontal Cortex

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Abstract

Background: In our previous studies, we investigated the right-left asymmetry (RLA) of cerebral tissue oxygenation (StO_2) at rest in humans and the influence of the individual chronotype (i.e. individual chronobiological disposition) on StO_2 . The aim of the current study was to investigate (i) whether the RLA exists during a cognitive task and coloured light exposure (CLE), and (ii) how changes in StO_2 induced by CLE and cognitive performance during a 2-back task are related to the subject's chronotype. **Methods:** 36 healthy subjects (22 female, 14 male, age 26.3 ± 5.7 years) were studied twice on two different days. They were exposed to a sequence of blue followed by red light or vice versa in a randomised crossover study design. During CLE,

subjects were asked to perform a 2-back task. We measured StO_2 of the right and left prefrontal cortex (PFC) as well as the right and left visual cortex with functional near-infrared spectroscopy (fNIRS). At the behavioural level, we recorded the number of correct and incorrect answers given by the subjects. The chronotype was determined with the Horne and Östberg morningness-eveningness questionnaire. **Results:** (i) We found that the blue and red light caused a RLA in the PFC. For red light exposure, the 2-back performance was negatively correlated with StO_2 in the right PFC ($r = -0.283, p = 0.016$), and for blue light, exposure in the left PFC ($r = -0.326, p = 0.005$). (ii) 83% of subjects who performed the 2-back task at their optimal time of day according to their chronotype showed increased and higher changes in StO_2 ($\Delta\text{StO}_2 > 1\%$) compared to subjects who did not exercise at their optimal time of day. (iii) No correlation was found between chronotype and 2-back task performance (red: $p = 0.38$; blue: $p = 0.42$). **Conclusions:** We found for the first time that blue and red light exposure target different regions of the PFC during performance of a 2-back task, which can be explained by the approach and withdrawal model. These results illustrate that studying the subregions

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(i.e. right, left, and even centre) of the cortex provides a better understanding of the CLE effects in the human brain. Our study also shows that individual chronotype plays an important role in the individual changes in StO₂ induced by CLE.

Keywords

Coloured light exposure · 2-back task ·
Chronotype · Cerebral tissue oxygenation ·
Functional near-infrared spectroscopy`

1 Introduction

In our previous functional near-infrared spectroscopy (fNIRS) studies, we have repeatedly observed a right-left asymmetry (RLA) of cerebral tissue oxygenation (StO₂) at rest in humans [1, 2]. We have shown that the RLA is a real and remarkable phenomenon that reflects the real properties of the brain. The first aim of this study was to determine whether the RLA also exists during a complex experimental paradigm involving coloured light exposure (CLE) and a cognitive (2-back) task. The rationale for integrating a cognitive task into CLE is to examine the effects of coloured light in learning environments. Colours and coloured lights serve as discretionary design components in physical learning spaces, potentially influencing behaviour and learning outcomes in educational settings.

There are numerous factors that influence our daily cognitive performance. In addition to social, environmental, and situational factors that can alter our cognitive functioning, inter-individual differences in regulating sleep-wake regulation affect the optimal timing for performing cognitive tasks [3, 4]. Chronotype, the behavioural manifestation of the underlying physiological phase of sleep-wake behaviour and a measure of individual differences in rest/activity or circadian timing [5, 6], has been shown to influence performance on a variety of cognitive tasks [7, 8]. Light exposure is another important factor influencing physiology and cognition [9–11]. However, it is not yet fully understood how different coloured

light exposures affect cognitive performance and how brain response is modulated by the subject's chronotype depending on the wavelength of the light exposure. Therefore, the second aim of this study was to investigate how changes in StO₂ induced by CLE and cognitive performance during a 2-back task are related to the subject's chronotype.

2 Subjects and Methods

Thirty-six healthy subjects (22 female, 14 male, age 26.3 ± 5.7 years) were studied twice on two different days but at the same time of the day. They were exposed to a sequence of blue followed by red light or vice versa in a randomised crossover study design. We opted for blue and red since they represent two of the three primary colours situated at opposite ends of the visible light spectrum in humans. During the CLE, subjects were asked to perform a 2-back task at defined intervals. A series of letters was presented auditorially and subjects had to respond quickly each time the current letter matched the letter presented two positions back in the sequence. The subjects were in darkness for 7 min (baseline). After this phase, the first exposure to coloured light (blue or red) was executed for 14 min; the 2-back task was performed during the first 7 min of these 14 min. During the following 7 min of the first coloured light exposure, the subjects were asked to relax (recovery phase). In the last 14 min of the measurement, exactly this procedure was repeated with the second coloured light exposure (red or blue). We applied long-term CLE because it provides a more comprehensive understanding of the cumulative effects of CLE on cerebral haemodynamics and oxygenation. Moreover, long-term CLE better mimics real-world scenarios in which individuals are exposed to coloured light for prolonged periods of time in a variety of settings, including education, the workplace, and healthcare.

We measured StO₂ of the right and left prefrontal cortex (PFC) as well as the right and left visual cortex (VC) with fNIRS (Imagent, ISS Inc., Champaign, IL, USA). Prior to data

analysis, excessively noisy data and data with inappropriately high numerical values (e.g. $\text{StO}_2 > 100\%$) were removed and the StO_2 signals were low pass filtered with a moving average filter having a window length of 1 minute. The area under the curve of StO_2 changes during the 2-back/CLE phases was calculated for each subject. At the behavioural level, the number of correct (#Hits) and incorrect (#FalseAlarms) responses of the subjects was recorded. A net difference score was calculated from these two values (Net score = #Hits – #FalseAlarms). Pearson's correlation coefficients were calculated to examine the relationship between ΔStO_2 in subregions of the cortices and task performance. The Wilcoxon signed-rank test was used to determine the significance of task performance during blue and red light exposure. Chronotype was determined using the Horne and Östberg morningness-eveningness questionnaire. Scores ranged from 16 to 86, including evening (16–41), neither (42–58), and morning chronotypes (59–86).

3 Results

- (i) With red light exposure, 2-back performance was negatively correlated with StO_2 in the right PFC ($r = -0.283$, $p = 0.016$), whereas such correlation was observed in the left PFC with blue light exposure ($r = -0.326$, $p = 0.005$). No correlation was observed between performance in the 2-back task and changes in StO_2 in the VC (Fig. 1).
- (ii) Twenty-one subjects were neither evening nor morning type, i.e. “no type”, eight were morning type, and seven were evening type.
- (iii) Five of the six subjects (83% of subjects) who performed the 2-back task at the optimal time of day according to their chronotype (i.e. evening type subjects performing in the evening, and morning type in the morning) showed increased and higher changes in StO_2 ($\Delta\text{StO}_2 > 1\%$). In contrast, more than half of the subjects (red: 67%, blue: 56%) who did not exercise at their

optimal time of day showed lower or less positive changes in StO_2 ($\Delta\text{StO}_2 < 1\%$) (Fig. 2).

- (iv) No correlation was found between chronotype and 2-back task performance (red: $p = 0.38$; blue: $p = 0.42$).
- (v) No statistically significant difference ($p = 0.46$) was found in the 2-back task performance net score between blue and red light exposure (blue: 89 ± 11 (mean $\pm$ SD), red: 90 ± 10).

4 Discussion and Conclusions

RLA has been described for a wide range of brain functions [12]. RLA was first introduced in the field of language functions, where it was shown that the left hemisphere is dominant in language processing [13, 14]. In our previous studies, we also found the RLA of StO_2 at rest [1, 2]. In the current study, we aimed to determine whether the RLA also exists during an experimental paradigm with CLE and a 2-back task. We found that the blue and red light elicited brain activation in the left and right PFC, respectively, which was negatively correlated with performance. Regardless of light colour, the PFC is closely related to behavioural performance and behavioural adaptation to external stimuli [15, 16]. The higher positive changes in StO_2 were due to the increased task engagement during poor or moderate performance. On the other hand, the better the subject's performance, the smaller the changes in StO_2 . Although a 2-back task generally influences the PFC, this task in combination with specific coloured light exposures targeted different regions of the PFC, i.e. blue: left PFC, red: right PFC. This can be explained by the approach-withdrawal model, where the left PFC activity is associated with the approach-positive affect and the right PFC activity associated with the withdrawal-negative affect [1, 17]. Blue light appeals to almost everyone and is associated with generally more positive concepts (e.g. calm, comfort, and contentment), possibly leading to significant correlations in the left PFC, while red light is associated with negative meaning in most

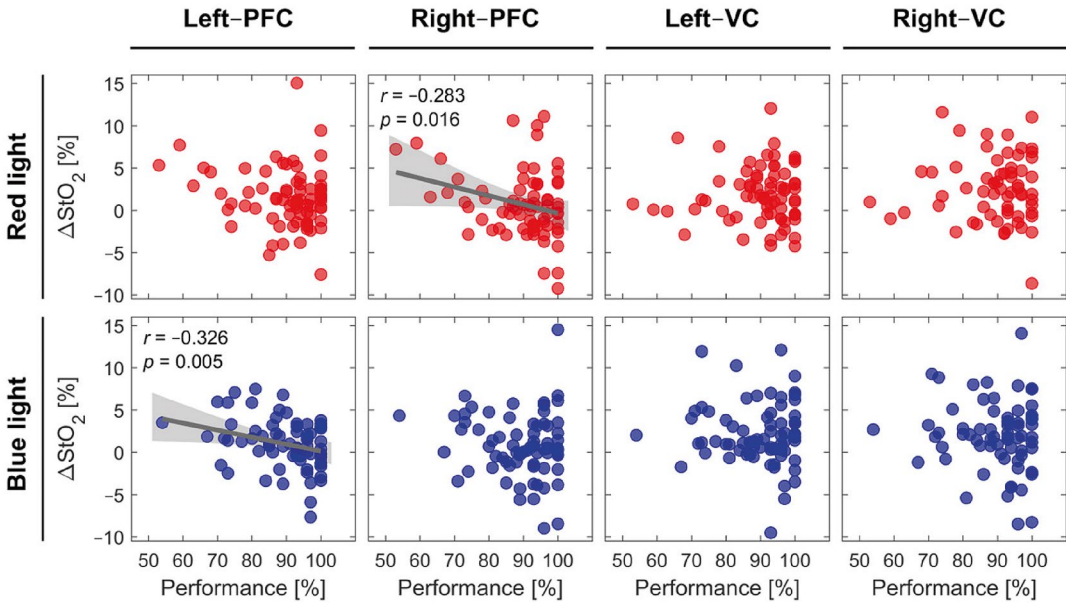


Fig. 1 Scatter plots displaying changes in StO_2 (ΔStO_2) at the PFC and VC compared to the subject's performance during the 2-back/CLE phases. The linear fit is shown for

pairs with a significant correlation. The grey-shaded areas show 95% confidence intervals

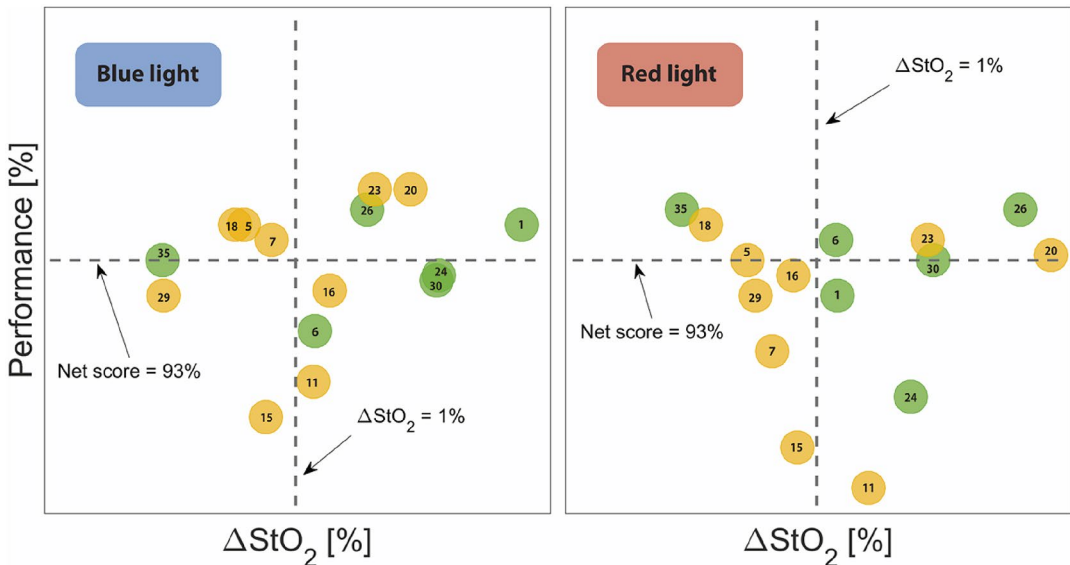


Fig. 2 Scatter plots showing ΔStO_2 in the whole PFC (left and right PFC are averaged) compared to the subject's performance during the 2-back/CLE phases for subjects who are "morning type" and "evening type". Green circles: Subjects performed the 2-back task at their preferred time of day. (i.e. morning types performing in the morning, and evening types in the evening). Orange cir-

cles: Subjects did not perform at their preferred time of day. Shown are the median values of task performance and StO_2 changes (net score = 93%, $\Delta\text{StO}_2 = 1\%$). The numbers within the data points indicate the subjects' identification codes. Morning type: #1, #6, #11, #18, #20, #24, #29, and #30. Evening type: #5, #7, #15, #16, #23, #26, and #35

cases (e.g. anger, danger, and fear), possibly leading to lateralisation to the right.

As the second aim of this study, we investigated how changes in StO_2 induced by CLE and a 2-back task are linked to the subject's chronotype. No correlation was found between chronotype and 2-back task performance for both conditions. This is in line with the findings of a recent study [4]. It is known that the chronotype depends on the individual subject, age, and genetic and environmental factors [18]. With the individual level analyses, "no type" subjects were first excluded and the effects of chronotype on the subject's performance and ΔStO_2 were investigated for data from each CLE. We revealed higher brain activity in the PFC for subjects who performed the 2-back task at their optimal time of day according to their chronotype (i.e. evening-type subjects performing in the evening). The findings of a functional magnetic resonance imaging study also showed increased task-related response and higher cerebral activity in evening as compared to morning chronotypes in the subjective evening hours for optimal sustained attention during the performance of a psychomotor vigilance task [19]. To some extent, our results are in line with findings that evening types exhibited higher thalamic activity than morning types in the subjective evening hours and morning-type individuals exhibited higher activity than evening-type subjects in the middle frontal gyrus during the morning session [3]. This could be due to increased strategic or attentional recruitment of prefrontal areas, implicated in compensating working memory load [3, 4].

The present investigation is subject to the following limitations: Although the number of participants was calculated using a power analysis, more correlations and more robust causal relationships might have been found if the sample size had been larger. Moreover, RLA may indeed have biological effects that were not investigated in this study. Genetic and behavioural genetic research may also contribute to a better understanding of the biological mechanisms associated with RLA.

We found for the first time that blue and red-light exposure targeted different regions of the

PFC when performing the 2-back task, which can be explained by the approach-withdrawal model. These results illustrated that investigating the subregions (i.e. right, left, and even central) of the cortex provides a better understanding of the CLE effects in the human brain. Our study highlights that the individual chronotype plays a role in the individual changes in cerebrovascular oxygenation induced by CLE. Moreover, our findings contribute to a better clarification of the relationship between chronotype and school performance, and it can help to identify how, when, and what aspects of the chronotype can be targeted via education or even therapeutic interventions. The effects of chronotype on school performance could occur during the learning and evaluation phases. The impact of chronotype on school achievements could also be mediated by other factors relevant to performance (e.g. conscientiousness, mood, and alertness) [20]. Moreover, if chronotype is modifiable and involved in the pathogenesis of mental health disorders, it could be a target for therapeutic interventions designed to prevent or treat mental illness [6]. Finally, our findings are important for future neuropsychological studies.

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References

1. Zohdi H, Scholkmann F, Wolf U (2020) Frontal cerebral oxygenation asymmetry: Intersubject variability and dependence on systemic physiology, season, and time of day. *Neurophotonics* 7:025006
2. Scholkmann F, Zohdi H, Wolf U (2020) Right-left asymmetry of prefrontal cerebral oxygenation: does it depend on systemic physiological activity, absolute tissue oxygenation or hemoglobin concentration? *Adv Exp Med Biol* 1232:105–112
3. Schmidt C, Collette F, Reichert CF et al (2015) Pushing the limits: Chronotype and time of day modulate working memory-dependent cerebral activity. *Front Neurol* 6:199
4. Kossowski B, Drożdżel D, Rode K et al (2021) The influence of light exposure and chronotype on working memory in humans. *Acta Neurobiol Exp (Wars)* 81:111–120

5. Lunn RM, Blask DE, Coogan AN et al (2017) Health consequences of electric lighting practices in the modern world: a report on the National Toxicology Program's workshop on shift work at night, artificial light at night, and circadian disruption. *Sci Total Environ* 607–608:1073–1084
6. Taylor BJ, Hasler BP (2018) Chronotype and mental health: recent advances. *Curr Psychiatry Rep* 20:1–10
7. Hartstein LE, Durniak MT, Karlicek RF, Berthier NE (2018) A comparison of the effects of correlated colour temperature and gender on cognitive task performance. *Light Res Technol* 50:1057–1069
8. Schmidt C, Collette F, Cajochen C, Peigneux P (2007) A time to think: circadian rhythms in human cognition. *Cogn Neuropsychol* 24:755–789
9. Zohdi H, Scholkmann F, Wolf U (2021) Individual differences in hemodynamic responses measured on the head due to a long-term stimulation involving colored light exposure and a cognitive task: a SPA-fNIRS Study. *Brain Sci* 11:54
10. Zohdi H, Egli R, Guthruf D et al (2021) Color-dependent changes in humans during a verbal fluency task under colored light exposure assessed by SPA-fNIRS. *Sci Rep* 11:9654
11. Zohdi H, Natale L, Scholkmann F, Wolf U (2022) Intersubject variability in cerebrovascular hemodynamics and systemic physiology during a verbal fluency task under colored light exposure: clustering of subjects by unsupervised machine learning. *Brain Sci* 12:1449
12. Hougaard A, Jensen BH, Amin FM et al (2015) Cerebral asymmetry of fMRI-BOLD responses to visual stimulation. *PLoS One* 10:1–16
13. Liu H, Stufflebeam SM, Sepulcre J et al (2009) Evidence from intrinsic activity that asymmetry of the human brain is controlled by multiple factors. *Proc Natl Acad Sci U S A* 106:20499–20503
14. Homae F (2014) A brain of two halves: insights into interhemispheric organization provided by near-infrared spectroscopy. *Neuroimage* 85:354–362
15. Nissen A (2020) Psychological and physiological effects of color use on ecommerce websites: a neural study using fnirs. *Int Conf Inf Syst ICIS 2020 – Mak Digit Incl Blending Local Glob* 1–17
16. Taren AA, Venkatraman V, Huettel SA (2011) A parallel functional topography between medial and lateral prefrontal cortex: evidence and implications for cognitive control. *J Neurosci* 31:5026–5031
17. Gable PA, Neal LB, Threadgill AH (2018) Regulatory behavior and frontal activity: considering the role of revised-BIS in relative right frontal asymmetry. *Psychophysiology* 55:e12910
18. Roenneberg T, Kuehnle T, Pramstaller PP et al (2004) A marker for the end of adolescence. *Curr Biol* 14:R1038–R1039
19. Cajochen C, Chellappa S, Schmidt C (2010) What keeps us awake?-the role of clocks and hourglasses, light, and melatonin. Elsevier Inc.
20. Zerbini G, Mellow M (2017) Time to learn: how chronotype impacts education. *PsyCh J* 6:263–276

Differences in Brain Activity During Real and Virtual Reality Motor Tasks in Normal Healthy Individuals

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Abstract

This study aimed to clarify the differences between brain activity during virtual reality and real motor tasks. Twelve healthy adults participated in this study. Near-infrared spectroscopy (NIRS) was used to measure brain activity, and a total of 34 channels, 17 channels each, centred on the bilateral motor cortex, were used as measurement sites. Two

types of motor tasks were used: a real Box and Block test (R-BBT) and a virtual reality Box and Block test (VR-BBT). In both motor tasks, an increase in oxy-haemoglobin (O_2Hb) and a slight decrease in deoxy-haemoglobin (HHb) levels were observed in the central region of the participant's motor cortex. The O_2Hb in the motor cortex during R-BBT increased at the beginning of the task, rapidly decreased, and remained at a low value. Conversely, the amount of O_2Hb in the motor cortex during the VR-BBT remained high throughout the task. In addition, O_2Hb in the dorsal prefrontal cortex during the VR-BBT was significantly higher than during the R-BBT. This study indicates that brain activity differs significantly between real and virtual reality motor tasks, even for similar tasks.

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Keywords

Functional near-infrared spectroscopy · Virtual reality · Box and Block test · Ventrolateral prefrontal cortex · Healthy individuals

1 Background

In recent years, novel strategies for lower- and upper-limb motor rehabilitation have been developed to take advantage of the brain reorganisa-

tion aspects of neuroplasticity. Virtual reality (VR) is a computer-based technology that allows the creation of multisensory simulated environments in which users can interact and receive real-time feedback regarding their performance. Some systematic reviews [1–3] have reported that VR training focusing on motor control, balance, gait, and muscle strength is more effective than conventional rehabilitation. Reports demonstrating the effectiveness of VR training for upper extremity motor dysfunction range from early onset to recovery and home rehabilitation [4–6]. Although these reports indicate that rehabilitation using VR improves functional disabilities, few studies have focused on how VR causes changes in cortical activation.

Therefore, this study aimed to investigate, first in a healthy population, the characteristics of cerebral cortical haemodynamics during a semi-immersive VR motor task using functional near-infrared spectroscopy (fNIRS). The motor task was the Box and Block test (BBT) utilised in upper limb rehabilitation of patients with stroke, and differences in brain activity while performing the BBT in real and VR environments will be examined. If reasonable results are obtained in this study, clinical application of BBT in VR is expected.

2 Methods

Twelve right-handed healthy volunteers (age: 21.2 ± 0.4 y, female: 8) participated in the study. According to the tenets of the latest Declaration of Helsinki, written informed consent was obtained from each participant before recording, after a full explanation of the protocol and the non-invasiveness of the study. The Medical Ethics Committee of Kanazawa University approved this study.

We prepared a conventional real-world Box and Block test (R-BBT) and a virtual reality Box and Block test (VR-BBT). As a 3D hand-sensing device, the Leap Motion IR170 (LEAP Motion, Inc., San Francisco, CA) was utilised. The virtual version of the BBT was designed using Unity3D software (Unity Technologies, CA, USA). The

participants were then randomly administered the R-BBT and VR-BBT for 60 seconds with 40-second rest intervals between trials.

This study used a continuous-wave fNIRS imaging system (OMM-3000, Shimadzu Corp., Kyoto, Japan) utilising near-infrared light diode sources. The regions of interest (ROI) were the prefrontal cortex and sensory-motor-related areas, including the left Ventrolateral prefrontal cortex (VLPFC), left Premotor cortex (PMC), left Primary motor cortex (M1), and left Somatosensory area (Sa). (Fig. 1).

The peak values and the area under the curve (AUC) of O₂Hb and HHb were calculated through the 60 seconds of a task period [7].

The number of blocks transported per minute was compared between the R-BBT and VR-BBT. Furthermore, the relationship between the number of blocks carried by the R-BBT and the VR-BBT was analysed using Pearson's correlation analysis. The peak values and AUC of O₂Hb and HHb were also compared between R-BBT and VR-BBT for each channel. Statistical analyses were performed using the corresponding t-test, with a significance level of 5%.

3 Results

The number of blocks per minute for R-BBT was 49.1 ± 7.5 , and for VR-BBT, was 27.3 ± 9.4 . The number of blocks carried during the R-BBT was significantly higher than that during the VR-BBT (effect size: 4.7, $t = 16.2$, $p < 0.001$). In addition, the number of blocks carried in the R-BBT was significantly and positively correlated with the number carried in the VR-BBT ($r = 0.87$, $p < 0.01$).

In this study, we focused on the O₂Hb and HHb changes during both BBT motor tasks. In the NIRS data, a consistent and progressive increase in O₂Hb and a concomitant gradual decrease in HHb were observed in the left motor-related regions (M1, Sa, PMC), from the beginning of the task, and O₂Hb/HHb gradually returned to baseline values after the task ended in both the R-BBT task (Fig. 2a) and the VR-BBT task (Fig. 2b). O₂Hb for Ch1, Ch3, Ch8, Ch11,

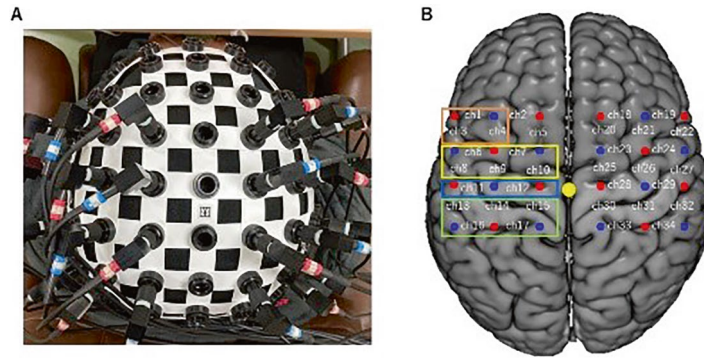


Fig. 1 Regions of interest in functional near-infrared spectroscopy. (a) The probe position was set to 34 channels for a total of 24 probes (12 sources, 12 detectors) in a 3×4 arrangement using a whole-head fibre holder (Flexible Adjustable Surface Holder; FLASH). (b) The probe distance was 30 mm, and the sampling rate was

10 Hz. The regions of interest were placed on the left Ventrolateral prefrontal cortex (VLPFC): channels 1, 3, 4, left Premotor cortex (PMC): channels 6, 7, 8, 9, 10, left Primary motor cortex (M1): channels 11, 12, and left Somatosensory are (Sa): channels 13, 14, 15, 16, 17.

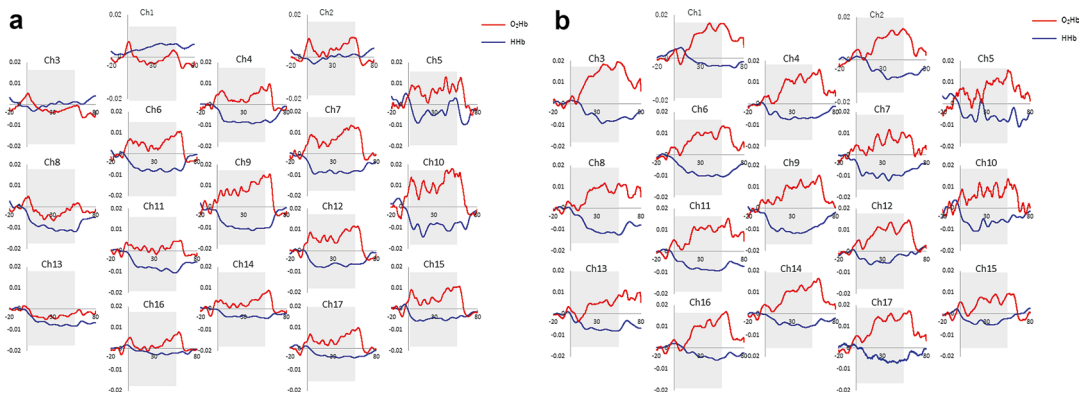


Fig. 2 Results of O_2Hb and HHb during R-BBT (a) and VR-BBT (b). Red line: O_2Hb , Blue line: HHb

and Ch13 increased during the task in the VR-BBT, but did not show an increased change in the R-BBT. In the left VLPFC (Ch3), O_2Hb maxima during the R-BBT task was 0.012 mM-mm, 95% confidence interval (CI) 0.001–0.024, and during the VR-BBT task was 0.026 mM-mm, 95% CI 0.013–0.040, indicating that O_2Hb was significantly higher during the VR-BBT task than during the R-BBT task (Table 1). Furthermore, in Ch3, the AUC of O_2Hb during the R-BBT task was -2.10 , 95% CI -7.10 – 3.90 which was not a steady increase, while the AUC during the VR-BBT task was 7.62 , 95% CI 3.11 – 12.11 , indicating a significant increase in O_2Hb throughout the motor task. In the left

somatosensory cortex (Ch13, 16, 17), the maximum of O_2Hb was significantly higher during the VR-BBT task than during the R-BBT task (Table 1). Similarly, the AUC of O_2Hb during the motor task was significantly higher for the VR-BBT than for the R-BBT in the left somatosensory cortex (Ch13, 16, 17).

In the left VLPFC (Ch3), minimum HHb during the VR-BBT task was significantly lower than during R-BBT task (R-BBT: -0.009 mM-mm, 95% CI -0.010 – 0.003 , VR-BBT: -0.017 mM-mm, 95% CI -0.023 – 0.011 , effect size: 0.89 , $p = 0.010$). In the left somatosensory cortex, minimum and AUC of HHb were lower during VR-BBT than

Table 1 Maximum O₂Hb in ROIs

Ch.	ROIs	R-BBT	VR-BBT	Effect size
1	VLPFC	0.013 ± 0.015	0.022 ± 0.015	0.020
3		0.012 ± 0.008	0.026 ± 0.017*	0.020
4		0.018 ± 0.012	0.020 ± 0.013	0.010
6	PMC	0.020 ± 0.010	0.019 ± 0.010	0.012
7		0.021 ± 0.013	0.018 ± 0.014	0.010
8		0.014 ± 0.017	0.020 ± 0.016	0.015
9		0.022 ± 0.013	0.021 ± 0.015	0.008
10		0.030 ± 0.016	0.028 ± 0.020	0.018
11	M1	0.013 ± 0.010	0.019 ± 0.013	0.013
12		0.020 ± 0.011	0.022 ± 0.015	0.011
13	Sa	0.008 ± 0.013	0.015 ± 0.013*	0.011
14		0.014 ± 0.007	0.020 ± 0.011	0.011
15		0.018 ± 0.010	0.018 ± 0.011	0.011
16		0.014 ± 0.007	0.021 ± 0.008*	0.011
17		0.016 ± 0.009	0.024 ± 0.01*	0.011

* $p < 0.05$

VR-BBT, but no significant differences between tasks.

4 Discussion

To our knowledge, this is the first study to combine a high-resolution, low-cost 3D hand-sensing device with non-invasive fNIRS measurements of haemodynamic changes to reveal new regions of brain activity in VR motor tasks during BBT.

The results of this study revealed that activation of the left VLPFC increased significantly in the BBT task in the VR environment compared to that in the BBT task in a real environment. This is similar to reports using NIRS [8] and EEG [9] for brain activity during VR tasks. Therefore, it was thought that the brain activity of the VLPFC would increase because it was involved in the association between visual and motor information in the VR motion task in this study. This typical activation suggests the existence of a mirror neuron system during VR tasks [10]. Furthermore, activation in VLPFC during BBT in the VR environment remained high. Learning new associations between visual contextual cues and motor goal behaviours is considered important for performing motor tasks in VR, and the VLPFC consistently shows high

activity in maintaining and retrieving goal-relevant information.

In the present study, no obvious differences in cerebral oxygenation dynamics in M1 occurred between real and virtual BBT, but brain activity during BBT in the VR environment in Sa was significantly increased compared with BBT in the real environment. Interestingly, although the motor task of the BBT involved significantly more repetitive movements in the real environment, there was no difference in M1 activity. In contrast, Sa activity increased in the VR environment. An fMRI analysis revealed an increase in sensory-motor cortical activity, enhanced connectivity between the sensory and motor cortices, and improved interhemispheric asymmetry after VR [11]. In TMS-based assessments, VR has been shown to increase corticospinal tract excitability [12]. By contrast, Holper et al. [13] reported an fNIRS assessment of brain activity in a VR environment and found reduced activation in the motor cortex. Participants were tasked with observing movement and motor imagery in VR videos, which may have been lacking in the immersive experience and possible embodiment of the virtual upper limb. It was thought that a sense of immersion and understanding that the VR hand can perform the motor task in first-person virtual reality, as in this study, would be important. In addition, increased sensory-motor cortical activity and improved upper extremity function have been reported after four weeks of VR training in patients with stroke [14]. Increased brain activity in the sensory-motor cortex and improved interhemispheric balance are important for functional recovery after stroke, and VR training may improve motor function after stroke.

On the other hand, we could not exclude the possibility that the brain-blood volume might have increased with the motor task, which was a limitation of the present study. The accuracy with which the changes in oxygen dynamics in this study represent the vascular responses associated with neural activity was not clear enough and should be analysed in the future by utilising other measurement modalities. The limitation of this study was that the instrument did not include the short-channel regression. The short-channel regression could increase the sensitivity of the brain.

5 Conclusion

The present study demonstrated that brain activity in the sensory cortex and VLPFC is maintained at high levels when performing BBT tasks in a semi-immersive VR environment in healthy individuals.

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COI The authors declare no conflicts of interest associated with this manuscript.

References

- Howard MC (2017) A meta-analysis and systematic literature review of virtual reality rehabilitation programs. *Comput Human Behav* 70:317–327
- Kim W-S et al (2020) Clinical application of virtual reality for upper limb motor rehabilitation in stroke: review of technologies and clinical evidence. *J Clin Med Res* 9
- Chen J, Or CK, Chen T (2022) Effectiveness of using virtual reality-supported exercise therapy for upper extremity motor rehabilitation in patients with stroke: systematic review and meta-analysis of randomized controlled trials. *J Med Internet Res* 24:e24111
- Tsoupikova D et al (2015) Virtual immersion for post-stroke hand rehabilitation therapy. *Ann Biomed Eng* 43:467–477
- Thielbar KO et al (2020) Home-based upper extremity stroke therapy using a multiuser virtual reality environment: a randomized trial. *Arch Phys Med Rehabil* 101:196–203
- da Silva Cameirão M et al (2011) Virtual reality based rehabilitation speeds up functional recovery of the upper extremities after stroke: a randomized controlled pilot study in the acute phase of stroke. *Restor Neurol Neurosci* 29:287–298
- Takizawa R et al (2008) Reduced frontopolar activation during verbal fluency task in schizophrenia: a multi-channel near-infrared spectroscopy study. *Schizophr Res* 99:250–262
- Moro SB et al (2016) A novel semi-immersive virtual reality visuo-motor task activates ventrolateral prefrontal cortex: a functional near-infrared spectroscopy study. *J Neural Eng* 13:036002
- Calabrò RS et al (2017) The role of virtual reality in improving motor performance as revealed by EEG: a randomized clinical trial. *J Neuroeng Rehabil* 14:53
- Hao J, Xie H, Harp K, Chen Z, Siu K-C (2022) Effects of virtual reality intervention on neural plasticity in stroke rehabilitation: a systematic review. *Arch Phys Med Rehabil* 103:523–541
- Saleh S et al (2011) Mechanisms of neural reorganization in chronic stroke subjects after virtual reality training. *Conf Proc IEEE Eng Med Biol Soc* 2011:8118–8121
- Bao X et al (2013) Mechanism of kinect-based virtual reality training for motor functional recovery of upper limbs after subacute stroke. *Neural Regener Res* 8:2904–2913
- Holper L et al (2010) Testing the potential of a virtual reality neurorehabilitation system during the performance of observation, imagery, and imitation of motor actions recorded by wireless functional near-infrared spectroscopy (fNIRS). *J Neuroeng Rehabil* 7:1–13
- Wang Z-R et al (2017) Leap motion-based virtual reality training for improving motor functional recovery of upper limbs and neural reorganization in subacute stroke patients. *Neural Regener Res* 12:1823–1831

Effects of Head Elevation on Cerebral Oxygenation in Acute Cerebral Infarction

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Abstract

This study clarifies the effects of head elevation on cerebral oxygenation in patients with acute cerebral infarction. We included 76 patients with anterior circulation cerebral infarction admitted to the stroke care unit within 7 days of symptom onset. Using near-infrared spectroscopy (NIRS), we measured regional saturation oxygen (rSO₂), oxygenated haemoglobin (O₂Hb), deoxygenated haemoglobin (HHb), and total haemoglobin (THb) in both the affected hemisphere (AH) and unaffected hemisphere (UH) after raising the head to 0°, 30°, and 60° for 1 minute each. The mean arterial pressure (MAP) was measured non-invasively at each position. Our

result demonstrated a significant decrease in O₂Hb as the head elevation angle (HEA) increased, with no observable interaction between the AH and UH. A significant decrease in rSO₂ interaction was observed. In contrast, the HHb and THb levels changed significantly as the HEA increased, although no interactions were identified. No significant correlations existed between the observed changes in O₂Hb, HHb, THb, or rSO₂ and changes in MAP at each HEA.

Keywords

Cerebral infarction · Head elevation · Prefrontal cortex · Cerebral oxygenation · Wearable near-infrared spectroscopy

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1 Introduction

Early mobilisation improves the ability of patients with acute cerebral infarction (CI) to perform daily activities [1]. However, during the acute phase of early mobilisation, the progression of ischaemia is associated with decreased cerebral blood flow (CBF), making the management of CBF important [2]. Near-infrared spectroscopy (NIRS) can be used to monitor CBF by measuring cerebral oxygenation [3]. A previous

study indicated that changing the head elevation angle (HEA) from 0° to 30° resulted in decreased cerebral oxygenation [4]. However, although patients with CI have their HEA at 60° in daily life [5], few studies have explored changes in cerebral oxygenation at HEAs of 60° in patients with acute CI. Therefore, this study determines the effects of HEA on cerebral oxygenation in patients with acute CI.

2 Methods

2.1 Participants

This single-centre, prospective, observational study included 76 patients with CI who were admitted to the stroke care unit of Kobe City Medical Center General Hospital between August 2022 and April 2023. The inclusion criteria were as follows: patients with CI in the anterior circulation who were admitted within 7 days of symptom onset and those who could mobilise. The exclusion criteria included patients for whom applying the NIRS plate was impractical, those aged <18 years, and individuals with respiratory instability (requiring oxygen therapy or exhibiting abnormal breathing patterns [6]). In addition, patients who had difficulty understanding the instructions or maintaining a resting position due to agitation were excluded. Patients who did not provide informed consent for participation were excluded. This study was approved by the Research Ethics Review Committee of the Kobe City Medical Center General Hospital (number: zh230304). Information about the study was provided to all patients, and they were informed of the option to withdraw from the study at any time.

2.2 Measurement Protocol

Cerebral oxygenation was measured using an NIRS device (Brain Activity Monitor Hb133; ASTEM, Kanagawa, Japan). Hb133 is equipped with two optodes, with their placement corresponding to frontal pole (Fp) 1 and Fp 2 based on

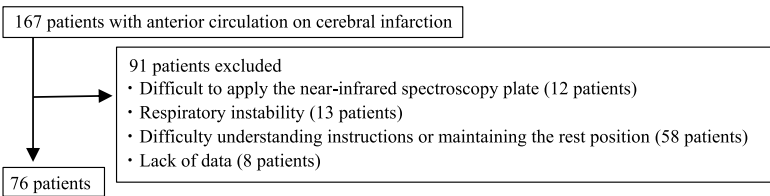
the international 10–20 system, targeting the left and right prefrontal cortex (PFC) as regions of interest. Considering the ease of measurement in clinical settings, we measured one channel each of the left and right PFC. The interoptode distances were 30 and 35 mm, corresponding to the distance between the source and detector. Near-infrared light with wavelengths of 770 and 830 nm was employed, and data were sampled at a rate of 1.0 Hz.

The starting posture was an HEA of 0°, and the HEA was sequentially adjusted from 0° to 30° to 60° after 1 min at each position [7]. Cerebral oxygenated haemoglobin (O₂Hb), deoxygenated haemoglobin (HHb), total haemoglobin (THb), and regional saturation of oxygen (rSO₂) were measured in both hemispheres at all HEAs. The hemispheres were classified into the affected hemisphere (AH) and unaffected hemisphere (UH) based on the imaging diagnosis of magnetic resonance imaging. Considering the influence of autoregulatory capacity [8], the mean values were calculated for each 1-minute condition based on data from 30 seconds to 1 min, and the difference (Δ) between the mean values of cerebral oxygenation associated with each HEA was calculated. The mean arterial pressure (MAP) was measured using non-intuitive blood pressure measurements at each postural change.

2.3 Statistical Analysis

Continuous variables are expressed as mean and standard deviation (SD), while qualitative variables are represented by number and frequency. The mean changes in AH and UH cerebral oxygenation associated with HEA were analysed using repeated measures two-way analysis of variance (ANOVA), with Shaffer's modified sequentially rejective Bonferroni procedure as a post hoc test. Spearman's rank correlation coefficient was used to analyse the relationship between MAP and cerebral oxygenation associated with each change in HEA. Statistical analyses were performed using R software (version 4.2.1; CRAN, Freeware,

Fig. 1 Patient flowchart



New Zealand). P-values of <0.05 were considered statistically significant.

3 Results

3.1 Patient Characteristics

The final analysis included 76 patients (Fig. 1). The median patient age was 72.5 years, and 25 patients were female. The mean National Institutes of Health Stroke Scale score was 4.8 (Table 1).

3.2 Effect of HEA on Cerebral Oxygenation

O₂Hb, HHb, and THb significantly differed within each HEA; however, no significant differences were observed within each hemisphere nor was there a significant interaction between HEA and hemisphere. A significant difference in HEA and interaction was observed in rSO₂, although no significant difference was observed between the hemispheres (Table 2). The post hoc test result indicated significant differences within each HEA in cerebral oxygenation measurements (Fig. 2).

3.3 Relationship Between Cerebral Oxygenation and MAP

No significant correlations were identified between the changes in O₂Hb, HHb, THb, or rSO₂ in the AH and changes in the MAP at each HEA (Table 3).

Table 1 Patient characteristics

Characteristics	
Age (years)	72.5 (14.5)
Women	25 (33)
Past medical history	
Diabetes mellitus	19 (25)
Hypertension	55 (72)
Stroke	23 (30)
Trials of Org 10,172 in acute Stroke treatment classification	
Small vessel occlusion	11 (14)
Large artery atherosclerosis	24 (32)
Cardioembolism	18 (24)
Other determined aetiology	5 (6)
Undetermined	18 (24)
National Institutes of Health stroke scale at admission (points)	4.8 (4.3)
Haemoglobin at admission (g/dL)	13.8 (1.7)
Number of days from the date of stroke onset to the date of measurement (days)	2.2 (1.2)

Data are presented as mean (SD) or numbers (percentages)

4 Discussion

In this study, we compared the cerebral oxygenation of patients with acute CI at different HEAs of 0°, 30°, and 60°. We observed a decrease in both O₂Hb and rSO₂ in both the AH and UH as the HEA increased, although no significant changes in the MAP were observed.

These findings may be attributed to impaired cerebral autoregulation in patients with acute CI. O₂Hb [3] and rSO₂ [9], which are related to CBF, typically decrease immediately after orthostasis in healthy patients, but the levels increase within 30 seconds due to cerebral autoregulation [8]. On the contrary, cerebral autoregulation is impaired in patients with CI, affecting either the AH in severe cases or both the AH and UH in mild cases

Table 2 Effect of head elevation on cerebral oxygenation

	Main effect (HEA)		Main effect (hemisphere)		Interaction	
	F ratio	P value	F ratio	P value	F ratio	P value
rSO ₂	47.75	<0.001	0.01	0.976	4.28	0.026
ΔO ₂ Hb	24.78	<0.001	0.06	0.812	0.31	0.624
ΔHHb	9.48	<0.001	0.96	0.331	2.87	0.084
ΔTHb	9.21	0.001	0.05	0.825	0.90	0.365

A two-way repeated measures analysis of variance with Greenhouse-Geisser corrections was used
Abbreviations: *HEA* head elevation angle, *rSO₂* regional saturation of oxygen, *O₂Hb* oxygenated haemoglobin, *HHb* deoxygenated haemoglobin, *THb* total haemoglobin

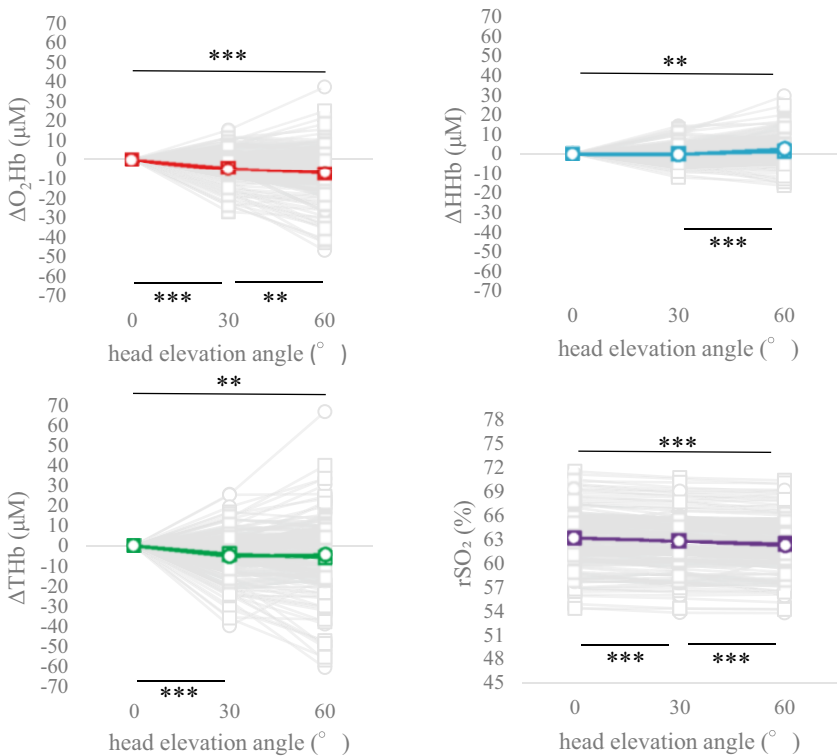


Fig. 2 Effect of head elevation on cerebral oxygenation. (Post hoc test: Shaffer's Modified Sequentially Rejective Bonferroni Procedure was used. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Abbreviations: *O₂Hb* oxygenated haemoglobin, *HHb* deoxygenated haemoglobin, *THb* total haemoglobin, *rSO₂* regional saturation of oxygen)

Table 3 Relationship between cerebral oxygenation and MAP

	HEA	ρ value	95% Confidence interval	p value
rSO ₂	0° to 30°	0.037	−0.190 – 0.260	0.751
	30° to 60°	0.195	−0.032 – 0.402	0.092
ΔO ₂ Hb	0° to 30°	−0.082	−0.302 – 0.146	0.479
	30° to 60°	0.162	−0.066 – 0.374	0.163
ΔHHb	0° to 30°	−0.221	−0.426 – 0.004	0.055
	30° to 60°	0.026	−0.201 – 0.250	0.823
ΔTHb	0° to 30°	−0.101	−0.319 – 0.128	0.387
	30° to 60°	0.135	−0.094 – 0.350	0.246

Spearman's rank correlation coefficient was used. Abbreviations: *HEA* head elevation angle, *rSO₂* regional saturation of oxygen, *O₂Hb* oxygenated haemoglobin, *HHb* deoxygenated haemoglobin, *THb* total haemoglobin, *MAP* mean arterial pressure

[10]. The bilateral impairment observed in our study may be due to the inclusion of mild cases.

Further, a previous study that included both healthy patients and patients with CI at HEA of 0° showed that changes in MAP corresponded to changes in cerebral oxygenation among patients with CI [11]. Reports on the effects of head elevation on CBF in healthy patients suggested that head elevation results in a shift of blood flow to the lower extremities, leading to decreased CBF [12]. This study compared cerebral oxygenation and MAP between HEA 0°, 30°, and 60° in patients with CI. The authors found that O₂Hb and rSO₂ were presumably decreased in response to head elevation, independent of MAP.

This study had a limitation. NIRS signals are affected by skin blood flow [13], and it would be difficult to exclude the effect of these signals.

In conclusion, regardless of MAP, we observed a decrease in rSO₂ and O₂Hb in the AH and UH as HEA increased in patients with acute CI.

References

1. Li Z, Zhang X, Wang K, Wen J (2018) Effects of Early Mobilization after Acute Stroke: a meta-analysis of randomized control trials. *J Stroke Cerebrovasc Dis* 27:1326–1337. <https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.12.021>
2. Park TH, Lee J-K, Park M-S, Park S-S, Hong K-S, Ryu W-S et al (2020) Neurologic deterioration in patients with acute ischemic stroke or transient ischemic attack. *Neurology* 95:e2178–e2191. <https://doi.org/10.1212/WNL.00000000000010603>
3. Hoshi Y, Kobayashi N, Tamura M (1985) Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *J Appl Physiol* 2001(90):1657–1662. <https://doi.org/10.1152/jappl.2001.90.5.1657>
4. Kim MN, Edlow BL, Durduran T, Frangos S, Mesquita RC, Levine JM et al (2014) Continuous optical monitoring of cerebral hemodynamics during head-of bed manipulation in brain-injured adults. *Neurocrit Care* 20:443–453. <https://doi.org/10.1007/s12028-013-9849-7>
5. Kubota S, Endo Y, Kubota M, Shigemasa T (2017) Assessment of effects of differences in trunk posture during Flower's position on hemodynamics and cardiovascular regulation in older and younger subjects. *Clin Interv Aging* 12:603–610. <https://doi.org/10.2147/CIA.S132399>
6. Boulding R, Stacey R, Niven R, Fowler S (2016) Dysfunctional breathing: a review of the literature and proposal for classification. *Eur Respir Rev* 25:287–294. <https://doi.org/10.1183/16000617.0088-2015>
7. Mukli P, Nagy Z, Racz FS, Portoro I, Hartmann A, Stylianou O et al (2021) Two-tiered response of cardiorespiratory-cerebrovascular network to orthostatic challenge. *Front Physiol* 12:622569. <https://doi.org/10.3389/fphys.2021.622569>
8. Mol A, Claassen JAHR, Maier A, van Wezel RJA, Meskers CGM (2022) Determinants of orthostatic cerebral oxygenation assessed using near-infrared spectroscopy. *Auton Neurosci* 238:102942. <https://doi.org/10.1016/j.autneu.2022.102942>
9. Krakow K, Ries S, Daffertshofer M, Hennerici M (2000) Simultaneous assessment of brain tissue oxygenation and cerebral perfusion during orthostatic stress. *Eur Neurol* 43:39–46. <https://doi.org/10.1159/000008127>
10. Castro P, Azevedo E, Sorond F (2018) Cerebral autoregulation in stroke. *Curr Atheroscler Rep* 20:37. <https://doi.org/10.1007/s11883-018-0739-5>
11. Su H, Huo C, Wang B, Li W, Xu G, Liu Q et al (2018) Alterations in the coupling functions between cerebral oxyhaemoglobin and arterial blood pressure signals in post-stroke subjects. *PLoS One* 13:e0195936. <https://doi.org/10.1371/journal.pone.0195936>
12. Olufsen MS, Smith B, Mehlsen J, Ottesen J (2011) The impact of gravity during head-up tilt. *Annue Int Conf IEEE Eng Med Biol Soc* 2011:2399–2402. <https://doi.org/10.1109/IEMBS.2011.6090669>
13. Hirasawa A, Yanagisawa S, Tanaka N, Funane T, Kiguchi M, Sorensen H et al (2015) Influence of skin blood flow and source-detector distance on near-infrared spectroscopy-determined cerebral oxygenation in humans. *Clin Physiol Funct Imaging* 35:237–244. <https://doi.org/10.1111/cpf.12156>

Evaluating the Status of the Injured Brain: Cerebrovascular Reserve (CVR) Is Not Equivalent to Induced Cerebrovascular Reactivity (iCVRx) and Induced Pressure Reactivity (iPRx) in Defining the Critical Cerebral Perfusion Pressure (CPP)

Edwin M. Nemoto, Denis E. Bragin,
and Howard Yonas

Abstract

Methods evaluating the status of the injured brain have evolved over the past 63 years since Lundberg first reported clinical measurement of intracranial pressure (ICP) to evaluate the status of the injured brain (Lundberg, *Acta Psychiatr Scand Suppl.* 36:1–193, 1960). Subsequent evaluation involved measurement of the autoregulatory capacity of the brain by measuring cerebral blood flow (CBF) with decreasing mean arterial pressure (MAP) to define the critical CPP where the vasodilatory capacity of the cerebral circulation is exceeded

and CBF begins to fall (CPP of 50 mmHg). A seminal advance was made by Marmarou (Marmarou et al., *J Neurosurg.* 48:332–344, 1978) who measured brain compliance by injecting a bolus of saline into the intracranial catheter while measuring the rise in intracranial pressure (ICP) otherwise known as induced pressure reactivity (iPRx). Seeking to utilise continuous measurement of iPRx in traumatic brain injury (TBI) patients with continuous monitoring of ICP, the ICP response to arterial pulsations was developed to evaluate the optimal CPP patients with raised ICP by the arterial pulsations-based iPRx. A similar approach was made with Doppler measurement of CBF with arterial pulsations for iCVRx to guide optimal CPP (CPPopt). Both iPRx and iCVRx are associated with microvascular shunts (MVS) and can accurately measure the critical CPP, whereas the CBF autoregulation curve by decreasing MAP does not. Sophisticated continuous multimodal monitoring established with ICM+ algorithms successfully identifies CPPopt for ICP control and identifies CBF dysregulation as related to outcome, but does not provide insights into

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the mechanisms involved in the loss of CBF autoregulation as related to increased ICP and potentially effective treatments (Froese et al., *Neurocrit Care*. 34:325–335, 2021).

Keywords

Intracranial pressure · CBF autoregulation · Microvascular shunts · Cerebral blood flow (CBF) · Vasodilatory capacity

where the vasodilatory capacity of cerebral blood vessels is exceeded or the “critical CPP” nominally defined at a CPP of 50 mmHg and a breakthrough of CBF autoregulation at a CPP > 150 mmHg (Fig. 1). CBF autoregulation curves were routinely determined by decreasing mean arterial pressure (MAP). Flow compromised regions showed an increase in the critical CPP to higher values and a decreased response to a vasodilatory stimulus with CO₂.

1 Cerebral Blood Flow (CBF) Autoregulation

Methods in the development of measurement of cerebral blood flow (CBF) evolved in the 1950s–1970s, initiated most significantly by the development of the inert gas measurement of CBF reported by Kety and Schmidt [4], revolutionising CBF measurements especially clinically. Not long thereafter, inert radioactive gas measurements with external radiation detection were developed primarily by the Scandinavians led by Lassen and colleagues [5]. These advances allowed the precise definition of the critical cerebral perfusion pressure (CPP), i.e. the point

2 Defining the Critical CPP by Decreasing MAP or Increasing ICP

The CBF autoregulation curve with decreasing CPP was historically done by decreasing mean arterial pressure (MAP), but Miller et al. [6] realised that patients had increased ICP to decrease CPP and asked if the critical CPP was the same if CPP was decreased by increasing ICP instead of decreasing MAP. In a study on dogs, he found that the critical CPP decreased from 50 mmHg to 30 mmHg when ICP was increased as opposed to decreasing MAP to decrease CPP (Fig. 2). Grubb et al. [7] observed the same in

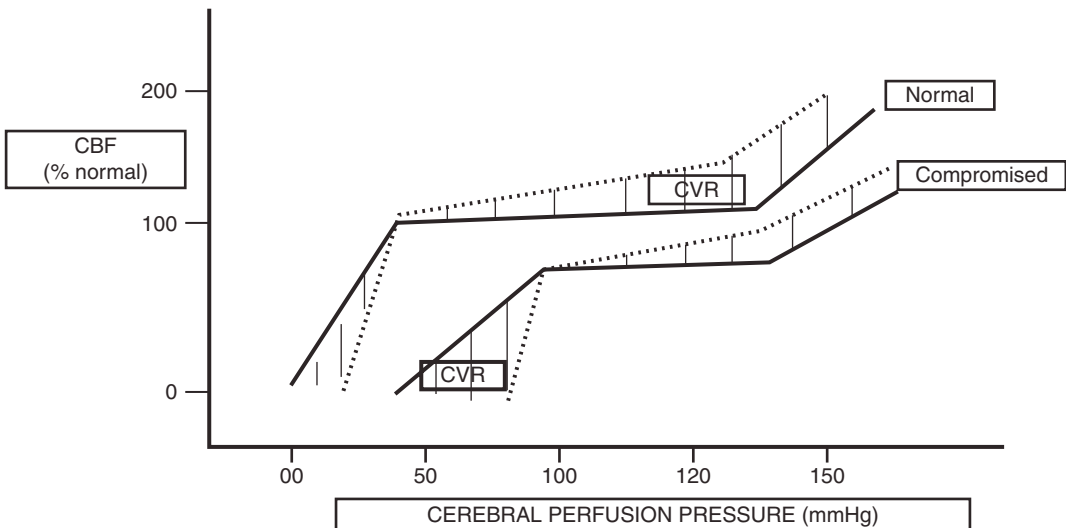


Fig. 1 Hypothetical Cerebral blood flow (CBF) autoregulation curved in normal and compromised brain regions with their cerebrovascular response to a vasodilatory stim-

ulus by CO₂. Illustration shows the increase in the critical CPP in the compromised brain region and the attenuated CO₂ response

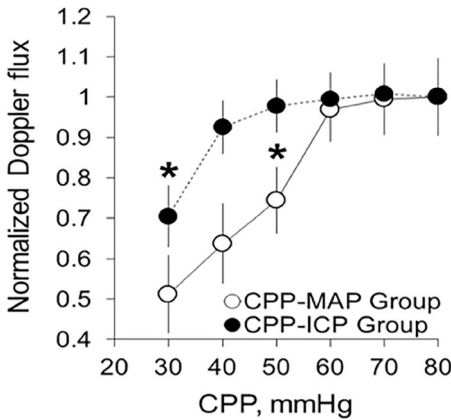


Fig. 2 Static autoregulation curves for cerebral perfusion pressure (CPP)-intracranial pressure (ICP) group ($n = 12$ rats) and CPP-mean arterial pressure (MAP) group, ($n = 7$ rats). Data normalised to baseline CPP of 70 mm Hg obtained before any manipulation (mean $\pm$ SEM, $*p < 0.05$) [11, 13]

monkeys. The reason for this decrease in critical CPP was unexplained for nearly half a century when we hypothesised and showed that it was due to the opening of microvascular shunts (MVS) with increased ICP, which we proved to be the case in subsequent studies on rats with increased ICP using two-photon laser scanning microscopy (2PLSM) [8–10].

3 Induced Pressure Reactivity (iPRx) and Induced Cerebrovascular Reactivity (CVRx)

To describe the compliance of the injured brain with raised intracranial pressure (ICP), Marmarou [2] developed the concept and method of induced pressure reactivity (iPRx) by the injection of small (1 ml) volume of saline into the brain through the ICP catheter in the lateral ventricle being used for continuous measurement of ICP. He also used the measurement of the response in CBF to the transient increase in ICP (iCVRx) [13], which is more cumbersome than the ICP response (iPRx)

which became the standardised method of monitoring intracranial compliance. Both iCVRx and iPRx are induced measurements of the status of the cerebrovascular autoregulation that accurately reflects the critical CPP in contrast to the passive changes in CBF with decreasing CPP in measuring the vasodilatory status of cerebral blood vessels (Fig. 3).

4 Microvascular Shunts and Intracranial Pressure in Induced Cerebrovascular Reactivity and Induced Pressure Reactivity

Induced cerebrovascular reactivity (iCVRx) and pressure reactivity (iPRx) are associated with the opening of MVS as indicated by their relationship to the increase in MVS/CAP ratio or increase in MVS (Fig. 4) that is mitigated by increasing CPP [8, 9] (Fig. 5). Changes in Doppler flow with increasing ICP compared to decreasing MAP show that flow is maintained at lower CPP in the ICP compared to the MAP group due to MVS flow just as it is maintained with decreasing CPP by increasing ICP [13].

Both iPRx and iCVRx increase with decreasing CPP in contrast to the decrease in CBF with decreasing CPP in the CBF autoregulation curve where cerebrovascular dilation maintains CBF constant as CPP decreases until maximum vasodilation occurs and CBF begins to fall. The dilation of cerebral blood vessels correlates with an increase in iPRx and iCVRx, reflecting the effect of cerebrovascular dilation on the arterial pulsations on the induced increase in intracranial pressure reactivity. The increase in iCVRx and iPRx is directly related to an increase in the ratio of MVS/CAP or increasing MVS flow. The same relationship is revealed in the diversion of the increase in MVS/CAP ratio with decreasing CPP in the CPP-ICP compared to the CPP-MAP groups (Fig. 5).

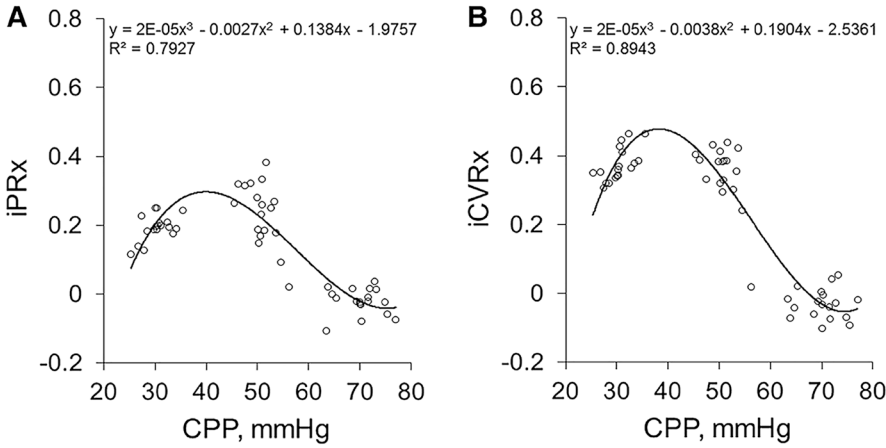


Fig. 3 Dopamine-induced ICP reactivity (iPRx) (a) and cerebrovascular reactivity (iCVRx) (b) in the cerebral perfusion pressure (CPP)-mean arterial pressure (MAP) group (CPP reduced by MAP decrease). Each circle represents one test (nine tests per rat, total seven rats). Trend line is the fitted polynomial (third order) to all data points [12]

Fig. 4 Correlation between MVS/CAP flow ratio and iCVRx (a) and iPRx (b) plotted for every hour after TBI with high ICP. Open circles—baseline. Red dotted line—linear fit [11]

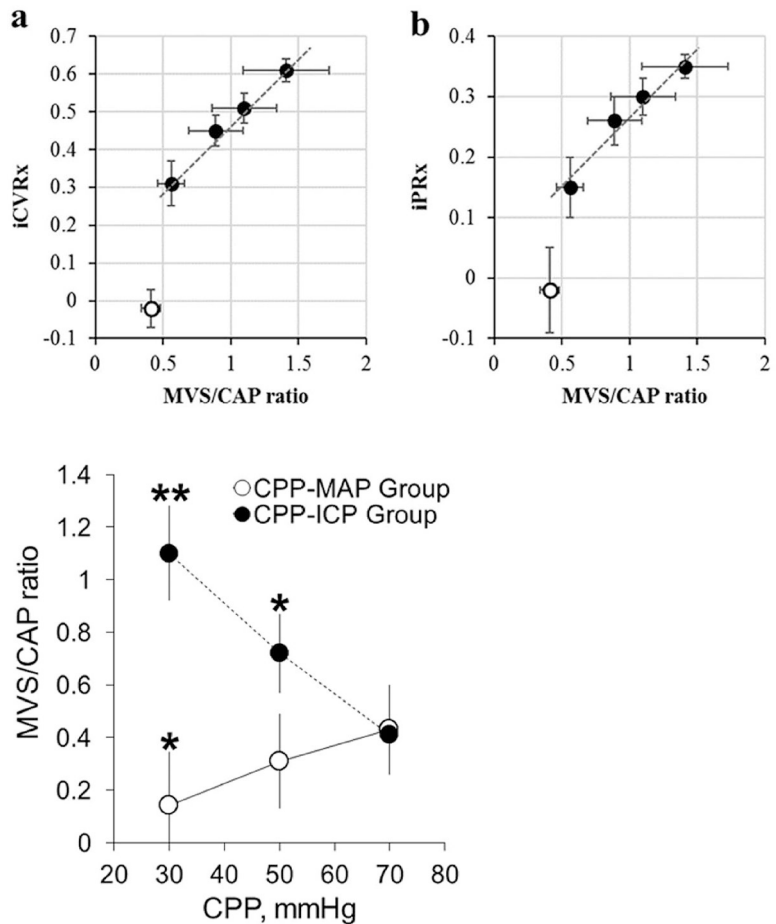


Fig. 5 Microvascular shunt (MVS)/capillary (CAP) flow ratio showing decreased cerebral perfusion pressure (CPP) by increased intracranial pressure (ICP) causes stagnation of CAP flow and an increase of MVS flow (CPP-ICP group, $n = 12$). The CPP decrease by mean arterial pressure (MAP) reduction decreased MVS/CAP ratio. (CPP-MAP group, $n = 7$). Mean $\pm$ SEM, $*p < 0.05$, $**p < 0.01$, compared to a baseline CPP of 70 mmHg [13]

5 Summary

Our results show that critical CPP in the injured brain with raised ICP is not accurately determined by the conventional method of decreasing CPP by decreasing MAP because of the role of MVS and can only be accurately measured by iCVRx or iPRx.

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References

1. Lundberg N (1960) Continuous recording and control of ventricular fluid pressure in neurosurgical practice. *Acta Psychiatr Scand Suppl* 36:1–193
2. Marmarou A, Shulman K, Rosende RM (1978) A nonlinear analysis of the cerebrospinal fluid system and intracranial pressure dynamics. *J Neurosurg* 48(3):332–344
3. Froese L, Batson C, Gomez A, Dian J, Zeiler FA (2021) The limited impact of current therapeutic interventions on cerebrovascular reactivity in traumatic brain injury: a narrative overview. *Neurocrit Care* 34(1):325–335
4. Kety SS, Schmidt CF (1948) The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J Clin Invest* 27(4):476–483
5. Ingvar DH, Lassen NA (1965) Methods for cerebral blood flow measurements in man. *Br J Anaesth* 37:216–224
6. Miller JD, Stanek A, Langfitt TW (1972) Concepts of cerebral perfusion pressure and vascular compression during intracranial hypertension. *Prog Brain Res* 35:411–432
7. Grubb RL Jr, Raichle ME, Phelps ME, Ratcheson RA (1975) Effects of increased intracranial pressure on cerebral blood volume, blood flow, and oxygen utilization in monkeys. *J Neurosurg* 43:385–398
8. Nemoto EM (2006) Dynamics of cerebral venous and intracranial pressures. *Acta Neurochir Suppl* 96:435–437
9. Bragin DE, Bush RC, Müller WS, Nemoto EM (2011) High intracranial pressure effects on cerebral cortical microvascular flow in rats. *J Neurotrauma* 28:775–785
10. Bragin DE, Bush RC, Nemoto EM (2013) Effect of cerebral perfusion pressure on cerebral cortical microvascular shunting at high intracranial pressure in rats. *Stroke* 44:177–181
11. Yoshihara M, Bandoh K, Marmarou A (1995) Cerebrovascular carbon dioxide reactivity assessed by intracranial pressure dynamics in severely head injured patients. *J Neurosurg* 82(3):386–393
12. Bragin DE, Statom GL, Nemoto EM (2018) Induced dynamic intracranial pressure and cerebrovascular reactivity assessment of cerebrovascular autoregulation after traumatic brain injury with high intracranial pressure in rats. *Acta Neurochir Suppl* 126:309–312
13. Bragin DE, Statom GL, Yonas H, Dai X, Nemoto EM (2014) Critical cerebral perfusion pressure at high intracranial pressure measured by induced cerebrovascular and intracranial pressure reactivity. *Crit Care Med* 42(12):2582–2590

Haemodynamic Responses to Spontaneous Neural Activity on the Electroencephalogram in Preterm Infants

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Abstract

Delta brushes are spontaneous neural activities observed in preterm electroencephalograms (EEGs) and are thought to reflect the activities of subplate neurons in the developing brain. We investigated the haemodynamic responses associated with delta brushes in five preterm infants at two time points (at 33 or 34,

and 36 weeks of postmenstrual age), using simultaneous EEG-functional near-infrared spectroscopy (NIRS). An automated detection algorithm was developed to identify the brush components of delta brushes in the bipolar EEG envelope; we placed eight EEG electrodes. An eight-channel NIRS device was placed around the head of each infant to measure changes in oxy- and deoxy-haemoglobin (Hb) concentrations. Haemodynamic grand averages were calculated for local brushes in each NIRS channel. We classified the responses into five patterns based on changes in oxy- and deoxy-Hb signals (positive in-phase/anti-phase, negative in-phase/anti-phase, and unclassified) and evaluated the relationship between the locations of NIRS measurements and those of brushes, as well as the haemodynamic response patterns and infant age at the time of recording. In all the

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10 recordings, we found that positive responses (oxy-Hb increases) predominated, not only in the corresponding areas but also in remote areas. Particularly, notable responses were observed in the bilateral temporal areas. Among the positive responses, the proportion exhibiting an anti-phase pattern was 12% at 33 to 34 weeks of postmenstrual age and 54% at 36 weeks of postmenstrual age. Our unexpected finding of remarkable temporal responses to localised neuronal activity supports the hypothesis that the insula is the most strongly interconnected hub in the developing brain.

Keywords

Delta brush · Preterm infants · Hemodynamic response · EEG and fNIRS

1 Introduction

Spontaneous neural activities play crucial roles in the structural and functional maturation of the developing brain. Such activities can be observed in the electroencephalograms (EEGs) of preterm infants. One of the most characteristic and well-known features is the “delta brush” (Fig. 1) [1].

Animal studies have indicated that delta brushes, also termed spindle bursts, reflect the neuronal activity of the subplate located beneath

the cortical plate. The subplate is required for early brain development, including neuronal migration and synaptogenesis [2]. However, data on the relationship between delta brushes and subplate neuronal activities in humans are limited. Delta brushes begin to increase in number around 28 weeks, peak at 32–34 weeks, and gradually decline toward 40 weeks of postmenstrual age (PMA), in alignment with the developmental trajectory of the human subplate.

Arichi et al. demonstrated that posterior-temporal delta brushes in human late preterm infants were associated with ipsilateral blood oxygen level-dependent activation [3]. Such activation was observed in the temporal and insular cortices, which are highly interconnected hubs within the developing cortex [3]. However, neurovascular coupling in preterm infants is still poorly understood, and the haemodynamic responses to subplate neuronal activities remain unknown. This study investigated the haemodynamic responses associated with local delta brushes using simultaneous EEG-functional near-infrared spectroscopy recordings (EEG-fNIRS).

2 Methods

2.1 Participants

We recruited a total of five preterm infants (two females) who were hospitalised in the neonatal intensive care unit of Nagoya University Hospital. They were born between 31 and 33 weeks of gestational age and underwent EEG-fNIRS recordings at 33 or 34, and 36, weeks of PMA. The head circumference ranged from 28.0 to 32.4 cm at the first session and 29.6 to 34.0 cm at the second session. None of the infants had any major congenital anomalies, required surgical treatments, or exhibited brain injuries detected by neuroimaging during hospitalisation. This study was approved by the ethical committee of Nagoya University Hospital (approval no. 2019–0506). Written informed consent was obtained from the parents.

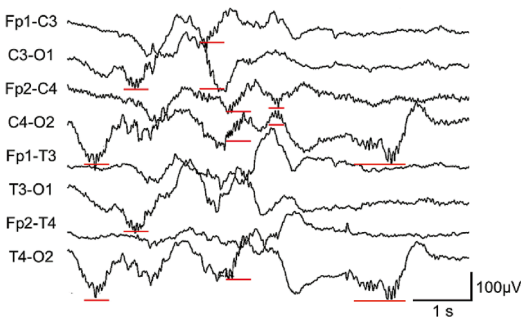


Fig. 1 Delta brushes in the EEG. A representative EEG recorded at 33 weeks of postmenstrual age. Delta waves with superimposed alpha or beta rhythms (brushes) are seen in O2, O1, C3, and C4 (red lines)

2.2 EEG-fNIRS Recordings

The duration of each recording was more than 40 min and included periods of both active sleep (AS) and quiet sleep (QS).

EEGs (EEG-1200 platform, Nihon Kohden, Tokyo, Japan) were recorded using at least eight electrodes (Fp1, Fp2, C3, C4, O1, O2, T3, and T4) placed according to the international 10–20 system with a 0.002 s time resolution. In addition to an EEG, an electro-oculogram, abdominal respiratory movements, a chin electromyogram, an electrocardiogram, and a video were recorded simultaneously.

An eight-channel NIRS device (ETG-100, Hitachi Medical Corporation, Tokyo, Japan) was placed around each infant's head covering the frontal, left and right temporal, and occipital areas (Fig. 2). This instrument generated near-infrared light of two wavelengths (780 and 830 nm) and measured the time courses of the relative changes in oxy- and deoxy-haemoglobin

(Hb) levels at a 0.1 s time resolution. The distance between the source and the detector was set to 2.0 cm as in a previous study [4].

2.3 Classification of Behavioural States

Behavioural states were classified every 30 s following the criteria of the American Clinical Neurophysiology Society [5]. Classification was based on the EEG patterns, rapid eye movements, respiratory patterns, and eye opening/closing. The behavioural states included AS, QS, indeterminate sleep, and awake. All data segments of AS and QS lasting at least 2 min were included in analyses.

2.4 EEG Preprocessing and Automated Detection of Delta Brushes

An automated detection algorithm was developed to identify the brush component of the delta brush. Initially, the bipolar EEG envelope was filtered over the 8–25 Hz frequency range, which corresponds to that of brushes. Subsequently, the algorithm detected brush events of duration ranging from 0.3 to 1.5 s and of average amplitude $>10 \mu\text{V}$ within a sliding window of 0.3 s; this met the defined criteria for brushes [1, 6]. Only local brushes, i.e., those that did not duplicate in any other electrode during the durations of brushes appearing in one electrode, were selected.

2.5 NIRS Preprocessing and the Grand Averages of the Oxy- and Deoxy-Hb Signals

First, we detected motion artifacts in each channel, defined as changes in the sums of oxy- and deoxy-Hb signals that exceeded 0.15 mM·mm

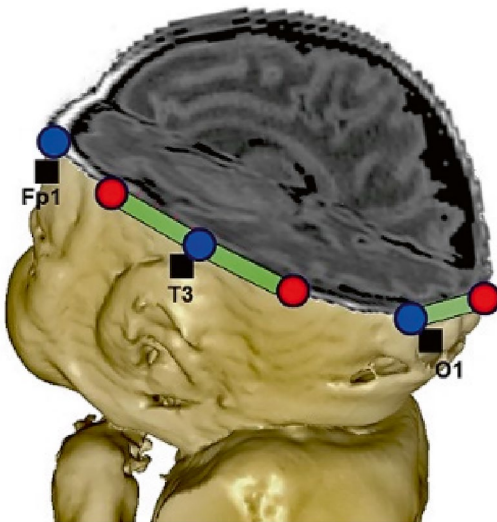


Fig. 2 Placement of the NIRS and EEG equipment. The NIRS channels were placed just above the Fp1-T3-O1-O2-T4-Fp2 line of the EEG electrodes. Red circles indicate NIRS sources, blue circles are NIRS detectors, and black squares are EEG electrodes

between the means of four successive samples and the means of the next four samples. The segments of recordings containing motion artifacts were excluded from the analyses of the channel. Next, the oxy- and deoxy-Hb time-course signals were band-pass filtered at 0.05–0.1 Hz to remove physiological noise and high frequency measurement noise.

The grand averages of the oxy- and deoxy-Hb signals for each NIRS channel after preprocessing were calculated for all brush onsets that were localised to each EEG electrode, as detected by the algorithm, throughout all AS and QS of each recording.

2.6 Evaluation of Grand Averages of Oxy- and Deoxy-Hb Signals

The grand averages of the oxy- and deoxy-Hb signals for each local brush in the NIRS channels were separately qualitatively assessed during AS and QS. The haemodynamic responses were classified into five patterns (Fig. 3): positive (in-phase and anti-phase), negative (in-phase and anti-phase), and unclassified. The NIRS channels were further analysed by dividing them into corresponding and remote areas (non-correspondence). Corresponding areas were defined as follows: frontal NIRS channels for ipsilateral brushes on frontal pole electrodes, temporal NIRS channels for ipsilateral brushes on central or temporal electrodes, and occipital NIRS channels for ipsilateral brushes on occipital electrodes. In addition, the in-phase/

anti-phase patterns within the positive responses (Fig. 3a, b) were evaluated based on the recorded infant age and the locations of delta brushes.

3 Results

The median (range) total recording time for the 10 recordings was 72.0 (43.0–85.0) min. The AS time analysed was 34.0 (21.0–59.5) min, and the QS time analysed was 18.0 (8.5–27.5) min. The median (range) mean brush occurrence rates (events/min/electrode) for the eight electrodes during AS and QS were 7.1 (3.8–7.6) and 7.3 (5.4–8.3) for the five recordings taken at 33–34 weeks of PMA, respectively. Similarly, for the five recordings taken at 36 weeks of PMA, the median (range) mean brush occurrence rates were 3.2 (1.2–5.4) and 4.9 (3.6–8.1) during AS and QS, respectively.

In all 10 recordings, positive responses predominated among the grand averages for local brushes (positive in-phase 41%, positive anti-phase 18%, negative in-phase 11%, negative anti-phase 11%, and unclassified 19%). Positive responses were observed for all local brushes, not only in the corresponding areas but also in remote areas (Table 1), with particularly remarkable responses in the bilateral temporal areas (Fig. 4). Similar responses were observed during both AS and QS.

In total, 46 of 372 positive responses (12%) exhibited an anti-phase pattern at 33 to 34 weeks of PMA, and 158 of 293 positive responses (54%) showed an anti-phase pattern at 36 weeks of PMA (Table 1).

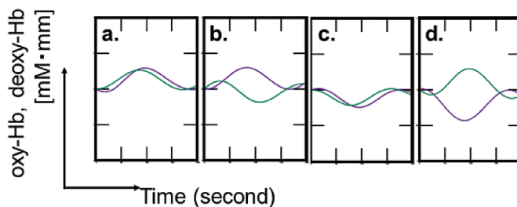


Fig. 3 Representative haemodynamic response patterns. Purple lines show the grand averages of oxy-Hb signals, and green lines are those of deoxy-Hb signals. (a) Positive in-phase, (b) positive anti-phase, (c) negative in-phase, and (d) negative anti-phase responses

4 Discussions and Conclusions

We investigated the haemodynamic responses to spontaneous neural activities of delta brushes apparent in EEGs of preterm infants; we used EEG-fNIRS recordings. We found that haemodynamic responses to local delta brushes were apparent at both time points during the preterm period. Contrary to expectations, we observed

Table 1 Haemoglobin phases (oxygenation and deoxygenation) within the positive responses (increased oxy-Hb signals) of local brushes

Location of delta brush		33/34 weeks		36 weeks	
		Corresponding	Remote	Corresponding	Remote
Frontal	In-phase	11 (58%)	79 (62%)	8 (62%)	26 (29%)
	Anti-phase	2 (11%)	13 (10%)	4 (31%)	30 (34%)
Central/temporal	In-phase	50 (64%)	108 (47%)	20 (26%)	55 (24%)
	Anti-phase	4 (5%)	12 (5%)	29 (38%)	56 (24%)
Occipital	In-phase	8 (47%)	70 (54%)	3 (23%)	23 (23%)
	Anti-phase	1 (6%)	14 (11%)	1 (8%)	38 (38%)
Total	In-phase	326 (55%)		135 (26%)	
	Anti-phase	46 (8%)		158 (30%)	

Data are presented as the original numbers (%). The percentages represent the proportions of all responses, excluding those with fewer than 10 NIRS data points, used when calculating the grand averages

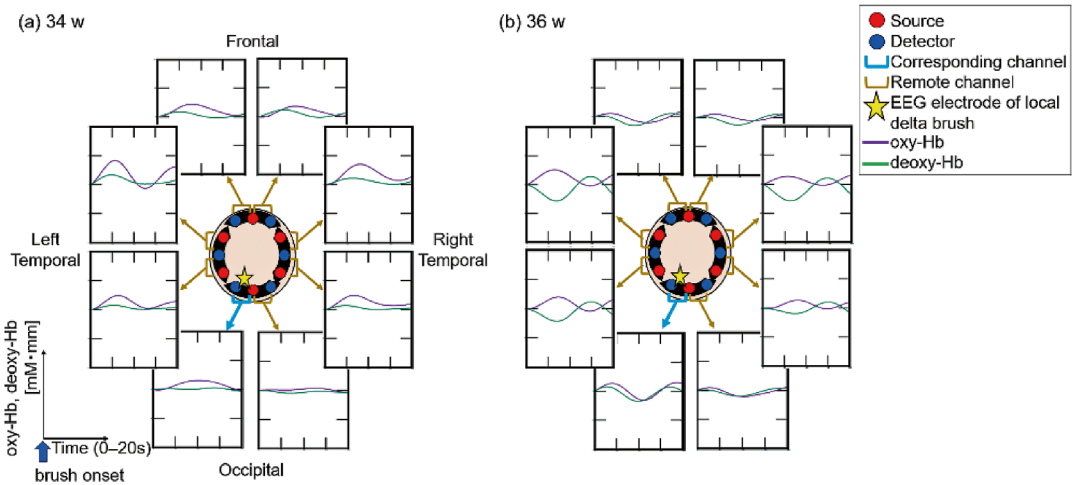


Fig. 4 Grand averages of each NIRS channel for local brushes in the left occipital EEG electrode of one infant. (a) Grand averages recorded at 34 weeks and (b) those recorded at 36 weeks of postmenstrual age during quiet sleep

notable, bilateral, temporal neurovascular coupling associated even with delta brushes localised to the frontal or occipital EEG channels. These findings are consistent with those of a previous study that reported activation of temporal areas [3] and support the hypothesis that the insula is the most strongly interconnected hub in the developing brain [7].

As previously reported in human preterm infants [8], we observed various types of haemodynamic responses. Among these, our data suggest that haemodynamic responses associated with delta brushes develop in patterns that range from in-phase to anti-phase. The trajectories were similar to those of the time-averaged phase differences between spontaneous low-frequency

oscillatory changes in oxy-Hb and deoxy-Hb, thus the haemoglobin phase of oxygenation and deoxygenation, in infants at rest [9]. Given that delta brushes are the most common spontaneous activities during the preterm period, a hypothesis that the haemoglobin phase of oxygenation and deoxygenation develops along with chronological age seems reasonable.

Our study had several strengths. The use of EEG-fNIRS recordings enabled direct investigation of the haemodynamic responses to spontaneous electrical activities in younger preterm infants; it was possible to obtain repeated measurements and to differentiate the sleep states. Additionally, a reliable, automated detection algorithm for delta brushes was developed. The

algorithm detected brushes with an accuracy similar to that of a previous study that evaluated brushes manually; brushes were more common at 33–34 weeks than 36 weeks of PMA, and more frequent during QS compared to AS at 36 weeks of PMA [10].

However, this study had the limitation of a small sample size. To address this, it is necessary to increase the number of recordings and to conduct quantitative evaluations via group analyses in future studies. Furthermore, the amplitudes of the oxy-Hb and deoxy-Hb changes were affected by both signal changes and the optical pathlengths. Although notable haemodynamic changes were observed in temporal areas, such responses cannot yet be conclusively regarded as significant.

In conclusion, we determined the haemodynamic responses associated with the intrinsic neural activities of delta brushes in preterm infants. Although the distribution of the haemodynamic responses at 33–34 weeks resembled that at 36 weeks of PMA, the haemoglobin phases differed. Further study is needed to elucidate the functional roles played by delta brushes.

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References

1. Kidokoro H (2021) Delta brushes are not just a hallmark of EEG in human preterm infants. *Pediatr Int* 63(2):130–136
2. Molnar Z, Luhmann HJ, Kanold PO (2020) Transient cortical circuits match spontaneous and sensory-driven activity during development. *Science* 370(6514):eabb2153
3. Arichi T, Whitehead K, Barone G, Pressler R, Padormo F, Edwards AD et al (2017) Localization of spontaneous bursting neuronal activity in the pre-term human brain with simultaneous EEG-fMRI. *elife* 6:e27814
4. Taga G, Homae F, Watanabe H (2007) Effects of source-detector distance of near infrared spectroscopy on the measurement of the cortical hemodynamic response in infants. *NeuroImage* 38(3):452–460
5. Tsuchida TN, Wusthoff CJ, Shellhaas RA, Abend NS, Hahn CD, Sullivan JE et al (2013) American Clinical Neurophysiology Society Standardized EEG Terminology and Categorization for the Description of Continuous EEG Monitoring in Neonates: Report of the American Clinical Neurophysiology Society Critical Care Monitoring Committee. *J Clin Neurophysiol* 30:161–173
6. Whitehead K, Pressler R, Fabrizi L (2016) Characteristics and clinical significance of delta brushes in the EEG of premature infants. *Clin Neurophysiol Pract* 2:12–18
7. Ball G, Aljabar P, Zebari S, Tusor N, Arichi T, Merchant N et al (2014) Rich-club organization of the newborn human brain. *Proc Natl Acad Sci USA* 111(20):7456–7461
8. Nourhashemi M, Mahmoudzadeh M, Goudjil S, Kongolo G, Wallois F (2020) Neurovascular coupling in the developing neonatal brain at rest. *Hum Brain Mapp* 41(2):503–519
9. Watanabe H, Shitara Y, Aoki Y, Inoue T, Tsuchida S, Takahashi N et al (2017) Hemoglobin phase of oxygenation and deoxygenation in early brain development measured using fNIRS. *Proc Natl Acad Sci USA* 114(9):E1737–E1E44
10. Watanabe K, Iwase K (1972) Spindle-like fast rhythms in the EEGs of low-birthweight Infants. *Dev Med Child Neurol* 14(3):373–381

Making Neuroendovascular Therapy for Cerebrovascular Disease Using Distal/Trans-radial Artery Access Safer

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Abstract

Neuroendovascular therapy using distal/trans-radial artery access (d/TRA) has attracted attention as a less invasive procedure. We have selected dTRA or TRA in all cases of carotid artery stenting (CAS). In recent years, TRA has been actively selected for mechanical thrombectomy for acute ischaemic stroke (MT for AIS) and Onyx embolisation for arteriovenous malformations. We compared the patient background, surgical strategy, perioperative complications, and outcome in 41 patients who underwent CAS in the first period (Apr 2017-Feb 2019) and 12 patients in the second period (Apr 2020-Feb 2022) avoiding trans-femoral artery access (TFA) as possible. We

compared the patient background, surgical strategy, perioperative complications, and outcome in 46 patients who underwent MT for AIS via TFA from Apr 2022 to Dec 2022 as the first period and five patients who underwent MT for AIS via TRA from Jan 2023 to Sep 2023 as the second period. Concerning CAS, the second period included significantly more symptomatic cases, with a higher rate of edaravone use to prevent hyperperfusion and a significantly smaller sheath diameter. In the second period, CAS was performed in severe conditions; nevertheless, there was no significant difference between the two groups in terms of either the ratio of cases detected by postoperative diffusion-weighted imaging positive or the ratio of cases with puncture site-related complications (PSCs). Concerning MT for AIS, there were no significant differences between the two groups with the patient backgrounds. The percentage of effective reperfusion, time from puncture to recanalisation, and outcome in the second period were all non-inferior to those in the first period when conventional MT for AIS was performed. Even though we introduced d/TRA in patients with cognitive decline and inability to remain at rest during the perioperative period, we completely avoided PSCs via d/TRA. In

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particular, cerebral hyperperfusion syndrome after CAS and ischaemia-reperfusion injury or re-occlusion after MT were rare but critical perioperative complications, and near-infrared spectroscopy (NIRS) may be used to monitor these problems. We introduced intensive evaluation by NIRS if we had time to spare.

Keywords

Distal/trans-radial artery access (d/TRA) · Neuroendovascular therapy (NET) · Carotid artery stenting (CAS) · Mechanical thrombectomy for acute ischemic stroke (MT for AIS) · Onyx

1 Introduction

Neuroendovascular surgeons tend to be familiar with trans-femoral access (TFA) and avoid distal/trans-radial artery access (d/TRA) because of unfamiliarity with the procedure, concern for the use of large access catheters in the narrower radial artery, and lack of devices specifically designed for d/TRA [1]. In recent years, the technological innovation of flexible guiding sheaths and distal access catheters has made it easier to perform d/TRA and allowed procedures to be completed even when the outer diameter (O.D.) of the system is almost the same as the arterial inner diameter (I.D.) with no trouble. In this article, we report the carotid artery stenting (CAS), mechanical thrombectomy for acute ischaemic stroke (MT for AIS), and Onyx infusion for arteriovenous malformations (Onyx for AVM), where a brain oximeter might be helpful.

2 Methods

2.1 Common Procedures for Neuroendovascular Therapy Via d/TRA

Our institutional review board approved this retrospective study. All patients provided informed consent for us to use their data. In scheduled cases, patients started to receive dual antiplatelet

medication at least two weeks before the procedure. We evaluated perioperative platelet aggregation using the screen filtration pressure method with Hematracer ZEN (DS Medical, Tokyo, Japan). During procedures, bilateral regional cerebral oxygen saturation was usually monitored with INVOS (5100C Cerebral/Somatic Oximeter; Medtronic, Minneapolis, USA), which served as the method of near-infrared spectroscopy (NIRS) [2]. Xylocaine tape was applied to the planned puncture site and brachial artery 1 hour before the procedure. We also used EDF tape (Kinesio Medical Taping, Albuquerque, USA) for pain management of the cubital portion. A homemade vacuum cushion was used to secure the right or left hand in the most appropriate surgical position for puncture. Under echo guidance, the distal radial artery or radial artery was punctured, a short sheath was placed, and 1.5 mg isosorbide nitrate was injected. After each neuroendovascular therapy (NET), reliable haemostasis was also critical and one of the strongest points of d/TRA. BLEED SAFE DTRA (Medikit, Tokyo, Japan) for dual or Prelude (Merit Medical Systems, South Jordan, USA) for right or left was used for puncture site haemostasis, and a TR Band (Terumo Medical, Tokyo, Japan) was applied for rescue (Fig. 1).

2.2 Statistical Analysis

We report the data as means. We used Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. All statistical analyses were conducted using JMP (version 10.1 software: SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically significant.

3 Results

3.1 Carotid Artery Stenting

According to individual risk, we used conventional prevention options such as preoperative injection of edaravone (a free radical scavenger), total anaesthesia, strict control of blood pressure,



Fig. 1 How to prepare and finish the neuroendovascular therapy via distal/trans-distal access

deep sedation with ventilator, and period angioplasty. Systemic heparinisation was established. In procedures, a 5-French (Fr) guiding sheath (AxcelGuide stiff-J; Medikit, Tokyo, Japan) was inserted into d/TRA, and the tip was placed at the common carotid artery. We used the embolic protection (EP) balloon before 2022 spring and now used the EP filter. After surgery, imaging studies, such as magnetic resonance imaging, were performed within three days. We defined periprocedural ischaemic stroke, including intracerebral haemorrhage, as well as acute myocardial infarction, permanent complications, and death from any cause as adverse events. We compared the patient background, surgical strategy, sheath O.D., postoperative diffusion-weighted imaging (DWI) findings, and puncture site-related complications (PSCs), etc. in 41 patients who underwent CAS in the first period (Apr 2017–Feb 2019) and 12 patients in the second period (Apr 2020–Feb 2022) with the increase in the number of patients with arteriosclerosis obliterans and high obesity complications, we started to avoid TFA where possible. The procedure was completed in all patients. As for overall CAS, TFA was selected significantly less frequently in the second period and instead a dual layer stent was used. The second period included significantly more symptomatic cases, with a higher rate of edaravone use to prevent hyperperfusion, with a significantly smaller sheath diameter. Due to the setting being a regional hospital, not a single case of CAS resulted in the induction of total anaesthesia. In the second period, CAS was performed in as severe conditions as in the first period, but

Table 1 The baseline characteristics in patients who underwent carotid artery stenting (CAS) during first period (1st) and in patients who underwent CAS during second period (2nd)

	1st (n = 41)	2nd (n = 12)	P value
Age (years)	77	70	n.s.
Male	85%	75%	n.s.
Symptomatic	44%	100%	< 0.01
Pre Edaravone	44%	100%	< 0.01
General anaesthesia	44%	0%	< 0.05
Sheath O.D.	6.3Fr	5.0Fr	< 0.01
OGT	49%	100%	< 0.01
DWI positive	32%	25%	n.s.
PSCs	9.8%	0%	n.s.

Pre-edaravone preoperative administration of edaravone; *OGT* outlet gate technique; *Symptomatic* symptomatic lesion; *Time* operative time; *DWI positive* lesion detected on postoperative diffusion-weighted imaging; *PSCs* puncture site-related complications; *n.s.* not significant

despite this, there was no significant difference between the two groups in terms of either the ratio of cases detected by postoperative DWI positive or the ratio of cases with PSCs. In particular, no case of PSCs was observed in the second period. Especially in the second period, we introduced the outlet gate technique (OGT) in all the patients, in which the embolic balloon was deflated step by step in accordance with the ratio of cerebral blood oxygenation (CBO), measured by an INVOS Cerebral/Somatic Oximeter [2] (Table 1).

3.2 Mechanical Thrombectomy for Acute Ischaemic Stroke

Because all cases were emergencies, a brain oximeter was performed intraoperatively in only one case (Fig. 2), and perioperative platelet aggregation was not performed preoperatively in all cases. Until 2022, in accordance with the Japanese stroke guidelines, balloon guiding catheters were used in principle, and all cases were performed under local anaesthesia. From 2023, we began to select TRA in cases where circumstances warranted avoiding TFA. We compared the patient background, MT strategy, sheath O.D., aspiration catheter I.D., the ratio of thrombolysis in cerebral infarction (TICI) over 2b as effective reperfusion, time from puncture to recanalisation (P to R), modified Rankin Scale (mRS) score at 14 days after onset, and PSCs, in 46 patients who underwent MT for AIS via TFA from Apr 2022 to Dec 2022 as the first period and five patients who underwent MT for AIS via TRA from Jan 2023 to Sep 2023 as the second period. There were no sig-

nificant differences between the two groups with regard to either age or sex. For the MT strategy we chose a direct aspiration first pass technique (ADAPT) or stent retriever and aspiration catheter combined technique, there was also no significant difference between the two groups regarding MT strategy. Both the sheath O.D. and aspiration catheter I.D. were significantly smaller in the second period to complete the procedure in TRA. There was no significant difference between the two groups, in particular, there was no PSCs in the second period. Most importantly, the percentage of TICI over 2b, time P to R, and mRS in the second period were all non-inferior to those in the first period when conventional MT for AIS were performed (Fig. 2 and Table 2). In order to prevent the time required to prepare the NIRS, it was limited to only those cases in work zones with sufficient manpower, by measuring CBO over time with the INVOS Cerebral/Somatic Oximeter, we were able to rule out the presence of post-MT ischaemia-reperfusion injury and re-occlusion [2, 3].

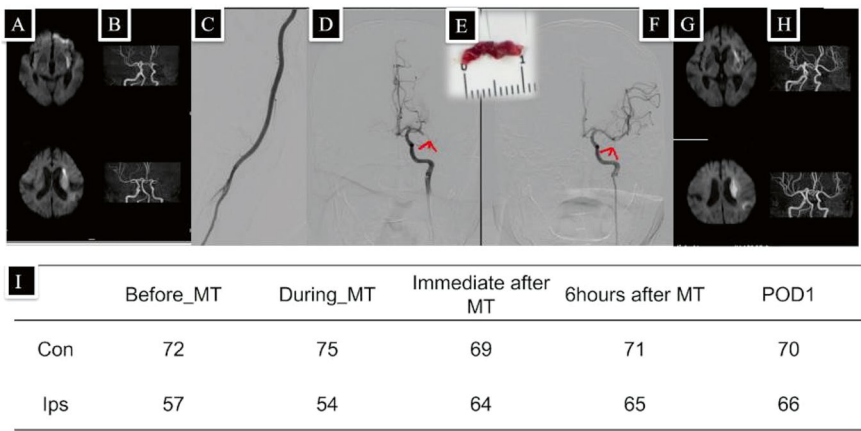


Fig. 2 Magnetic resonance (MR) imaging of onset (a) showing the left cerebral infarction. MR angiography of onset (b) showing the left middle cerebral artery (MCA) occlusion. After checking the cubital portion under digital subtraction angiography (DSA) (c), DSA showed left MCA occlusion in the M1 (d). We chose mechanical thrombectomy (MT) for acute ischaemic stroke strategy, a direct aspiration first pass technique (ADAPT). We achieved complete recanalisation (thrombolysis in cere-

bral infarction grade 3) with 1 cm red thrombus under first pass (e, f). MR imaging and angiography one day after MT (g, h) showed no new cerebral infarction and complete recanalisation of left MCA. (i) The INVOS Cerebral/Somatic Oximeter was linked to the perioperative MT. It showed us the cerebral blood oxygenation trend, allowing us to evaluate whether there was ischaemia-reperfusion injury or re-occlusion dynamically. Con: contralateral side (=right); Ips: Ipsilateral side (=left)

Table 2 The baseline characteristics in patients who underwent mechanical thrombectomy for acute ischaemic stroke (MT for AIS) from Apr 2022 to Dec 2022 as the first period and 5 patients who underwent MT for AIS via trans-radial artery access from Jan 2023 to Sep 2023 as the second period

	1st (n = 46)	2nd (n = 5)	P value
Age (years)	79	84	n.s.
% of combined technique	80%	60%	n.s.
Sheath O.D.	8.2Fr	5.0Fr	< 0.01
Aspiration catheter I.D.	0.064inch	0.059inch	n.s.
% of effective reperfusion	93%	100%	n.s.
Time P to R (minutes)	45	39	n.s.
mRS at 14 days	3.2	3.4	n.s.
PSCs	4.3%	0%	n.s.

O.D. outer diameter; *I.D.* inner diameter; *Time P to R* time from puncture to recanalisation; *mRS* modified Rankin Scale score; *PSCs* puncture site-related complications; *n.s.* not significant

3.3 Onyx Embolisation for AVM

From 2023, we also began to choose TRA in Onyx Liquid Embolic agent (Medtronic, Minneapolis, USA) embolisation for AVM cases where circumstances warranted avoiding TFA. However, because AVM is a rare disease, only one case has been performed in dTRA and one in TRA. Both were Spetzler-Martin grade 3 ruptured AVMs with the main feeder coming from the left middle cerebral artery. Onyx embolization via dTRA was performed with the system of 3 Fr O.D. guiding sheath (AxcelGuide; Medikit, Tokyo, Japan), distal access catheter (GuidePost; Tokai Medical Products, Aichi, Japan), and flow-directed microcatheter (Marathon; Medtronic, Minneapolis, USA). Onyx embolisation via TRA was performed with the system of 5 Fr O.D. guiding sheath (AxcelGuide stiff-J; Medikit, Tokyo, Japan), distal access catheter (Soft torqueable catheter optimised for intracranial access; Microvention, Tustin, USA), and a dual-lumen balloon catheter

(Scepter XC; Microvention, Tustin, USA) DMSO resistant. Both procedures were completed, and no perioperative complications were observed.

4 Discussion

Generally speaking, arterial I.D. of at least 2.0 mm and 2.3 mm were required to accommodate a 5Fr O.D. and 6Fr O.D. guiding sheath, respectively [4]. Therefore, the application of the guiding sheath for RA or dRA was limited in elderly patients. However, recent technological innovations in guiding sheaths have greatly improved their flexibility, pushability, and accessibility making it possible to perform the NET even if the sheath O.D. is slightly larger than the arterial I.D. PSCs after NET occurred in 0.73–9.7% in the world. CAS and taking antiplatelet drugs were associated with significantly higher incidence of PSCs. 40% of the PSCs were treated conservatively, but 20% were needed to treat with endovascular or open surgery [5]. Elderly patients with cognitive impairment might not reasonably cooperate with NET under local anaesthesia and even postoperative bed rest, which might lead PSCs [6]. Additionally, the coexistence rate of carotid artery stenosis and coronary artery disease in arteriosclerosis obliterans (ASO) is about 20–30% and 50%, respectively. In general, ASO and obesity significantly affect PSCs. Dual layer stent (Casper stent; Microvention, Tustin, USA) officially demonstrates its compatibility with the 5 Fr O.D. (I.D. > 0.074 inch) system, which is slimmer than conventional other stent system. All NET via TFA attack the complex aortic arch, which is a risk factor for technical failures, and type III aortic arch with friable atheroma is the riskiest feature. The highest prevalence of atherosclerosis distribution is in the descending aorta especially with increasing age [4]. TRA may minimise catheter contact in the arch. In the past, TFA has been associated with lower mortality and improved functional outcomes in MT for AIS, along with higher recanalisation rates. The increased success rate of reperfusion with larger aspiration catheters and better flow control with the use of balloon guiding catheters

via TFA were considered to lead TFA to advantage lower mortality and more favourable outcomes [7]. However, with recent innovations in guiding sheath and aspiration catheter technology, aspiration catheters as large as 0.062-inch I.D. (RED62, Penumbra, TX, USA) can be used with 5Fr O.D. guiding sheaths, and even 0.055-inch I.D. (Esperance, Wallaby Medical, CA, USA) (Sofia, Microvention, Tustin, USA) aspiration catheters have shown improved aspiration power. Moreover, the combined technique aspiration catheter with the appropriate stent retriever is sufficient to achieve a first pass effect. The use of TRA is slowly but surely increasing. In 2017, Dutch cardiologists reported the world's first cardiac catheterisation using dTRA, which punctures the radial artery just below the anatomical snuffbox. The application of dTRA to endovascular treatment of cranial nerves has been discussed in recent years. dTRA has been reported in shorter haemostasis times and significantly fewer radial artery occlusions in a prospective study published in cardiovascular medicine [8]. The platelet aggregation test using Hematracer ZEN may help regulate antiplatelet drugs and NIRS *may help monitor rare but critical perioperative complications*. This makes neuroendovascular treatment safer and less invasive. In particular, cerebral hyperperfusion syndrome after CAS and ischaemia-reperfusion injury or re-occlusion after MT were rare but occur critical perioperative complications [9, 10], and NIRS may be used to measure CBO over time to evaluate the occurrence of these problems [2]. Because of its minimally invasive, we recommended evaluation by NIRS, if we have time to spare.

5 Conclusions

d/TRA can become the valuable approach route for less invasive neuroendovascular surgery while reducing complications related to puncture sites.

References

1. Bhatia V, Kumar A, Arafath MY (2023) Transradial approach in neurointervention: part-I: patient selection, preparation, and access site considerations. *J Neurosci Rural Pract* 14(1):7–15. <https://doi.org/10.25259/jnpr-2022-5-26>
2. Okuma Y, Hirotsune N, Shinozaki K et al (2021) Near-infrared spectroscopy might help prevent onset of cerebral hyperperfusion syndrome. *Adv Exp Med Biol* 1269:63–67. https://doi.org/10.1007/978-3-030-48238-1_10
3. Okuma Y, Hirotsune N, Ahmed U et al (2022) A case of intracranial vertebral artery stenosis treated with percutaneous transluminal angioplasty and stenting guided by brain oximetry. *Adv Exp Med Biol* 1395:127–131. https://doi.org/10.1007/978-3-031-14190-4_22
4. Barranco P, Rodriguez C, Dios L (2020) Radial Access for Neurointerventions. In: *Vascular and endovascular review*, vol 3. Open Access. <https://doi.org/10.15420/ver.2020.13>
5. Sato M, Matsumaru Y, Sakai N et al (2020) Analysis of puncture site-related complications in Japanese registry of Neuroendovascular therapy (JR-NET)3. *Neurol Med Chir* 60(5):271–275. <https://doi.org/10.2176/nmc.oa.2019-0243>
6. Guan F, Peng J, Hou S et al (2021) Periprocedural complications of cardiac implantable electronic device implantation in very elderly patients with cognitive impairment: a prospective study. *Medicine (Baltimore)* 100(46):e27837. <https://doi.org/10.1097/md.00000000000027837>
7. Siddiqui AH, Waqas M, Neumaier J et al (2021) Radial first or patient first: a case series and meta-analysis of transradial versus transfemoral access for acute ischemic stroke intervention. *Journal of NeuroInterventional Surgery* 13(8):687–692. <https://doi.org/10.1136/neurintsurg-2020-017225>
8. Aminian A, Sgueglia GA, Wiemer M et al (2022) Distal versus conventional radial access for coronary angiography and intervention: design and rationale of DISCO RADIAL study. *Am Heart J* 244:19–30. <https://doi.org/10.1016/j.ahj.2021.10.180>
9. Gauberti M, Lapergue B, Martinez de Lizarrondo S et al (2018) Ischemia-reperfusion injury after endovascular thrombectomy for ischemic stroke. *Stroke* 49(12):3071–3074. <https://doi.org/10.1161/strokeaha.118.022015>
10. Zohdy YM, Saad H, Howard BM et al (2024) Repeat thrombectomy after large vessel re-occlusion: a propensity score matched analysis of technical and clinical outcomes. *J Neurointerv Surg*. <https://doi.org/10.1136/jnis-2023-021197>

Mental Workload and Prefrontal Brain Activity During Silent Reading Task in University Students with Problematic Smartphone Use

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Abstract

Problematic smartphone use (PSU) is common among young people and linked to poor academic performance. However, how PSU affects learning processes remains unclear. This study investigated the influence of auditory cue stimulation during a reading task on the mental workload and prefrontal brain activity of young individuals with PSU. Sixteen university students with PSU and 14 healthy controls (HC) performed a silent reading task, during which fake notification sounds were introduced. Their mental workload was assessed using the NASA Task Load Index (NASA-TLX), and prefrontal brain activity was measured using functional near-infrared spectroscopy (fNIRS). The

results of the NASA-TLX suggest that the PSU group experienced greater frustration than the HC group. The fNIRS results showed that in the right medial prefrontal region of the HC group, O₂Hb levels increased following cue stimulation, whereas no change was observed in the PSU group. Moreover, in the HC group, HHb levels in the left lateral prefrontal region decreased after cue stimulation. The findings of the present study demonstrate that university students with PSU experience frustration and exhibit deactivation in the prefrontal regions associated with the executive control network during silent reading in realistic learning situations.

Keywords

Problematic smartphone use ·
Functional near-infrared spectroscopy ·
Prefrontal cortex · Mental workload ·
Smartphone addiction

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1 Introduction

Problematic smartphone use (PSU) is excessive smartphone use associated with addiction-like behaviours, which leads to dysfunction in daily life [1]. Particularly, young people with PSU may

exhibit a loss of cognitive control, resulting in continued PSU that resembles addictive behaviour [2]. PSU is common among university students and can cause physical, learning, and sleep problems [3].

Cue stimulation plays a central role in various addictive disorders. Current research has primarily explored addictive behaviours linked to substances, gambling, and internet use [4]. Some studies have investigated cue-reactive brain activity in the context of PSU using functional MRI scans [5, 6]. Students frequently engage in instant messaging during academic activities, which has been shown to negatively affect their academic performance [7]. Therefore, understanding how smartphone cue stimulation affects their behaviour and brain activity during their study routines is imperative.

This study aimed to investigate the influence of cue-auditory stimulation, specifically related to instant messaging, on the mental workload and prefrontal brain activity of university students with PSU in their natural environment.

2 Methods

2.1 Participants

Fifty right-handed university students were recruited for the study and completed the Smartphone Addiction Scale—Short Version (SAS-SV) [8]. Sixteen students with scores above the SAS-SV cutoff (31) constituted the PSU group (12 females; age: 20.6 ± 1.0 ; SAS-SV: 37.5 ± 4.1). Fourteen students with SAS-SV scores below the cutoff comprised the healthy control (HC) group (8 females; age: 20.7 ± 1.1 ; SAS-SV: 20.3 ± 3.4). No participants reported serious academic issues or undergoing pharmacological treatments. This study was approved by the Ethics Committee of the Niigata University of Health and Welfare (approval no. 18747–211012) and adheres to the standards outlined in the Declaration of Helsinki.

2.2 Experimental Procedure

First, the participants recited hiragana syllables (/a/ /i/ /u/ /e/ and /o/) for 30 seconds as a pre-task. Subsequently, they engaged in a 60-second silent reading task of a Japanese newspaper column on social topics. Participants were seated at a desk, simulating a typical study setting, with their smartphones placed in front of them. Smartphone use was prohibited during the experiments. A small wireless speaker at the back of the top board emitted fake notification sounds, mimicking those of an instant messaging app, three times for three seconds as a distraction, representing cue-auditory stimulation related to PSU (Fig. 1a).

2.3 Mental Workload Measurement

Mental workload was assessed using the NASA Task Load Index (NASA-TLX) [9] with six components: mental demand, physical demand, temporal demand, performance, effort, and frustration.

2.4 fNIRS Measurement

Frontal brain activity was recorded using an eight-channel wireless fNIRS device (Fig. 1b) (OctaMon; Artinis Medical Systems, Netherlands). Oxygenated haemoglobin (O_2Hb) and deoxygenated haemoglobin (HHb) levels were monitored in the bilateral prefrontal cortex (PFC). The device was set to a wavelength of 752 and 852 nm, source-detector distance of 35 mm, bandpass filter range of 0.01 to 0.5 Hz, and sampling frequency of 10 Hz.

2.5 Analysis

Mean values for the fNIRS data were calculated for 25 seconds, starting 15 seconds after the task began to its completion in the pre-task, which served as the baseline. For the silent reading task, the mean values were calculated for 10 seconds

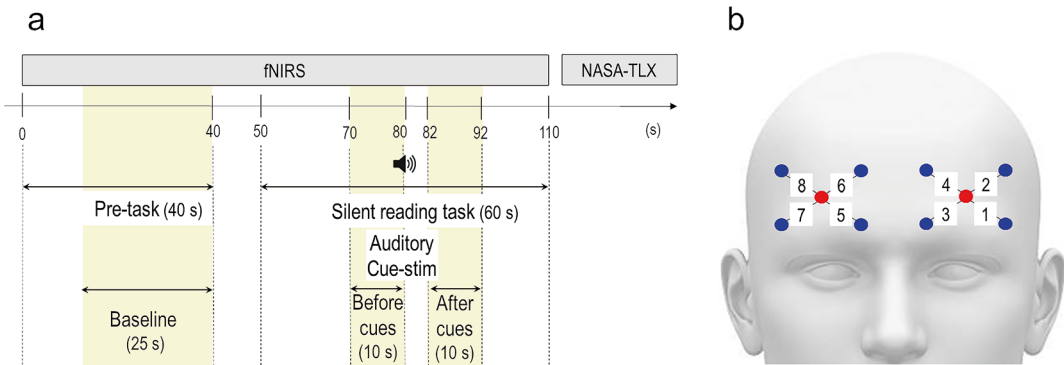


Fig. 1 The time course of the present experiment. As described, the range for analysis is light yellow (a), and the layout of fNIRS optodes is on the right (b). In this

layout, the red dots represent the sources, while the blue dots represent the detectors

before the auditory cues (before cues) and for 10 seconds after the cues (after cues) (Fig. 1a). The fNIRS data were analysed using OxySoft (Artinis Medical Systems, Netherlands). Owing to the non-normal distribution of the dataset, the statistical analysis involved the Wilcoxon signed-rank test for within-group comparisons and the Mann–Whitney U test for between-group comparisons. The significance level was set at $p < 0.05$, with p -values adjusted using the Bonferroni correction for multiple comparisons.

3 Results

The NASA-TLX results revealed significantly higher levels of frustration in the PSU group than in the HC group ($p = 0.046$). No significant differences were observed in the other components ($p > 0.05$). The fNIRS data showed significant differences in specific channels (Fig. 2). Furthermore, the average waveforms of the fNIRS located in the right medial and left lateral PFC obtained during the task are illustrated in Fig. 3.

4 Discussion

The results revealed that university students in the PSU group experienced more frustration than the students in the HC group during the silent

reading task. Notably, PSU has been associated with mental health issues, such as depression and anxiety, in young adults [3]. Moreover, the presence of smartphones can distract attention and impair cognitive performance [10, 11].

The fNIRS results demonstrated differences in the two groups' right medial and left lateral PFCs. In the right medial PFC, O_2Hb levels in channels 5 and 6 increased following cue stimulation in the HC group. A neuroimaging study showed increased activation in the right PFC during multitasking with distracting stimuli, compared to an undistracted condition in healthy young participants [12]. However, no difference was observed in the right PFC in the PSU group, showing individual variability. This indicates that participants with PSU might fail to show a neural response to focus on the reading task, depending on the personal contexts of their smartphone usage.

The slight differences in HHb in channels 1 and 2 located in the left PFC suggest a diminished executive attention network in the PSU group participants when faced with cue stimulation. In the left lateral PFC, the HHb levels in channel 2 decreased in the HC group after cue stimulation, while no change was observed in the PSU group. This finding is consistent with a previous fNIRS study that investigated the distracting effects of auditory stimuli during a reading task [13]. Additionally, following cue stimulation, the HHb levels in Channel 1 were lower in

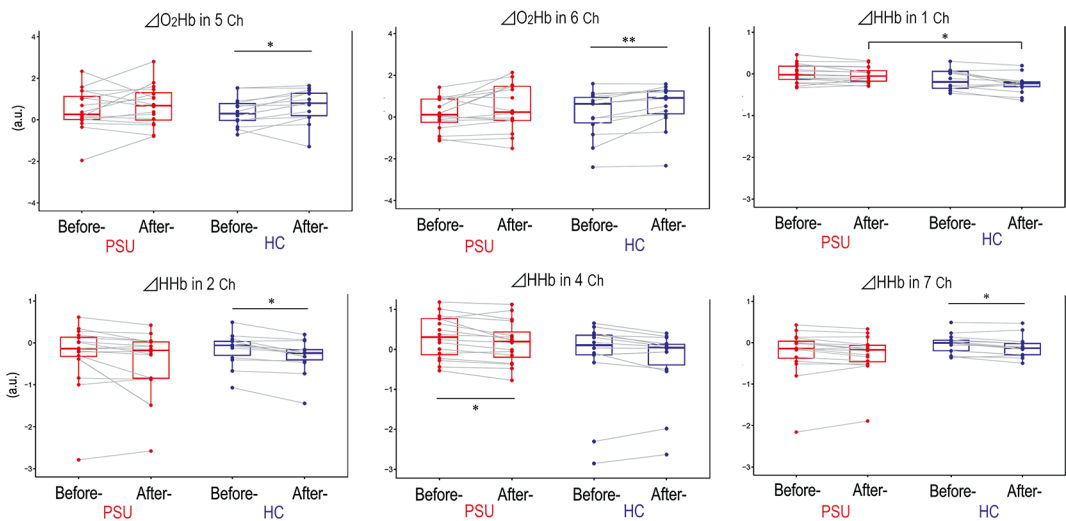


Fig. 2 ΔO_2Hb or ΔHHb values in each channel. Red and blue dots indicate mean values before and after cue stimulation for each participant in the problematic smartphone use (PSU) and healthy control (HC) groups. * $p < 0.05$, ** $p < 0.01$

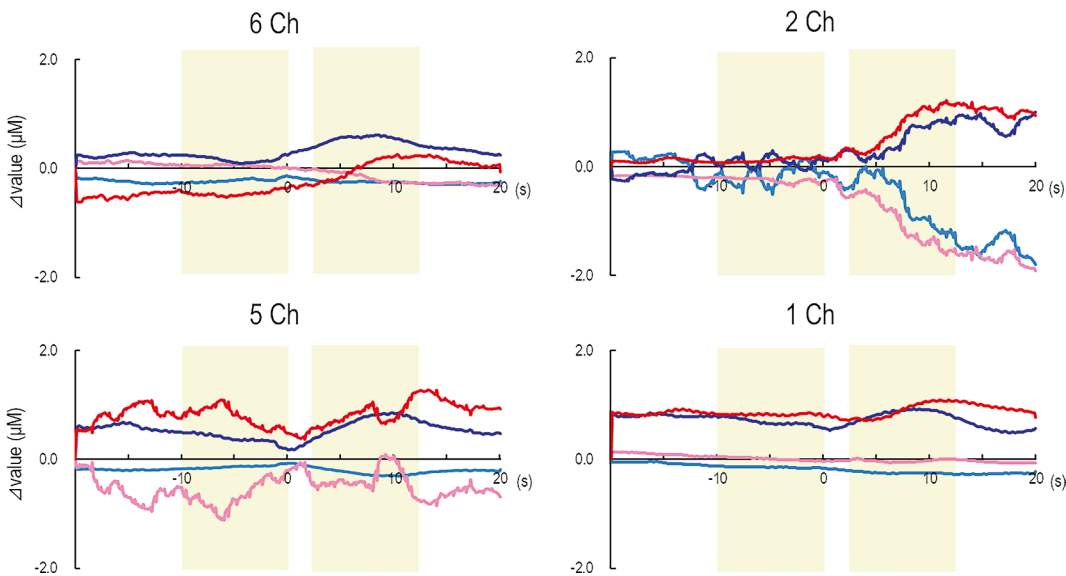


Fig. 3 Average waveforms of ΔO_2Hb or ΔHHb in 5,6 and 1,2 channels. Red and pink lines represent O_2Hb and HHb , respectively, in the PSU group. Blue and light blue lines represent O_2Hb and HHb , respectively, in the HC group. Yellow shadows indicate the analysis windows before and after cues

the HC group than in the PSU group. This indicates the deactivation of the left lateral PFC in young individuals with PSU. Deactivation of the dorsolateral prefrontal cortex (dlPFC) is observed in young individuals with PSU during attention and cognitive tasks. This suggests that deactivation of the left dlPFC may contribute to a decline

in inhibitory executive control [5, 14]. However, we did not observe these signs in O_2Hb levels in the left PFC. This study has certain limitations. While the present results suggest PFC deactivation during the silent reading task in university students with PSU, the specific brain activities related to their attentional function and academic

performance remain unclear. These investigations can help understand and address PSU by university students.

5 Conclusion

This study revealed that university students with PSU experienced frustration and deactivation in the PFC related to their executive control network during silent reading in their natural environments when faced with auditory cue stimulation. These results may inform coaching and environmental settings for learning in young adults with PSU.

References

- Elhai JD, Dvorak RD, Levine JC, Hall BJ (2017) Problematic smartphone use: a conceptual overview and systematic review of relations with anxiety and depression psychopathology. *J Affect Disord* 207:251–259. <https://doi.org/10.1016/j.jad.2016.08.030>
- Huang S, Lai X, Xue Y, Zhang C, Wang Y (2021) A network analysis of problematic smartphone use symptoms in a student sample. *J Behav Addict* 9(4):1032–1043. <https://doi.org/10.1556/2006.2020.00098>
- Candussi CJ, Kabir R, Sivasubramanian M (2023) Problematic smartphone usage, prevalence and patterns among university students: a systematic review. *J Affect Disord Rep* 14:100643. <https://doi.org/10.1016/j.jadr.2023.100643>
- Kim SN, Kim M, Lee TH, Lee JY, Park S, Park M, Kim DJ, Kwon JS, Choi JS (2018) Increased attentional bias toward visual cues in internet gaming disorder and obsessive-compulsive disorder: an event-related potential study. *Front Psych* 13(9):315. <https://doi.org/10.3389/fpsy.2018.00315>
- Nasser NS, Sharifat H, Rashid AA, Hamid SA, Rahim EA, Loh JL, Ching SM, Hoo FK, Ismail SIF, Tyagi R, Mohammad M, Suppiah S (2020) Cue-reactivity among young adults with problematic Instagram use in response to Instagram-themed risky behavior cues: a pilot fMRI study. *Front Psychol* 2(11):556060. <https://doi.org/10.3389/fpsyg.2020.556060>
- Schmitgen MM, Horvath J, Mundinger C, Wolf ND, Sambataro F, Hirjak D, Kubera KM, Koenig J, Wolf RC (2020) Neural correlates of cue reactivity in individuals with smartphone addiction. *Addict Behav* 108:106422. <https://doi.org/10.1016/j.addbeh.2020.106422>
- Junco R, Cotten SR (2012) No a 4 U: the relationship between multitasking and academic performance. *Comput Educ* 59(2):505–514. <https://doi.org/10.1016/j.compedu.2011.12.023>
- Kwon M, Kim DJ, Cho H, Yang S (2013) The Smartphone Addiction Scale: development and validation of a short version for adolescents. *PLoS One* 8(12):e83558. <https://doi.org/10.1371/journal.pone.0083558>
- Hart SG, Staveland LE (1988) Development of NASA-TLX (Task Load Index): Results of empirical and theoretical research. *Adv Psychol* 52:139–183
- Skowronek J, Seifert A, Lindberg S (2023) The mere presence of a smartphone reduces basal attentional performance. *Sci Rep* 13(1):1–9. <https://doi.org/10.1038/s41598-023-36256-4>
- Ward AF, Duke K, Gneezy A, Bos MW (2017) Brain drain: the mere presence of one's own smartphone reduces available cognitive capacity. *J Assoc Consum Res* 2(2):140–154. <https://doi.org/10.1086/691462>
- Moisala M, Salmela V, Hietajärvi L, Salo E, Carlson S, Salonen O, Lonka K, Hakkarainen K, Salmela-Aro K, Alho K (2016) Media multitasking is associated with distractibility and increased prefrontal activity in adolescents and young adults. *NeuroImage* 134:113–121. <https://doi.org/10.1016/j.neuroimage.2016.04.011>
- Midha S, Maior HA, Wilson ML, Sharples S (2021) Measuring mental workload variations in office work tasks using fNIRS. *Int J Human-Comput Stud* 147:102580. <https://doi.org/10.1016/j.ijhcs.2020.102580>
- Xiang MQ, Lin L, Song YT, Hu M, Hou XH (2023) Reduced left dorsolateral prefrontal activation in problematic smartphone users during the Stroop task: an fNIRS study. *Front Psych* 9(13):1097375. <https://doi.org/10.3389/fpsy.2022.1097375>

Monoacylglycerol Lipase Inhibition Using ABX-1431 Attenuates Cerebral Ischaemia Early After Traumatic Brain Injury

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Abstract

An early event in the pathology of traumatic brain injury (TBI) is a reduction in cerebral blood flow (CBF), which exacerbates secondary injury development and inhibits brain recovery. The endogenous cannabinoid system signalling (eCBs) might be critical in TBI recovery due to modulating synaptic activity and exerting neuroprotective and anti-inflammatory effects. In the brain, eCBs predominantly occur at cannabinoid receptor

type 1 via the eCB 2-arachidonoylglycerol (2-AG). The aim of this work was to test the efficacy of potentiating 2-AG signalling by monoacylglycerol lipase (MAGL) inhibition using ABX-1431 immediately following TBI. Laser speckle contrast imaging (LSCI) was used to create a high-resolution map of regional cerebral blood flow (CBF) over the pericontusion cortical surface. In-vivo two-photon laser scanning microscopy (2PLSM) was used to monitor cerebral microcirculation (i.v. fluorescein isothiocyanate dextran, FITC) and mitochondrial respiration and brain tissue oxygen supply (nicotinamide adenine dinucleotide autofluorescence, NADH) during 4 hours after CHI. After baseline imaging, male C57BL/6 J mice (10–12 weeks, >28 g) were subjected to a modified moderate Shohami weight-drop closed-head injury (CHI) followed by i.p. injection of ABX-1431 (5 mg/kg) or vehicle 30 min after the insult (10 mice per group). Differences between groups and between time points were determined using two-way repeated measures (ANOVA) for multiple comparisons and post hoc testing with the statistical significance level set at $p < 0.05$. Optical imaging revealed that CHI caused a decrease in regional CBF, arteriole diameters (vasospasm), and blood flow volume, leading to capillary microthrombosis and a reduction in capillary flow velocity. Compromised cerebral microcirculation

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led to the development of tissue hypoxia. ABX-1431 application, in a ~30-minute delay, mitigated the development of microvascular dysfunction, microthrombosis formation, and tissue hypoxia compared to the saline control group ($p < 0.05$, starting 1 hour after CHI). Therefore, MAGL inhibition by ABX-1431 attenuates cerebral ischaemia early after TBI. The observed 2-AG-mediated cerebrovascular relaxation might involve both a direct inhibition of smooth muscle contractility and a release of vasodilator mediator(s) from the endothelium.

Keywords

Sepsis · Ischaemia · Drag-reducing polymers

1 Introduction

An early event in the pathology of traumatic brain injury (TBI) is a reduction in cerebral blood flow (CBF), which exacerbates secondary injury and inhibits brain recovery by causing dampened oxygen and metabolic delivery to injured brain tissue, which is vital for its proper function and survival. A critical therapeutic strategy is to identify treatments that block the transition from primary to secondary injury in an attempt to prevent the progressive decline in brain function and the manifestation of neurologic disorder before it starts. The endogenous cannabinoid system signalling (eCBs) might be critical in TBI recovery due to its role in modulating synaptic activity and exerting neuroprotective and anti-inflammatory effects [1]. In the brain, eCB signalling predominantly occurs at cannabinoid type 1 receptor via the eCB 2-arachidonoylglycerol (2-AG). Potentiating 2-AG signalling by inhibiting its enzymatic degradation by monoacylglycerol lipase (MAGL) immediately following mTBI injury might attenuate pathological sequelae of TBI [2]. In this work, we tested the efficacy of potentiating 2-AG signalling using a novel potent and selective MAGL inhibitor ABX-1431 immediately following TBI.

2 Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Lovelace Biomedical Research Institute. Twenty laboratory-acclimated male wild-type C57BL/6 J mice (10–12 weeks, >28 g) were randomly assigned to the following groups: (1) TBI+ABX-1431; (2) TBI+vehicle (saline). Laser speckle contrast imaging (LSCI) was used to create a high-resolution map of regional cerebral blood flow on the cortical surface using an RFLSI III System (RWD Life Science, Shenzhen, China) [3]. In-vivo two-photon laser scanning microscopy (2PLSM) using a Prairie View Ultima System (Bruker, Billerica, MA) was utilised to monitor cerebral microcirculation (i.v. fluorescein isothiocyanate dextran, FITC), and mitochondrial respiration and brain tissue oxygen supply (nicotinamide adenine dinucleotide autofluorescence, NADH) at a baseline and up to 4 hours after TBI [4]. During the study, the mice were maintained on isoflurane (1%), nitrous oxide (69%), and oxygen (30%) face-mask anaesthesia. For imaging, the mouse's head was secured in a stereotactic frame, the skull was exposed through a midline scalp incision, and the incised skin was glued around the edges of the frontal and parietal bones using dental cement. To improve spatial resolution, the skull optical clearing treatment was performed in two steps before the experiment. Solution 1 (saturated supernatant solution of 75% (vol/vol) ethanol and urea) was applied to the exposed skull for about 10 min to allow the skull to turn transparent gradually. Then, Solution 1 was removed, and Solution 2 (0.7 M NaOH solution with dodecylbenzene sulfonic acid at a volume-mass ratio of 24:5, pH 7.2) was added to the same area for further clearing within 5 min [5]. After baseline imaging, the mice were subjected to a modified moderate Shohami's [6] weight-drop closed-head injury (CHI) followed by i.p. injection of ABX-1431 (5 mg/kg) or vehicle 30 min after the insult. This model induces a focal blunt injury over an intact skull that triggers a profound neuroinflammatory response within the intrathecal compartment with high consistency and repro-

ducibility, leading to neurological impairment and breakdown of the blood–brain barrier [6]. Where appropriate, statistical analyses were done using GraphPad Prism by Student's *t*-test or Kolmogorov-Smirnov test. Differences between groups were determined using a two-way analysis of variance (ANOVA) for multiple comparisons and post-hoc testing. Data are presented as a mean $\pm$ s.e.m., and the statistical significance level was $p < 0.05$.

3 Results

CHI led to cerebrovascular vasospasm, microcirculation dysfunction, and microthrombosis, associated with reduced mitochondrial respiration/tissue oxygen supply. The maximal effect was observed ~ 1 hour after the insult, with slight recovery by the end of the imaging. In the vehicle group, by the end of the monitoring period, LSCI revealed a significant decrease in regional cerebral blood flow (Fig. 1a, 0.81 ± 0.09 a.u., $p < 0.05$ from baseline). The diameters of the penetrating and precapillary arterioles (vasospasm) decreased from 17.4 ± 0.3 to 15.6 ± 0.2 μm (Fig. 1b), and blood flow volume reduced by 0.79 ± 0.05 a.u. (Fig. 1c, $p < 0.05$ from baseline). This led to a reduction in capillary red blood cell flow velocity from 1.12 ± 0.16 to 0.77 ± 0.15 mm/s in the contusion area of the cerebral cortex (Fig. 2a, $p < 0.05$ from baseline). Due to microthrombosis, the number of functioning capillaries decreased from

1009 ± 51 per mm^3 at a baseline to 811 ± 49 per mm^3 in the contusion area of the brain cortex (Fig. 2b, $p < 0.05$ from baseline). Microcirculation impairment reduced mitochondrial respiration and tissue oxygen supply, inversely reflected by an increase in NADH autofluorescence (Fig. 2c, 1.21 ± 0.06 normalised units, $p < 0.05$ from baseline). ABX-1431 application, in a ~ 30 -minute delay, mitigated the development of vasospasm, microvascular dysfunction, microthrombosis formation, and tissue hypoxia compared to the saline control group ($p < 0.05$, 1 hour after CHI from the vehicle group). By the end of the monitoring period, LSCI revealed a better preserved regional cerebral blood flow (Fig. 1a, 0.94 ± 0.01 a.u., $p < 0.05$ from baseline). The diameters of the penetrating and precapillary arterioles (vasospasm) decreased from 16.9 ± 0.4 μm (Fig. 1b), and blood flow volume reduced by 0.92 ± 0.4 a.u. (Fig. 1c, $p < 0.05$ from the vehicle group). Capillary red blood cell flow velocity was 0.97 ± 0.13 mm/s in the peri-contusion area of the cerebral cortex (Fig. 2a, $p < 0.05$ from the vehicle group). The number of functioning capillaries decreased only to 956 ± 42 per mm^3 in the peri-contusion area of the brain cortex (Fig. 2b, $p < 0.05$ from the vehicle group). Mitochondrial respiration and tissue oxygen supply were also better preserved than in the vehicle group (Fig. 2c, 1.09 ± 0.04 normalised units, $p < 0.05$).

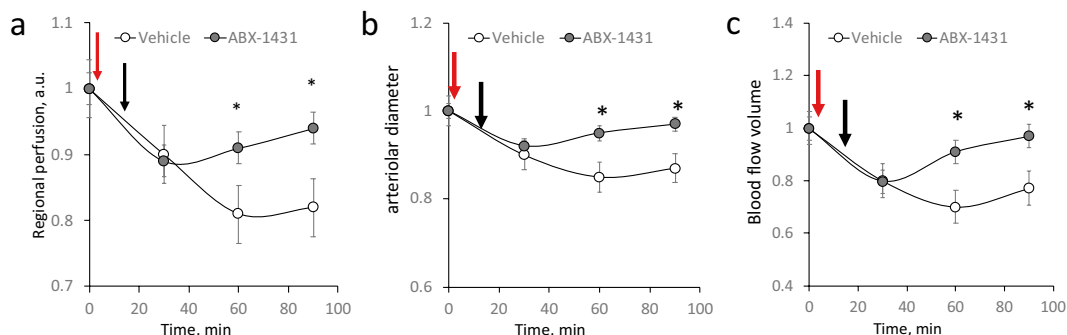


Fig. 1 ABX-1431, delivered 30 min after TBI, (a) mitigated a decrease in regional cerebral blood flow, (b) alleviated arteriolar vasospasm, and (c) restored blood flow

volume through arterioles in the pericontusional area compared to the saline control group, $n = 10$, $* = p < 0.05$. Red arrow—TBI, black arrow—ABX-1431 application

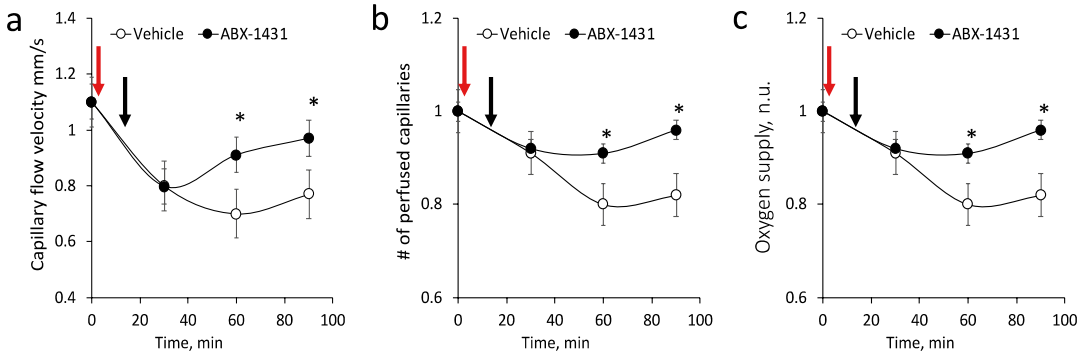


Fig. 2 ABX-1431, delivered 30 min after TBI, (a) restored capillary flow velocity, (b) re-recruited collapsed capillaries, thereby (c) preserving tissue oxygen supply in

pericontusional area compared to saline-treated animals, $n = 10$, $* = p < 0.05$. Red arrow—TBI, black arrow—ABX-1431 application

4 Discussion

ABX-1431, delivered 30 min after TBI, mitigated the development of cerebrovascular vasospasm, microvascular dysfunction, microthrombosis formation, and tissue hypoxia compared to the saline control group. The observed 2-AG-mediated cerebrovascular relaxation might involve both a direct inhibition of smooth muscle contractility and a release of vasodilator mediator(s) from the endothelium [7].

5 Conclusion

MAGL inhibition by ABX-1431 attenuates cerebral ischaemia early after TBI.

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References

1. Xu JY et al (2015) Endocannabinoids in synaptic plasticity and neuroprotection. *Neuroscientist* 21:152–168
2. Katz PS et al (2015) Endocannabinoid degradation inhibition improves neurobehavioral function, blood-brain barrier integrity, and neuroinflammation following mild traumatic brain injury. *J Neurotrauma* 2015(32):297–306
3. Bragina OA et al (2018) Increases in microvascular perfusion and tissue oxygenation via vasodilatation after anodal transcranial direct current stimulation in the healthy and traumatized mouse brain. *Adv Exp Med Biol* 1072:27–31
4. Bragin DE et al (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. *J Cereb Blood Flow Metab* 37(3):762–775
5. Zhang C et al (2018) A large, switchable optical clearing skull window for cerebrovascular imaging. *Theranostics* 8(10):2696–2708
6. Flierl MA, Stahel PF, Beauchamp KM, Morgan SJ, Smith WR, Shohami E (2009) Mouse closed head injury model induced by a weight-drop device. *Nat Protoc* 4(9):1328–1337
7. Benyó Z et al (2016) Endocannabinoids in cerebrovascular regulation. *Am J Physiol Heart Circ Physiol* 310(7):H785–H801

Neural Function Desynchronisation in Left and Right Dorsolateral Prefrontal Cortices During Virtual Reality Earthquake Video Viewing

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Abstract

During natural disasters such as earthquakes, individuals are required to evacuate calmly amidst significant emotional distress, presenting a considerable challenge. Very few studies have measured emotional responses during disasters, and the emotional responses and brain activity during natural disasters are poorly understood. Therefore, this study aimed to investigate emotional responses during an earthquake using immersive virtual reality (VR), focusing on changes in neural

connectivity in the left and right dorsolateral prefrontal cortices (DLPFCs). We measured changes in total haemoglobin concentration ($\Delta\text{total-Hb}$) using 2-channel near infrared spectroscopy (NIRS) while 24 healthy young adults viewed earthquake and neutral videos through a head-mounted display (HMD). Spearman's correlation analysis was applied to the time variation in $\Delta\text{total-Hb}$ in the left and right DLPFCs, independently for seismic or neutral video conditions. The findings revealed a negative correlation between the left and right total haemoglobin concentration changes during the earthquake video ($\rho = -0.53$). Conversely, individuals exposed to the neutral video exhibited a positive correlation ($\rho = 0.75$). The present steady-state analysis suggests that emotional changes induced by virtual earthquake videos disturbed steady-state neural synchronisation between the left and right DLPFCs.

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Keywords

Dorsolateral prefrontal cortices ·
Synchronization · Earthquakes · Virtual
reality · Near-infrared spectroscopy

1 Introduction

Japan has recently experienced numerous earthquakes with magnitude scores exceeding 5 according on the Japan Meteorological Agency seismic scale [1], spanning from Hokkaido to Okinawa. During natural disasters such as earthquakes, individuals are required to evacuate calmly, despite experiencing heightened emotional turmoil. While several studies have investigated the left-right asymmetry or synchrony of brain activity during emotion induction [2, 3], there remains a lack of research focusing on measuring emotional responses during disasters. Consequently, our understanding of emotional responses and brain activity during natural disasters remains incomplete.

This study aimed to investigate emotional responses during earthquakes using immersive virtual reality (VR), focusing on synchronising cerebral haemodynamics in the left and right dorsolateral prefrontal cortices (DLPFCs). To the best of our knowledge, this is the first study to observe synchronisation between the left and right DLPFCs during VR earthquake video viewing.

2 Methods

2.1 Subjects

Twenty-four healthy young adults (12 males, 12 females, 21.4 ± 0.2 years) participated in the study. The eligibility criteria included the absence of visual, auditory, or verbal comprehension impairments, as well as no history of mental disorders that could affect task performance. Participants were also required to have history of experiencing VR sickness prior to this experiment. Moreover, the Impact of Event Scale-Revised (IES-R) was administered to confirm the absence of prior trauma from the earthquake, and participants with a cutoff score of 24 were accepted. The Kyoto University Medical Ethics Committee approved this study. All participants were fully informed before the start of the experiment, and informed consent was obtained through verbal and written consent.

2.2 Materials and Procedure

Two types of video were used: three earthquake simulations and two neutral scenes. The earthquake simulations were created using a 3D reconstruction system to provide an immersive VR experience in three different environments [4, 5]: a living room, a conference room, and a laboratory office. The two neutral video clips were generated by overlaying pink noise to images of natural landscapes (rivers and grassland) [6]. A block design comprising earthquake and neutral video viewing conditions for each 100 s duration was employed.

First, the participants provided personal information, such as age, sex, education level, work history, dominant hand, and visual acuity. Next, they viewed the VR images while wearing a head-mounted display (HMD) with near-infrared spectroscopy (NIRS) and verbally responded to a subjective evaluation after viewing each video. For subjective evaluation, we used the Self-Assessment Manikin on a 9-point scale from 1 to 9 for valence, arousal, and dominance, and a 7-point Likert scale from 1 to 7 for fear, astonishment, anxiety, and panic. Each experiment lasted approximately 90 min and included an explanation.

2.3 NIRS Data Acquisition and Data Analysis

We used 2-channel NIRS (HOT-2000-VR, NeU, Japan) for change of total haemoglobin concentration. The sampling rate for the measurement was 0.1 s. It consists of a light-emitting diode (LED) with a wavelength of 810 nm, one on each side of the headset, and two sensor units (located approximately 1 and 3 cm from the LED). The NIRS probes were bilaterally positioned in the DLPFCs (left Fp1-F7 and right Fp2-F8), with the NIRS probe and the top of the face cushion of the HMD overlapping at a distance of approximately 3 cm above the nasion. Changes in total haemoglobin concentration ($\Delta\text{total-Hb}$) were calculated according

to the method described by Kiguchi and Funane [7]. The NIRS signal processing was performed according to a previous report [8]. Initially, a 3-s moving average (30 points) was employed to mitigate high-frequency noise in the

$\Delta\text{total-Hb}$ (measured values). Next, trends and periodic fluctuations were removed, and the data were processed using a moving median filter in College Analysis (CA) Ver. 8.4 [9], and derived the following equation:

$$t(n) = \left[\Delta Hb_{\text{total}}(n) - \{V_{\text{trend}}(n) + V_{\text{period}}(n)\} \right]_{\text{median filter}=200} \quad (1)$$

where $t(n)$ is the parameter obtained after applying the median filter (200) in Eq. (1), where $V_{\text{trend}}(n)$ and $V_{\text{period}}(n)$ denote the trend and periodic variations in the NIRS signal, respectively.

The left and right $t(n)$ values are called $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$, respectively. The units for $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ are expressed as arbitrary units (a.u.).

All results are presented as means $\pm$ standard error. Initially, the mean of the time-series data for $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ over a duration of 60 s, specifically from 20 to 80 s after the onset of each video-viewing session within 100 s, was calculated. This mean was determined either from the three earthquake videos or from the two neutral videos. Subsequently, after confirming the normality of the data using the Shapiro-Wilk test, Spearman's correlation coefficient (ρ) between $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ for both seismic and neutral videos was calculated. Wilcoxon signed-rank tests were performed for the subjective evaluations (valence, arousal, dominance, fear, astonishment, anxiety, and panic) of both earthquake and neutral videos. The analysis was performed using IBM SPSS Statistics Ver. 29 and CA Ver. 8.4 [9], with a significance level set at 5%.

3 Results

Three of the 24 participants were excluded from the analysis because they had difficulty viewing the videos continuously due to their physical condition or failure of data measurement. Twenty-one participants exhibited right-handedness, had a visual acuity of at least 0.7 in both eyes, and possessed an average education

duration of 14.8 ± 0.2 years. The IES-R was 1.4 ± 0.8 points, and no subjects were excluded due to seismic trauma.

Comparisons of subjective ratings between the earthquake and neutral conditions showed that valence, arousal, dominance, fear, astonishment, anxiety, and panic were significantly higher in the earthquake condition than in the neutral condition ($p < 0.001$).

The time series data of $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ during the earthquake video showed a significant negative correlation ($\rho = -0.53$, $p < 0.001$) (Fig. 1a, c), while those during the neutral video showed a significant positive correlation ($\rho = 0.75$, $p < 0.001$) (Fig. 1b, d).

4 Discussion

Previous studies that evaluated left-right hemispheric asymmetry related to emotion have reported that brain activity in the left hemisphere is generally predominant for positive stimuli. In contrast, the right hemisphere is activated by negative stimuli [2]. In addition, the right amygdala is associated with rapid processing of emotional stimuli, whereas the left amygdala is related to the maintenance of emotion [10]. In this study, temporal changes of total haemoglobin concentration (Fig. 1a) during the earthquake showed that the right hemisphere was dominant in the first half of the time, while the left hemisphere was dominant in the second half. This may be because negative emotions were rapidly evoked in the first half and maintained in the second half. In the neutral images, the right hemisphere was consistently dominant throughout (Fig. 1b), suggesting that the participants' feel-

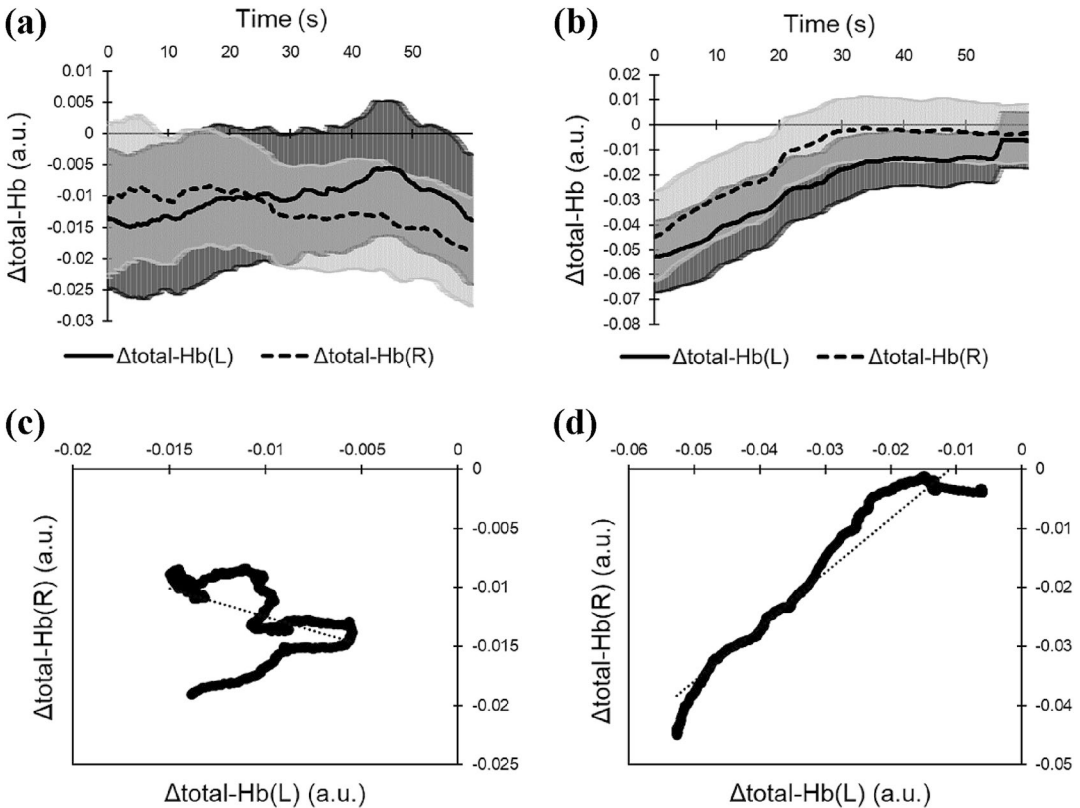


Fig. 1 $\Delta\text{total-Hb}$ time series data and relationships between both DLPFCs. (a, b) show mean and standard error of time-series data for the $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ ($n = 21$). The dark grey zone shows the standard

error of $\Delta\text{total-Hb(L)}$ and the light grey zone shows $\Delta\text{total-Hb(R)}$. (a) Earthquake and (b) neutral videos. (c, d) Scatter plots of the correlation between $\Delta\text{total-Hb(L)}$ and $\Delta\text{total-Hb(R)}$ during each video viewing

ings of boredom and tiredness were influenced by viewing natural scenery. However, the subjective evaluation showed that the earthquake evoked significantly more negative emotions than the neutral video and that the neutral video did not induce a reversal of the dominance of the left and right $\Delta\text{total-Hb}$, suggesting that the asynchrony between the left and right total haemoglobin concentration changes may reflect different emotional reactions. Emotional responses encompass alterations in heart rate and respiration, potentially influencing variations in brain haemoglobin concentration [11, 12]. Further research, incorporating additional biological signals, is warranted to elucidate the desynchronisation of left and right brain activity in response to emotional changes.

Tyagi et al. investigated the functional connectivity of brain regions during a VR disaster. They reported that the correlation between the left and right DLPFCs was higher during environmental experiences with a high cognitive stress load in emergencies [3], a result opposite to that of the present study. Our results may have been influenced by the difference in the task used in the present study, which did not require cognitive performance but only viewed video images. A previous study reported that reduced synchronisation strength in the left and right hemispheres is associated with reduced cognitive performance [13]. Therefore, further investigations, such as measurements under experimental conditions that require cognitive control, such as having participants think about simulated evacuation behav-

your, are needed. The dynamic interactions of brain networks have also been reported to change over time when various emotions are continuously experienced [14]. Therefore, further investigations focusing on a wider range of dynamic neural networks are required.

In conclusion, this study investigated the synchronisation of total haemoglobin concentration changes in the l- and r-DLPFCs during a virtual earthquake. While the l- and r-DLPFC showed significant positive correlations during neutral video viewing, they showed significant negative correlations during earthquakes. This result suggests that negative emotional arousal from the VR earthquake experience may interfere with the synchronisation of the left and right concentration change of total haemoglobin in the DLPFCs. The present results clarify human emotional reactions to disasters, such as earthquakes.

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References

1. Japan Meteorological Agency. Explanation of the seismic intensity. https://www.data.jma.go.jp/multi/quake/quake_advisory.html?lang=en. Accessed 14 June 2023
2. Güntürkün O, Ströckens F, Ocklenburg S (2020) Brain lateralization: a comparative perspective. *Physiol Rev* 100(3):1019–1063. <https://doi.org/10.1152/physrev.00006.2019>
3. Tyagi O, Hopko S, Kang J et al (2021) Modeling brain dynamics during virtual reality-based emergency response learning under stress. *Hum Factors*. <https://doi.org/10.1177/00187208211054894>
4. Asaba W et al (2021) Development of a VR disaster experience environment construction system with automatic object material recognition. STSS, Okayama, 15–17 Nov 2021
5. Asaba W et al (2023) Evaluation of the effect of VR disaster experience in familiar environment. In: Ahram T et al (eds) *Intelligent Human Systems Integration (IHSI 2023): Integrating People and Intelligent Systems*. AHFE 2023 International Conference. AHFE Open Access, vol 69. AHFE International. <https://doi.org/10.54941/ahfe1002889>
6. Unity Asset Store: Mountain Environment - Dynamic Nature. <https://assetstore.unity.com/packages/3d/vegetation/mountain-environment-dynamic-nature-191851>. Accessed 14 June 2023
7. Kiguchi M, Funane T (2014) Algorithm for removing scalp signals from functional near-infrared spectroscopy signals in real time using multidistance optodes. *J Biomed Opt* 19(11):110505. <https://doi.org/10.1117/1.JBO.19.11.110505>
8. Otsuka H, Okahashi S, Seiyama A (2022) Evaluation of emotional changes during earthquake video watching: a Wearable NIRS Study. *HIS* 24(4):239–248. https://doi.org/10.11184/his.24.4_239. (in Japanese)
9. Fukui M College analysis Ver. 8.4, Data Analysis Free Software. <https://www.heisei-u.ac.jp/ba/fukui/analysis.html>. Accessed 14 Jun 2023 (in Japanese)
10. Kohno S, Noriuchi M, Iguchi Y et al (2015) Emotional discrimination during viewing unpleasant pictures: timing in human anterior ventrolateral prefrontal cortex and amygdala. *Front Hum Neurosci* 9:51. <https://doi.org/10.3389/fnhum.2015.00051>
11. Balconi M, Grippa E, Vanutelli ME (2015) What hemodynamic (fNIRS), electrophysiological (EEG) and autonomic integrated measures can tell us about emotional processing. *Brain Cogn* 95:67–76. <https://doi.org/10.1016/j.bandc.2015.02.001>
12. Candia-Rivera D, Sappia MS, Horschig JM et al (2022) Confounding effects of heart rate, breathing rate, and frontal fNIRS on interoception. *Sci Rep* 12(1):20701. <https://doi.org/10.1038/s41598-022-25119-z>
13. Ding K, Wang H, Li C et al (2021) Decreased right prefrontal synchronization strength and asymmetry during joint attention in the left-behind children: a functional near-infrared spectroscopy study. *Front Physiol* 12:759788. <https://doi.org/10.3389/fphys.2021.759788>
14. Raz G, Touroutoglou A, Wilson-Mendenhall C et al (2016) Functional connectivity dynamics during film viewing reveal common networks for different emotional experiences. *Cogn Affect Behav Neurosci* 16:709–723. <https://doi.org/10.3758/s13415-016-0425-4>

Relationship of Cognitive Function with Oxygenated Haemoglobin Concentration Difference Between the Left and Right Prefrontal Cortex During 40-Min Moderate-Intensity Exercise

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Abstract

The high concentration of oxygenated haemoglobin (O₂Hb) in the prefrontal cortex (PFC) during exercise improves cognitive performance. In this study, we aimed to elucidate the relationship of cognitive function with the O₂Hb concentration difference between the left and right PFC (L-PFC and R-PFC, respectively) during sustained exercise. We enrolled 12 healthy adult males who, after a 4-min rest and warm-up, performed a 40-min exercise

regime at a workload corresponding to 50% maximal oxygen consumption. A 2-back task was performed, and the reaction times (RTs) were recorded before exercise, immediately after exercise, and 20 min after exercise. Near-infrared spectroscopy was used to monitor L-PFC and R-PFC. RT was shortened immediately and 20 min after exercise, and O₂Hb concentration difference between L-PFC and R-PFC positively correlated with RT 20 min after exercise. These findings suggest that 40-min exercise induced a phenomenon of neural compensation.

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Keywords

Functional near-infrared spectroscopy
(fNIRS) · Neural compensation · 2-back task
· Frontal cerebral oxygenation asymmetry

1 Introduction

Moderate-intensity exercise can improve cognitive function by enhancing prefrontal cortex (PFC) excitability, known to be accompanied by elevated regional oxygenated haemoglobin (O₂Hb) concentrations [1]. However, the functions of the left and right PFC (L-PFC and

R-PFC, respectively) differ. The L-PFC participates in cognitive, working memory, executive, and other functions [2], while the R-PFC contributes to inhibitory functions [3]. Improvement in cognitive performance is directly related to the O₂Hb concentration difference between the L-PFC and R-PFC [4], which may be related to the asymmetry between the left and right sides of the brain [5]. Therefore, we employed functional near-infrared spectroscopy (fNIRS) to monitor oxygenation changes in the L-PFC and R-PFC [6]. The effects of moderate-intensity exercise at 10, 20, and 45 min on cognitive function have been previously explored in young subjects, and the authors found that 20 min of exercise could elicit substantial cognitive improvement [7]. However, the impact of distinct exercise times on the asymmetry of the left and right sides of the brain, leading to differences in cognitive improvement, remains unexplored. Moreover, it needs to be established whether this cognitive improvement could persist beyond the original Stroop task, performed to observe cognitive and executive functions, to the 2-back task that assesses cognitive and working memory. The purpose of the current study was to verify potential improvements in cognitive function after 40 min of aerobic exercise and to clarify the correlation between O₂Hb concentration and cognitive function.

2 Methods

We enrolled 12 healthy right-handed adult males with a mean age of 22.2 ± 1.5 years. Before participation, subjects provided informed consent after receiving an explanation regarding the purpose and safety of the experiment. The study protocol was approved by the Ethics Committee of the Niigata University of Health and Welfare (approval number 17911-71110).

Before the experiment, cardiovascular exercise training was conducted to determine the maximum oxygen uptake (VO_{2max}) of each participant [8]. At the beginning of the experiment, the participants sat on a cycle ergometer (AEROBIKE 75XLII; Combi, Tokyo, Japan).

The load of the cycle ergometer was set to 20 W during warm-up and 50% VO_{2max} during exercise. After initiating the experiment, 4 min of rest and warm-up was followed by 40 min of exercise and 20 min of rest. A 2-back task was performed, and the reaction times (RT) before the 4-min rest (pre-RT), immediately after exercise (post1-RT), and after the 20-min rest (post2-RT) were recorded. We used fNIRS (OMM-3000; Shimadzu Corp, Kyoto, Japan) to monitor changes in the O₂Hb concentrations in the L-PFC and R-PFC (Fig. 1).

The 2-back task followed the pattern proposed by Deschuytener et al. 2006 [9]. The participants performed addition calculations from 0 to 9, memorising the last digit of the calculation result each time and selecting the last digit of the first two questions on the computer. A total of 12 questions were provided, and the RT was recorded as a parameter indicating cognitive function (Fig. 2).

We performed fNIRS using an 8-channel spectrometer, with the distance between the pairs of emission and detector probes set to 3.0 cm. Based on the international 10–20 system, the probes were located on the PFC. Changes in O₂Hb and deoxygenated haemoglobin (HHb) were monitored throughout the experimental period. We statistically analysed the difference in O₂Hb concentrations during exercise and rest, given that O₂Hb is considered the most reliable indicator of changes in regional cerebral blood flow [10]. The time resolution was set at 40 ms. First, a 0.1 Hz low-pass filter was employed; this filter has been shown to effectively remove noise from heart rate and breathing, although it cannot eliminate vascular oscillation noise below 0.1 Hz [11, 12]. After calculating the average values of the four channels of L-PFC and R-PFC per minute, the average value of the 4-min rest was subtracted from the O₂Hb per minute average of L-PFC and R-PFC to obtain the O₂Hb and HHb per minute change compared with the rest period in the experiment.

The impact of errors was minimised. The interquartile range of the 2-back task results was utilised for the analyses. The average pre-,

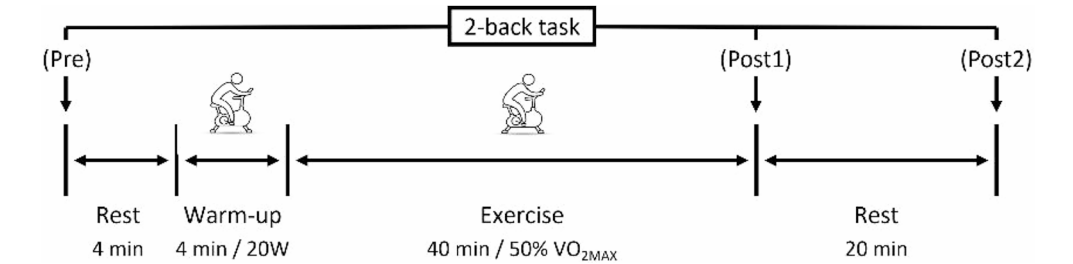


Fig. 1 Experimental protocol

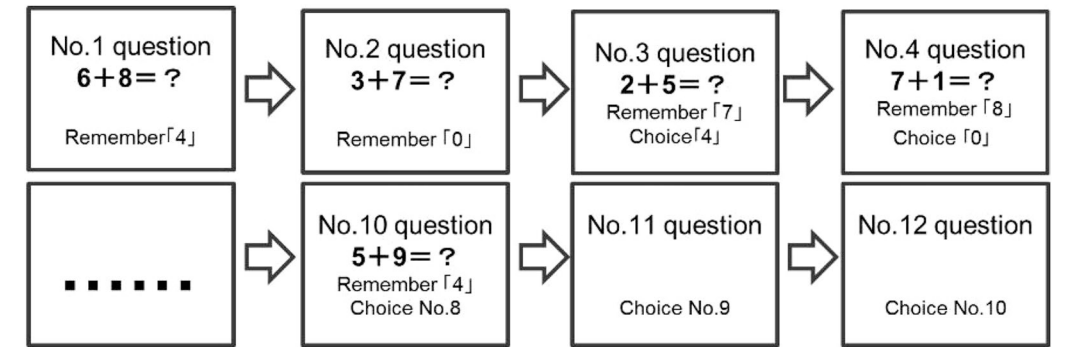


Fig. 2 The 2-back test protocol showing all 12 questions in sequence. At each step, the last number of the previous result must be remembered. Subsequently, the last numbers of the two previous results in the sequence should be reported

post1-, and post2-RTs were calculated. Only the post1- and post2-RTs were used for statistical analyses. Differences between pre-RT and post1- and post2-RTs were compared using the one-way analysis of variance. The pre-, post1-, and post2-RTs were also compared. The average O_2Hb concentrations in L-PFC and R-PFC were calculated during the 40 min of exercise. The O_2Hb concentration difference between the L-PFC and R-PFC was calculated. To establish the effect of the O_2Hb concentration difference on the RT to a question, the Pearson correlation coefficient was used to determine the correlation between the O_2Hb concentration difference and post1- and post2-RTs. The correlation between RTs and O_2Hb concentration differences was used to elucidate the effect of the O_2Hb concentration difference on cognitive improvement. Simultaneously, the correlation between VO_{2max} and RTs was calculated.

Table 1 Information on the subjects (mean $\pm$ SEM)	
Subjects	Numerical value
Age (y)	21.3 $\pm$ 1.1
Height (cm)	168.6 $\pm$ 5.2
Weight (kg)	63.4 $\pm$ 6.3
VO_{2max} (during 40 min exercise) (ml/min)	1438.2 $\pm$ 186.4

3 Results

The basic information of the subjects is shown in Table 1.

The 2-back task showed pre-, post1-, and post2-RTs of 2.02 $\pm$ 0.16, 1.51 $\pm$ 0.20, and 1.67 $\pm$ 0.17 s, respectively. Post1-RT and post2-RT were significantly faster than pre-RTs ($p < 0.01$).

Figure 3 shows the changes in the O_2Hb concentration in the L-PFC and R-PFC during the experiment. Although the O_2Hb concentration

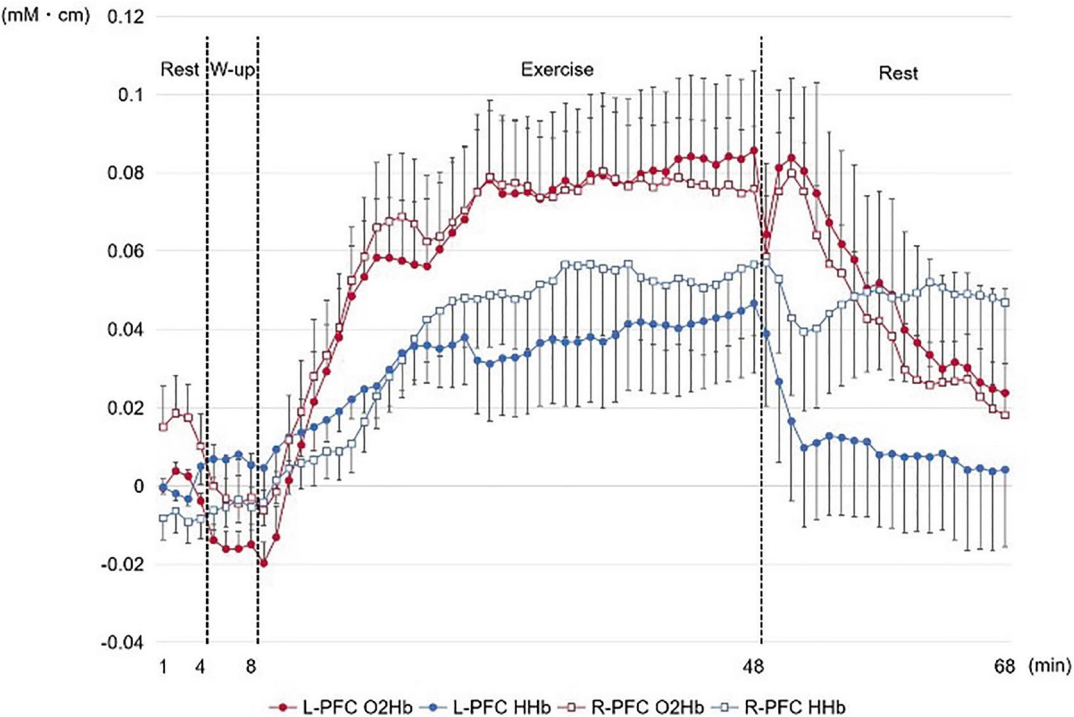


Fig. 3 Oxyhaemoglobin (O_2Hb) changes sampled in the left and right prefrontal cortex each minute. The red line represents O_2Hb , and the blue line represents deoxygenated haemoglobin (HHb). The circle represents left pre-

frontal cortex (L-PFC), and the square represents right prefrontal cortex (R-PFC). Values are presented as mean $\pm$ standard error of the mean (SEM)

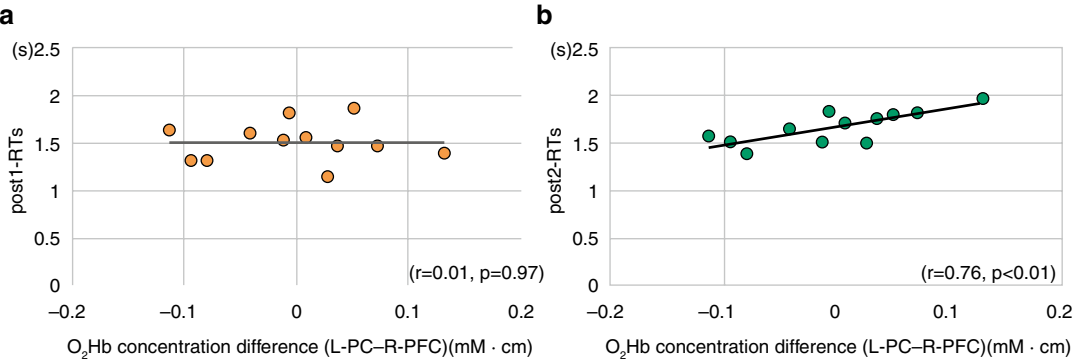


Fig. 4 (a) The correlation between post1-RTs and O_2Hb concentration difference (L-PFC-R-PFC). (b) The correlation between post2-RTs and O_2Hb concentration difference (L-PFC-R-PFC)

change did not correlate with the post1-RT ($r = 0.01$; $p = 0.97$; Fig. 4a), it positively correlated with the post2-RT ($r = 0.76$; $p < 0.01$; Fig. 4b). Furthermore, it was not correlated with the difference between pre- and post1-RTs ($r = 0.23$; $p = 0.46$) or between pre- and post2-RTs ($r = 0.37$; $p = 0.23$; Fig. 5).

The variation of VO_{2max} in the experiment is shown in Fig. 6. There is no correlation between RTs and VO_{2max} (post1-RTs vs VO_{2max} : $r = -0.38$; $p = 0.23$. post2-RTs vs VO_{2max} : $r = 0.06$, $p = 0.85$. post1-RTs vs VO_{2max} : $r = -0.38$, $p = 0.23$. post1-pre vs VO_{2max} : $r = -0.35$, $p = 0.26$. Post2-pre vs VO_{2max} : $r = -0.07$, $p = 0.83$).

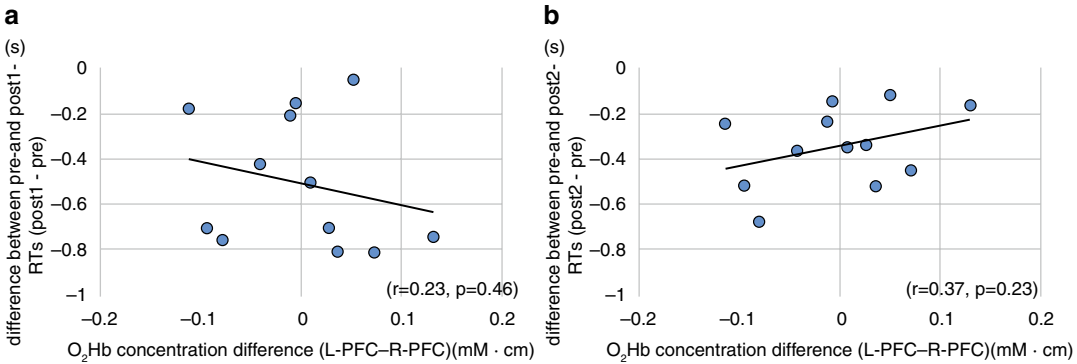


Fig. 5 (a) The correlation between difference between pre- and post1-RTs (post1 – pre) and O₂Hb concentration difference (L-PFC-R-PFC). (b) The correlation between difference between pre- and post2-RTs (post2 – pre) and O₂Hb concentration difference (L-PFC-R-PFC)

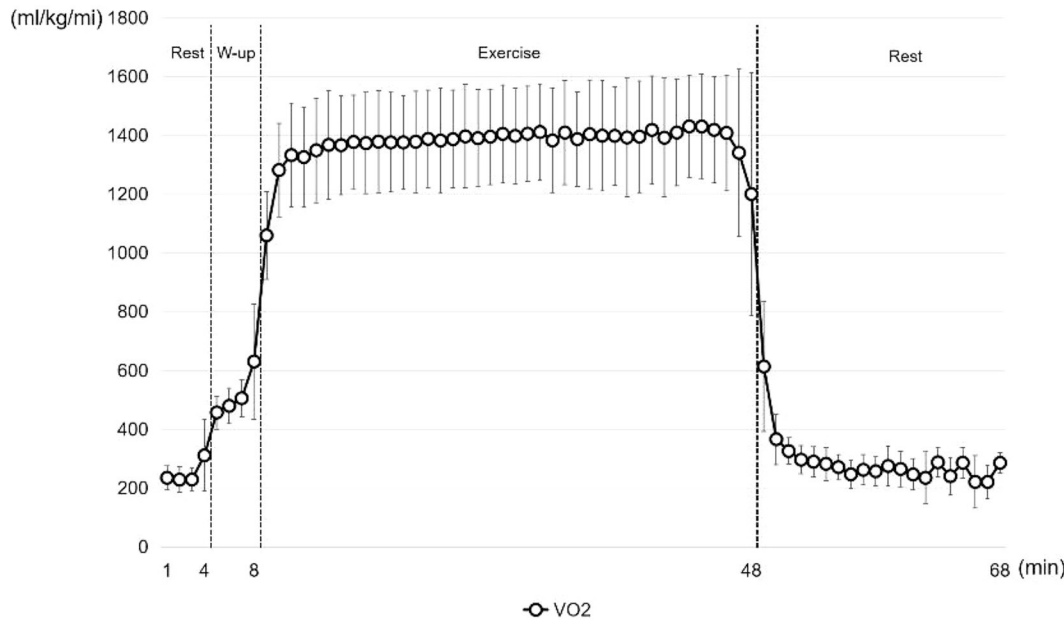


Fig. 6 Oxygen uptake (VO₂) changes. Values are presented as mean ± standard error of the mean (SEM)

4 Discussion

In the present study, moderate-intensity exercise for 40 min significantly improved cognitive function, which was sustained after 20 min of rest. The O₂Hb concentration difference was positively correlated with the post2-RT.

The 2-back task results in the present study were consistent with those of previous studies. Moderate-intensity exercise results in the activa-

tion of the L-PFC activation, and the reaction speed increases [13]. In addition, changes in the O₂Hb concentration support this conclusion. According to the neurovascular coupling theory, L-PFC activation is accompanied by an increase in the O₂Hb concentration in the respective region [14]. In the current study, O₂Hb concentrations were elevated in both the L-PFC and R-PFC after exercise initiation and remained at high levels. This finding reflects continuous PFC activation

on both sides, contrary to the findings reported previously. The cognitive improvement resulting from exercise may be related to L-PFC and not to R-PFC [13], possibly owing to neural compensation. According to the hemispheric asymmetry reduction in older adults (HAROLD) model, a lateralised activation mode may be more conducive to improving the efficiency of information processing [15]. When overactivated, compensation helps maintain brain function. In the present study, the exercise duration was 40 min, suggesting a phenomenon of neural compensation that activates the R-PFC.

The O₂Hb concentration difference was only positively correlated with the post2-RT. In other words, when L-PFC activation is more dominant during exercise, the post2-RT is longer. In contrast, when R-PFC activation is more dominant during exercise, the cognitive improvement may be greater. However, elevated O₂Hb concentrations in the L-PFC reduce the RT [4]. This may be related to the exercise duration, given that 40 min of moderate-intensity exercise can result in more fatigue than 20 min of the same. Central nervous system fatigue may decrease the efficiency of the L-PFC, resulting in dominant R-PFC activation [16, 17]. Exercise-induced central nervous system fatigue, particularly an increase in body temperature, can affect the efficiency of brain neurotransmitters [17]. However, these concepts warrant further research.

In addition, the O₂Hb concentration difference was not associated with the difference in RTs; this could be due to the smaller difference in RT. All participants showed negative RT differences; however, several factors affect the RT after exercise, such as elevated heart rate, increased respiratory rate, and muscle fatigue. Although the substantial effect of these factors on RT cannot be proven, they may affect small RT differences. In future research, limiting factors need to be carefully considered. Frontal cerebral oxygenation asymmetry has been found to exist not only after exercise but also during rest [18]. Individual differences [19], oxygen uptake status [20], and body position [21] of the subjects also need to be noted.

Due to the lack of sockets with a spacing of 7.5 mm in this study, it is not possible to conduct short channel measurements, which may lead to noise generated by blood flow in the head skin [22]. However, previous studies have shown that although there may be effects, post-exercise neurovascular coupling can still be significantly demonstrated [23].

5 Conclusions

The results of the current study revealed that the cognitive benefits of 40 min of sustained exercise could persist for 20 min after the exercise. Furthermore, the change in the O₂Hb concentration was correlated with the 2-back task results 20 min after exercise.

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References

1. Chang H, Kim K, Jung YJ, Kato M (2017) Effects of acute high-Intensity resistance exercise on cognitive function and oxygenation in prefrontal cortex. *J Exerc Nutr Biochem* 21(2):1
2. Redondo-Camós M, Cattaneo G, Perellón-Alfonso R, Alvarez-Schulze V, Morris TP, Solana-Sanchez J, España-Irla G, Delgado-Gallén S, Pachón-García C, Albu S, Zetterberg H, Tormos JM, Pascual-Leone A, Bartres-Faz D (2022) Local prefrontal cortex TMS-induced reactivity Is related to working memory and reasoning in middle-aged adults. *Front Psychol* 13:290
3. Aron AR, Robbins TW, Poldrack RA (2004) Inhibition and the right inferior frontal cortex. *Trends Cogn Sci* 8(4):170–177
4. Qin W, Kojima S, Yamazaki Y, Morishita S, Hotta K, Inoue T, Tsubaki A (2021) Relationship between the difference in oxygenated hemoglobin concentration changes in the left and right prefrontal cortex and cognitive function during moderate-intensity aerobic exercise. *Appl Sci* 11(4):1643
5. Stone MR, Gibson AS, Thompson KG (2016) Asymmetry of cerebral hemodynamic response to incremental cycling exercise. *Int J Sports Physiol Perform* 11(2):273–275

6. Byun K, Hyodo K, Suwabe K, Ochi G, Sakairi Y, Kato M, Dan I, Soya H (2014) Positive effect of acute mild exercise on executive function via arousal-related prefrontal activations: an fNIRS study. *NeuroImage* 98:336–345
7. Chang YK, Chu CH, Wang CC, Wang YC, Song TF, Tsai CL, Etner JL (2015) Dose-response relation between exercise duration and cognition. *Med Sci Sports Exerc* 47(1):159–165
8. Tran D (2018) Cardiopulmonary exercise testing. *Methods Mol Biol* 1735:285–295
9. Deschuyteneer M, Vandierendonck A, Muylaert I (2006) Does solution of mental arithmetic problems such as $2+6$ and 3×8 rely on the process of “memory updating”? *Exp Psychol* 53(3):198–208
10. Kono T, Matsuo K, Tsunashima K, Kasai K, Takizawa R, Rogers MA, Yamasue H, Yano T, Taketani Y, Kato N (2007) Multiple-time replicability of near-infrared spectroscopy recording during prefrontal activation task in healthy men. *Neurosci Res* 57(4):504–512
11. Zhou X, Sobczak G, McKay CM, Litovsky RY (2020) Comparing fNIRS signal qualities between approaches with and without short channels. *PLoS One* 15(12):e0244186
12. Huppert TJ, Diamond SG, Franceschini MA, Boas DA (2009) HomER: a review of time-series analysis methods for near-infrared spectroscopy of the brain. *Appl Opt* 48(10):D280–D298
13. Moriarty T, Bourbeau K, Bellovary B, Zuhl MN (2019) Exercise intensity influences prefrontal cortex oxygenation during cognitive testing. *Behav Sci* 9(8):83
14. Girouard H, Iadecola C (2006) Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *J Appl Physiol* 100(1):328–335
15. Cabeza R (2002) Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol Aging* 17(1):85
16. Davis JM, Bailey SP (1997) Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* 29(1):45–57
17. Taylor JL, Amann M, Duchateau J, Meeusen R, Rice CL (2016) Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Med Sci Sports Exerc* 48(11):2294
18. Scholkmann F, Zohdi H, Wolf U (2020) Right-left asymmetry of prefrontal cerebral oxygenation: does it depend on systemic physiological activity, absolute tissue oxygenation or hemoglobin concentration? *Oxygen Transport Tissue XLI*:105–112
19. Zohdi H, Scholkmann F, Wolf U (2020) Frontal cerebral oxygenation asymmetry: intersubject variability and dependence on systemic physiology, season, and time of day. *Neurophotonics* 7(2):025006
20. Ide K (2023) Acute hypoxia elicits prefrontal oxygenation asymmetry in young adults. *Neurophotonics* 10(4):045002–045002
21. Kamiyama Y, Fujita Y, Fuchigami T, Kamiyama H, Takahashi S, Sakatani K (2014) Asymmetrical changes in cerebral blood oxygenation induced by an active standing test in children with postural tachycardia syndrome. *Adv Exp Med Biol* 812:271–278
22. Wyser DG, Kanzler CM, Salzmann L, Lamercy O, Wolf M, Scholkmann F, Gassert R (2022) Characterizing reproducibility of cerebral hemodynamic responses when applying short-channel regression in functional near-infrared spectroscopy. *Neurophotonics* 9(1):015004–015004
23. Hiura M, Nariai T, Takahashi K, Muta A, Sakata M, Ishibashi K, Maehara T (2018) Dynamic exercise elicits dissociated changes between tissue oxygenation and cerebral blood flow in the prefrontal cortex: a study using NIRS and PET. *Oxygen Transport Tissue XL*:269–274

Part III

Clinical Application

Analysis of Changes over Time in Blood Amino Acids During Online Haemodiafiltration Dialysis in Acute Liver Failure Patients with Hepatic Encephalopathy

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Abstract

Acute liver failure (ALF) is a disease associated with severe symptoms, including rapid deterioration of liver function and impaired consciousness. Recently, online hemodiafiltration (OLHDF), an artificial liver replacement therapy, has attracted attention as a treatment option for comatose ALF. In this study, changes over time in blood aromatic amino acids (AAAs) and ammonia (NH_3), the causative agents of hepatic coma, during OLHDF in patients with ALF were analysed. Nine patients aged 20 years or older with high-grade hepatic encephalopathy admitted to the Kagoshima University Hospital Emergency Centre between October 2020 and September 2021 were included. OLHDF settings were blood flow 100 mL/min, dialysate flow 300 mL/min, and replacement fluid flow 100 mL/min. The analysis items were blood

NH_3 concentration before and after OLHDF, blood amino acid concentration from before to 24 hours after the start of OLHDF, and the presence or absence of conscious awakening after OLHDF. Of the 11 amino acids measured in this study, the AAAs (tyrosine and phenylalanine) had concentrations higher than the reference range before the start of OLHDF, but were within the reference range 24 hours after OLHDF. NH_3 was significantly reduced and the conscious awakening rate was 88.9%. When NH_3 and AAAs, which were considered causative agents of hepatic coma and whose concentrations were higher than the reference range, were removed by OLHDF, the level of consciousness improved significantly. Regarding branched chain amino acids (BCAAs: valine, isoleucine, and leucine), which is considered a protective factor in hepatic coma, the concentration range before starting OLHDF was within the reference range, but the concentration 24 hours after starting OLHDF was below the reference range. The Fisher ratio, the ratio of BCAAs to AAAs, increased from before to after 24 hours starting OLHDF, but was lower than the reference range. Therefore, supplementation should be considered if OLHDF is continued for a longer period of time. Changes over time of 11 amino acids and NH_3 in patients with

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ALF coma were analysed. NH_3 and AAAs, which were abnormally high, decreased to within the reference range 24 hours after the start of OLHDF and the level of consciousness improved. On the other hand, BCAAs, which is considered a protective factor in hepatic coma, the concentration 24 hours after starting OLHDF was below the reference range. Further studies are needed to elucidate the changes in biologically useful substances during OLHDF.

Keywords

Acute liver failure (ALF) · Intensive care unit (ICU) · Online haemodiafiltration (OLHDF) · Amino acids · Ammonia (NH_3)

1 Background

Acute liver failure (ALF) is a disease that causes severe symptoms such as impaired consciousness due to rapid deterioration of liver function. Plasma exchange (PE) therapy and continuous renal replacement therapy (CRRT) have been tried in the past, but the rate of recovery of consciousness has been insufficient. Recently, online haemodiafiltration dialysis (OLHDF) has attracted attention, and its introduction has dramatically improved the wakefulness [1]. On the other hand, because of its high dialysis efficiency, OLHDF has the theoretical problem that it simultaneously removes not only low and medium molecular weight toxic substances but also useful substances. For example, the reduction of amino acids in the blood may cause delayed wound healing and impaired immune function, which may adversely affect prognosis. However, there are few reports on the removal of low molecular weight substances such as amino acids by OLHDF. Therefore, the purpose of this study was to analyse the usefulness of OLHDF and changes in blood amino acids over time in patients with ALF coma.

2 Subjects and Methods

Nine patients with hepatic coma II or higher hepatic encephalopathy due to ALF aged 20 years or older who were admitted to the Kagoshima University Hospital Emergency Centre between October 2020 and September 2021 and gave written consent were included. Ammonia (NH_3) was measured before and after the start and end of OLHDF. Meanwhile, to identify trends in blood levels of many amino acids, blood levels of 11 amino acids [tyrosine (Tyr), phenylalanine (Phe), tryptophan (Trp), histidine (His), isoleucine (Ile), leucine (Leu), valine (Val), ornithine (Orn), methionine (Met), arginine (Arg), and lysine (Lys)] were measured before, immediately after, 0.5 h, 1 h, 4 h, 8 h, 16 h, and 24 h, the start of OLHDF by high-performance liquid chromatography at Clinical Pathology Laboratory Inc. Results are presented as median (Interquartile range: IQR). For statistical analysis, Wilcoxon's signed rank test was used to evaluate between the amino acid concentrations before OLHDF and 24 hours after OLHDF, and $p < 0.05$ was defined as statistically significant difference. Statistical analysis software was JMP Pro 16.1.0 for Windows (SAS Institute Inc., Cary, NC, USA).

The OLHDF procedure is shown in Fig. 1. The dialysis machine was DBG-03 (Nikkiso Co., Ltd., Tokyo), the dialysis water generator was MH500CX (Japan Water System Co., Ltd., Tokyo), the dialysate and replacement fluid were Kindaly AF-2 (Fuso Pharmaceutical Industries, Ltd., Osaka), and the hemodiafiltration machine was FIX-210Seco (NIPRO Corporation, Osaka). The vascular access was a quad lumen catheter (NIPRO Corporation, Osaka, Japan). The settings were blood flow rate of 100 mL/min, dialysate flow rate of 300 mL/min, and replacement fluid flow rate of 100 mL/min.

This study was approved by the Ethics Committee for Epidemiological Research, Graduate School of Medical and Dental Sciences, Kagoshima University (Approval No.: 200138 Epidemic).

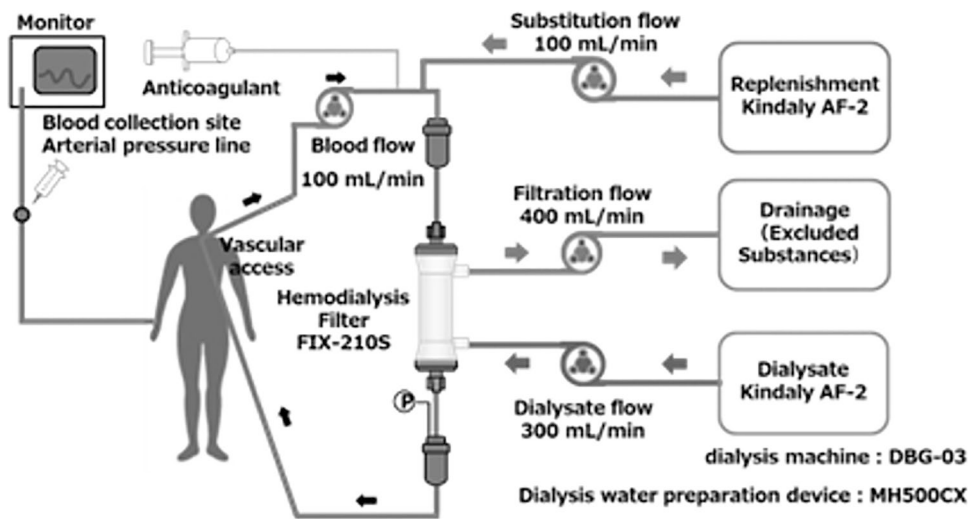


Fig. 1 OLHDF circuit

Table 1 Patient background

No	Age	Sex	BW (kg)	Causes of ALF (Point)	Child-Pugh classification	Blood purification volume (mL/kg/h)	OLHDF total time (h)	Awakening of consciousness ^a
1	61	M	54.0	Virus	13	111.1	16	Yes
2	87	M	60.9	Unknown	13	98.5	85	No
3	69	M	54.4	Drugs	13	110.3	42	Yes
4	61	F	58.0	Alcohol	15	103.4	100	Yes
5	48	M	79.6	Alcohol	15	75.4	138	Yes
6	51	F	48.9	Unknown	12	122.7	60	Yes
7	79	M	50.0	Drugs	12	120	63	Yes
8	44	M	68.1	Alcohol	11	103.3	92	Yes
9	60	M	40.3	Drugs	11	148.9	90	Yes
Median (IQR)	61.0 (49.5–74.0)		54.4 (49.5–64.5)		13 (11.5–14.0)	110.3 (100.9–121.4)	85 (510–96.0)	

^aAwake cases are defined as those whose coma severity has improved after completion of OLHDF according to the coma severity classification by psychiatric symptoms

3 Results

The patient background of the nine subjects is shown in Table 1.

Among the 11 types of amino acids measured this time (Fig. 2; Table 2), the concentration of aromatic amino acids (AAAs: Tyr, Phe) was abnormally high before the start of OLHDF, but it significantly decreased 24 hours after starting OLHDF, improving to within the reference range. Regarding branched chain amino acids (BCAAs: Val, Ile, Leu), the concentration range before

starting OLHDF was within the reference range, but the concentration 24 hours after starting OLHDF was below the reference range. The Fisher ratio, the ratio of BCAAs to AAAs, increased from 1.37 (0.97–1.92) before starting OLHDF to 1.55 (1.28–1.99) after 24 hours, but was lower than the reference range (2.31–4.29) (Table 2). NH₃ value decreased significantly from 112.0 (74.0–159.5) µg/dL to 65.0 (40.5–109.0) µg/dL. Improvement of consciousness after OLHDF was achieved in eight out of the nine patients (88.9%) (Table 1).

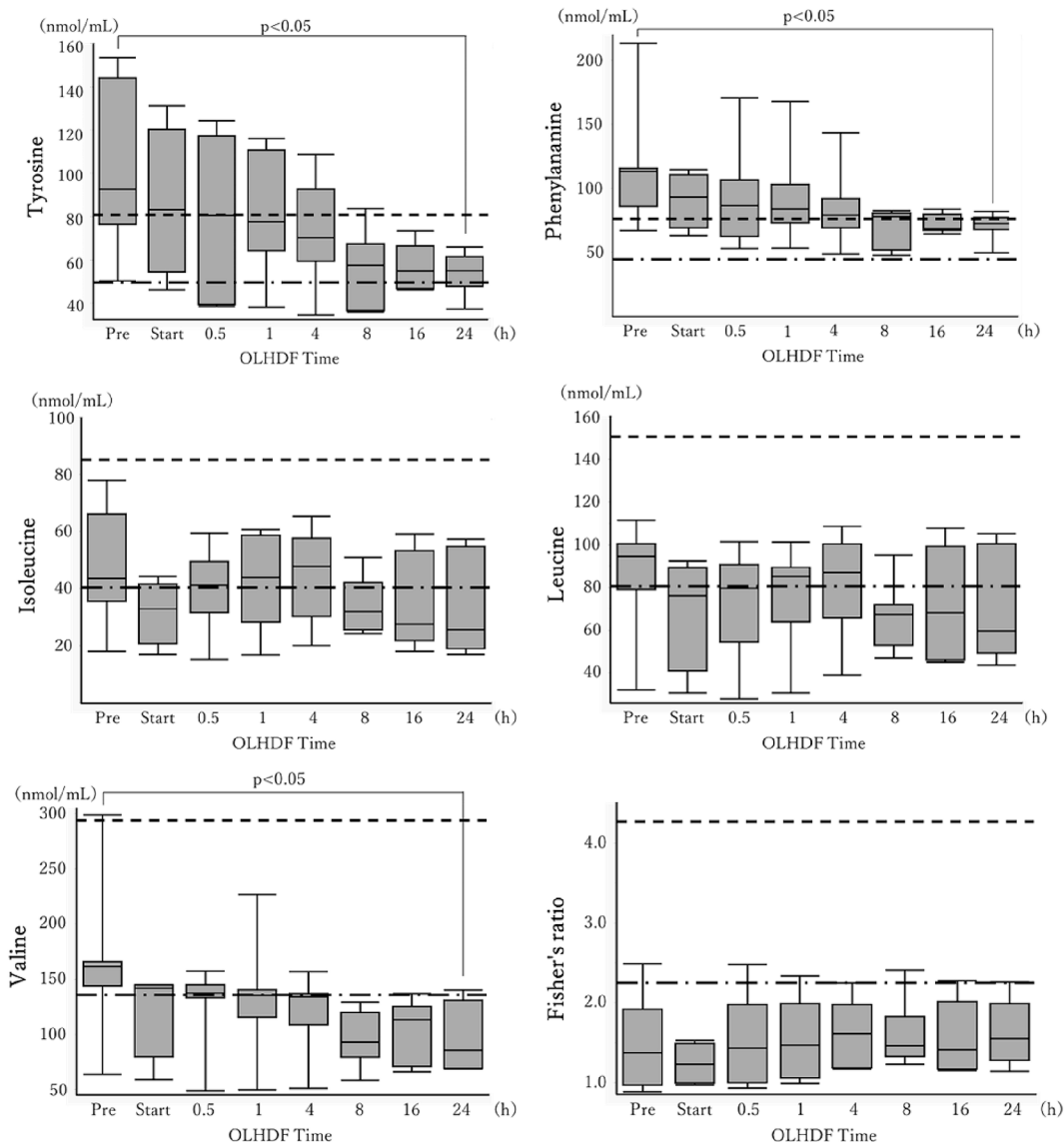


Fig. 2 Change over time in amino acid concentrations from before to 24 hours after OLHDF initiation ($n = 7$)

4 Discussion

In hepatic failure, toxic substances accumulate in the body due to the impaired detoxification function of the liver, which is thought to cause hepatic encephalopathy, and NH_3 [2] and AAAs [3] are considered to be typical substances that induce hepatic encephalopathy. Plasma amino acid concentrations in hepatic failure have attracted attention since Fischer et al. [3] reported arousal in patients with

hepatic encephalopathy treated with BCAA-rich amino acid preparations. In patients with hepatic failure, amino acid metabolism in the liver is reduced and amino acid metabolism in skeletal muscle is relatively increased, resulting in a decrease in BCAAs such as Ile, Leu, and Val, and conversely an excess of AAAs such as Phe and Tyr, which are not metabolised in the skeletal muscle. When AAAs cross the blood-brain barrier (BBB) and move into the brain, BCAAs compete with them, so the lower

Table 2 Amino acid concentrations before and 24 hours after OLHDF initiation

		MW (Da)	Pre OLHDF median (IQR) (nmol/mL) (n = 7)	Post OLHDF median (IRQ) (nmol/mL) (n = 7)	P-value	Reference range (nmol/mL)
AAA	Tyrosine	181.2	92.7 (76.5–144.0)	54.9 (47.6–61.6)	$p < 0.05$	50.2–82.6
	Phenylalanine	165.2	113.5 (86.0–115.9)	72.6 (68.0–77.4)	$p < 0.05$	45.7–76.5
	Tryptophan	204.2	29.3 (9.0–39.4)	20.8 (10.0–24.0)	$p < 0.05$	41.4–65.5
	Histidine	155.2	87.5 (59.8–113.7)	56.4 (50.0–60.7)	$p < 0.05$	67.9–97.1
BCAA	Isoleucine	131.2	43.5 (35.6–66.1)	25.5 (19.0–54.7)	NS	41.3–84.9
	Leucine	131.2	94.5 (79.1–100.5)	59.7 (49.3–100.5)	NS	80.9–154.3
	Valine	117.2	161.5 (143.6–166.0)	85.6 (69.1–130.9)	$p < 0.05$	158.4–287.7
	Ornithine	132.2	48.9 (43.0–79.7)	28.3 (23.6–46.0)	$p < 0.05$	43.2–95.7
	Methionine	149.2	37.1 (32.4–78.6)	29 (22.5–33.6)	NS	19.2–32.7
	Arginine	174.2	57.9 (29.0–59.7)	41.4 (14.7–50.5)	$p < 0.05$	46.0–121.7
	Lysine	146.2	204.6 (127.5–298.3)	150.3 (128.1–189.7)	NS	118.7–257.0
Fisher's ratio			1.37 (0.97–1.92)	1.55 (1.28–1.99)	NS	2.31–4.29

NS Not Significant, MW molecular weight

Fisher's ratio = BCAA/AAA (Tyrosine&\$\$\$; Phenylalanine)

the plasma BCAAs concentration in liver failure, the more easily AAAs move into the brain. Increased AAAs concentrations in the brain lead to increased production of pseudo-neurotransmitters, which is thought to contribute to hepatic encephalopathy. On the other hand, BCAAs also reduce blood ammonia levels [3].

Low molecular weight substances such as NH_3 (MW: 17 Da) and amino acids (MW: 110–210 Da) can be easily removed by haemodialysis. OLHDF (110 mL/kg/h) is a method that enables large-volume filtration by using part of the dialysate as a replacement fluid in the pre-dilution method, and has a dialysis efficiency nine times that of regular CRRT (12–13 mL/kg/h). In other words, OLHDF means excellent solute removal efficiency for low and medium molecular weight substances.

In the present study, the AAAs Tyr and Phe were abnormally high, but both decreased significantly by 24 hours after the start of OLHDF. As blood levels of amino acids were analysed up to 24 hours after the start of OLHDF, the correlation between the decrease in AAAs and improvement in the state of consciousness was unclear. The Fisher ratio, which represents the ratio of AAAs to BCAAs, improved slightly, and the rate of decrease of each amino acid by 24 h after OLHDF initiation correlated with the amino acid concen-

tration before OLHDF initiation for many amino acids (data not shown). On the other hand, when blood concentrations of amino acids were low, it was suggested that amino acids may have been supplied from the amino acid pool in tissues, especially the skeletal muscle. Because loss of muscle mass is a major obstacle to physical function and activities of daily living (ADL) acquisition in critically ill patients, supplementation with useful amino acids should be considered when OLHDF is performed for long periods of time.

This study had several limitations. First, the study had a small population. Larger validation studies are needed. Second, the actual amount of amino acids removed from the blood during OLHDF and changes in other useful substances, especially trace elements and vitamins, were unknown.

5 Conclusion

Changes over time of 11 amino acids and NH_3 in patients with ALF coma were analysed. Blood concentrations of NH_3 and AAAs considered to be hepatic coma-induced substances were high before the start of OLHDF, but improved to within the reference range 24 hours after the start of OLHDF, resulting in improved conscious

awakening. On the other hand, BCAAs, which is considered a protective factor in hepatic coma, the concentration 24 hours after starting OLHDF was below the reference range, suggesting that supplementation should be considered if OLHDF is continued beyond 24 hours. Further studies are needed to elucidate the changes of useful substances in vivo during OLHDF.

References

1. Inoue K, Kourin A, Watanabe T et al (2009) Artificial liver support system using large buffer volumes removes significant glutamine and is an ideal bridge to liver transplantation. *Transplant Proc* 41:259–261
2. Mousseau DD, Butterworth RF (1994) Current theories on the pathogenesis of hepatic encephalopathy. *Proc Soc Exp Biol Med* 206:329–344
3. Fischer JE, Rosen HM, Ebeid AM et al (1976) The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 80:77–91

Cerebral Hypoxia During Intermittent Hypoxic-Hyperoxic Training (IHHT): A Case Study Using Cerebral Oximetry Based on Time-Domain Near-Infrared Spectroscopy

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Abstract

Background: In intermittent hypoxia-hyperoxia training (IHHT), air is inhaled through a mask, with the O₂ content of the air varying at intervals. IHHT is used in sports training (e.g. to improve exercise tolerance), but also in medical-therapeutic applications (e.g. to improve cognitive performance and functional exercise capacity in geriatric patients). **Aim:** We aimed to evaluate the ability of a novel time-domain near-infrared spectroscopy (TD-NIRS) device to measure the effects of IHHT on cerebrovascular oxygenation and haemodynamics. **Subject and methods:** One subject (41 years old, male, athlete, colleague of the authors) performed an IHHT session as part of his regular training. In parallel, systemic physiological activity (arterial oxygenation (SpO₂) and pulse

rate (PR)) as well as cerebrovascular oxygenation (StO₂) and haemodynamics (total haemoglobin concentration, [tHb]) were measured. For the measurement of StO₂ and [tHb], a TD-NIRS device (NIRSBOX, PIONIRS, Italy) was employed. The TD-NIRS device uses two diode lasers (685 nm and 830 nm) as light sources and a solid-state light detector. The optode of the TD-NIRS device was placed over the left prefrontal cortex of the subject. The IHHT session had a total duration of 32 minutes and consisted of four cycles of hypoxia (5 min, O₂: 10%) followed by hyperoxia (3 min, O₂: 34%). **Results:** The IHHT session caused significant changes in SpO₂, HR, StO₂ and [tHb]. The hypoxia/hyperoxia challenges resulted in a decrease in SpO₂ from 97% to ~70% and decrease in StO₂ from ~70 to ~60%. During the hypoxia intervals, HR increased from ~50 to ~60, while [tHb] increased only moderately (from ~64 to ~66 µM). **Conclusions:** The case study presented here demonstrates the feasibility of the novel TD-NIRS device to measure changes in cerebrovascular haemodynamics and oxygenation during an IHHT session. It was observed that an intense IHHT session causes significant cerebral hypoxia (decrease of StO₂ by 10 percentage points). In contrast, cerebral haemodynamics (as indicated by changes in [tHb]) were

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only weakly influenced. Our study shows that IHHT can have a significant effect on the oxygen supply in the head, which should be taken into account in future applications of IHHT to prevent possible pathophysiological reactions that could be triggered by it.

Keywords

Cerebrovascular oxygenation ·
Haemodynamics · Oxygen supply ·
Intermittent hypoxia conditioning (IHC) ·
Total cerebrovascular haemoglobin concentration ([tHb])

1 Introduction

In intermittent hypoxic conditioning (IHC), a subject is intermittently exposed to oxygen (O_2) depleted air, with intervals between of air with normoxic (intermittent hypoxic-normoxic training) or hyperoxic (intermittent hypoxia-hyperoxia training, IHHT) gas mixtures. Introduced in 2005 [1], IHHT is a short-term periodic adaptive training method that is used in sports training (i.e. to improve exercise tolerance), but also in medical therapeutic applications (i.e. to improve cognitive performance and functional exercise capacity in geriatric patients [2–4] or to improve cardiorespiratory fitness in cardiac outpatients [5]).

Temporary systemic hypoxia occurs during IHC, especially IHHT, which is likely to cause cerebral hypoxia. Interestingly, to the best of our knowledge, no studies have been published on this issue in humans during IHHT.

Modern optical neuroimaging methods based on near-infrared spectroscopy (NIRS) enable to measure cerebrovascular perfusion and oxygenation non-invasively in humans [6, 7], and are well suited to investigate this issue. Multi-distance continuous wave (CW-NIRS) and time-domain NIRS (TD-NIRS) neuroimaging offer the advantage that absolute concentrations of cerebrovascular oxygenation and haemoglobin content ([tHb]) can be measured in a depth-dependent manner [8–11].

We aimed to evaluate the ability of a novel time-domain near-infrared spectroscopy (TD-NIRS) device to measure the effects of IHHT on cerebrovascular oxygenation and haemodynamics.

2 Subject and Methods

One subject (41 years old, male, athlete, colleague of the authors) performed an IHHT session as part of his regular training. In parallel, systemic physiological activity (arterial oxygenation (SpO_2) and pulse rate (PR)) as well as cerebrovascular oxygenation (StO_2) and haemodynamics (total haemoglobin concentration, [tHb]) were measured. StO_2 and [tHb] were measured using a novel TD-NIRS device (NIRSBOX, PIONIRS, Italy) [12], which uses two diode lasers (685 nm and 830 nm) as light sources and a solid-state light detector. The technical specifications of the instrument allow precise and reliable measurements (instrument response function: < 200 ps (FWHM), laser repetition frequency: 52 MHz, automated optical attenuators (5 optical density dynamic range), measurement stability: better than $\pm 1\%$ over more than 6 h of operation, direct-time-of-flight (DToF) curves measurement resolution (bin size): 9.77 ps, reproducibility: < 2% (coefficient of variation) on phantoms). The optode (G5-goccia, 2.5 cm source-detector separation [SDS]) of the TD-NIRS device was placed over the subject's left prefrontal cortex of the subject and was attached with a bandage.

The wavelength-dependent changes in the absorption coefficient and the reduced scattering coefficient were measured by the TD-NIRS device using a model of light propagation in turbid homogeneous semi-infinite media [13].

The IHHT session had a total duration of 32 minutes and consisted of four cycles of hypoxia (5 min, fraction of inspired O_2 (FiO_2): 10%) followed by hyperoxia (3 min, FiO_2 : 34%). The IHHT session was performed using the Cellgym device (CellAir Construction GmbH, Schorndorf, Germany).

The present report is a case report in which the non-invasive measurements were conducted on a colleague of our team who regularly performs IHHT; the measurement was done during such a regular training session. Furthermore, the author has approval by the ethics committee of the canton of Zurich that self-measurements of physiological signals (including NIRS) by the research team are allowed and are outside the scope necessary for approval (BASEC-Nr. Req-2018-00951).

Signal processing and data analysis was performed with MATLAB (R2017a, MathWorks, Inc., Massachusetts, USA).

3 Results

The IHHT session was associated with significant changes in SpO_2 , HR, StO_2 , and $[tHb]$ (Fig. 1). The hypoxia/hyperoxia challenges caused a decrease in SpO_2 from 97% to ~70%

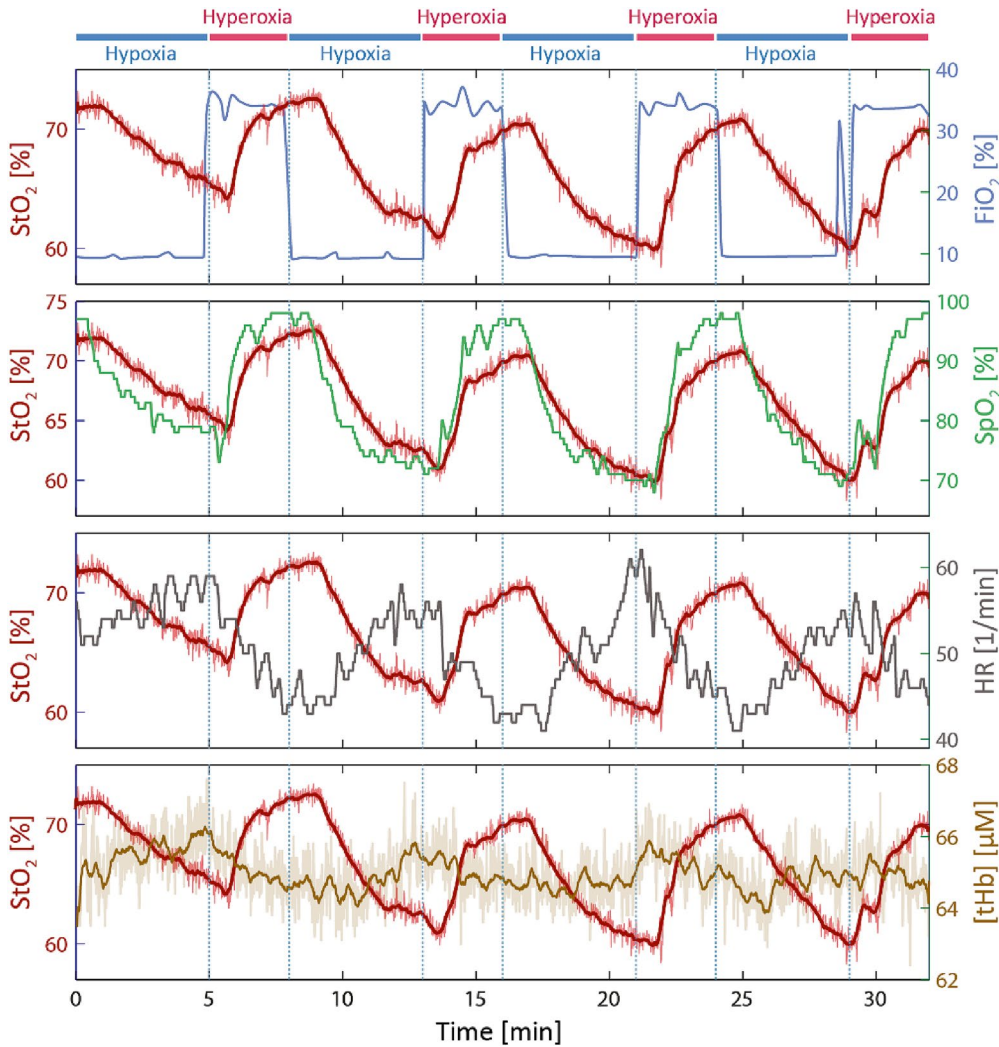


Fig. 1 Changes in inspired O₂ concentration (FiO₂), arterial oxygenation (SpO₂), heart rate (HR), total cerebrovascular haemoglobin concentration ([tHb]) and

cerebrovascular oxygenation (StO₂). The StO₂ and [tHb] and measurement are show as raw data and with a 10 second moving average applied (thicker line)

and a decrease in StO_2 from ~70% to ~60%. During the hypoxia intervals, HR increased from ~50 to ~60, while [tHb] increased only moderately (from ~64 to ~66 μM).

4 Discussion, Conclusion and Outlook

The case study presented here shows that an intense IHHT session causes significant cerebral hypoxia (decrease by 10 percentage points of StO_2). In contrast, cerebral haemodynamics (as indicated by changes in [tHb]) were only weakly affected. Our investigation shows that the novel TD-NIRS device was able to perform measurements of StO_2 and [tHb] of good quality, and that IHHT can have a significant effect on the oxygen supply in the head, which should be taken into account in future applications of IHHT in order to prevent possible pathophysiological reactions that could be triggered by it.

For future studies, it would be interesting to perform NIRS-based measurements during IHHT with a larger number of subjects and also with subjects that differ in certain characteristics (e.g. age, sex and health status). It would also be also valuable to measure the change in respiratory rate and the concentration of arterial CO_2 , as both parameters also influence cerebrovascular oxygenation [14–16].

In addition, it would be necessary to compare different NIRS-based measurement techniques (e.g. CW-NIRS and TD-NIRS) and to investigate how different light propagation models applied to TD-NIRS data affect the measured values. Furthermore, it would be interesting to calculate absolute values of cerebral blood flow during an IHHT session based on the recently developed method of Bale et al. [17] using time-series from NIRS-based measurements and SpO_2 .

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References

1. Arkhipenko YV, Sazontova TG, Zhukova AG (2005) Adaptation to periodic hypoxia and hyperoxia improves resistance of membrane structures in heart, liver, and brain. *Bull Exp Biol Med* 140(3):278–281
2. Serebrovska ZO et al (2019) Intermittent hypoxia-hyperoxia training improves cognitive function and decreases circulating biomarkers of Alzheimer's disease in patients with mild cognitive impairment: a pilot study. *Int J Mol Sci* 20(21):5405
3. Serebrovska ZO et al (2022) Response of circulating inflammatory markers to intermittent hypoxia-hyperoxia training in healthy elderly people and patients with mild cognitive impairment. *Life (Basel)* 12(3)
4. Behrendt T et al (2022) Effects of intermittent hypoxia-hyperoxia exposure prior to aerobic cycling exercise on physical and cognitive performance in geriatric patients—a randomized controlled trial. *Front Physiol* 13:899096
5. Dudnik E et al (2018) Intermittent hypoxia-hyperoxia conditioning improves cardiorespiratory fitness in older comorbid cardiac outpatients without hematological changes: a randomized controlled trial. *High Alt Med Biol* 19(4):339–343
6. Ayaz H et al (2022) Optical imaging and spectroscopy for the study of the human brain: status report. *Neurophotonics* 9(Suppl 2):S24001
7. Wolf M, Ferrari M, Quaresima V (2007) Progress of near-infrared spectroscopy and topography for brain and muscle clinical applications. *J Biomed Opt* 12(6):062104
8. Torricelli A et al (2014) Time domain functional NIRS imaging for human brain mapping. *NeuroImage* 85(Pt 1):28–50
9. Pifferi A et al (2016) New frontiers in time-domain diffuse optics, a review. *J Biomed Opt* 21(9):091310
10. Kleiser S et al (2018) In vivo precision assessment of a near-infrared spectroscopy-based tissue oximeter (OxyPrem v1.3) in neonates considering systemic hemodynamic fluctuations. *J Biomed Opt* 23(6):1–10
11. Hoshi Y (1955) Towards the next generation of near-infrared spectroscopy. *Philos Trans A Math Phys Eng Sci* 2011(369):4425–4439
12. Zhao F et al (2022) Reproducibility of identical solid phantoms. *J Biomed Opt*:27(07)
13. Zaccanti G et al (2009) Light propagation through biological tissue and other diffusive media: theory, solutions, and software. SPIE Press
14. Oyanagi K, Tsubaki A (2021) Effects of increased respiratory rate on cortical oxygenated hemoglobin during low-intensity exercise. *Respir Physiol Neurobiol* 291:103691
15. Scholkmann F et al (2013) End-tidal CO_2 : an important parameter for a correct interpretation in functional brain studies using speech tasks. *NeuroImage* 66:71–79

-
16. Caldwell M et al (2016) Modelling confounding effects from extracerebral contamination and systemic factors on functional near-infrared spectroscopy. *NeuroImage* 143:91–105
 17. Bale G et al (2020) Near-infrared spectroscopy measured cerebral blood flow from spontaneous oxygenation changes in neonatal brain injury. *Adv Exp Med Biol* 1232:3–9

Comparison of Eye-Tracking Parameters and Brain Oxygen Saturation in Patients with COVID-19 Moderate Pneumonia

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Abstract

The aim was to study the relationship between eye tracking (ET) parameters and cerebral tissue oxygen saturation (SctO₂) values in the acute period of moderate COVID-19 pneumonia. *Materials and Methods.* A single-centre, non-randomised study included 92 patients in the acute period of SARS-CoV-2 moderate pneumonia (Delta variant). The mean time from admission was 1.5 ± 0.9 days. M:49, W:43. The mean age was 34.7 ± 3.9 years. The

eye vergence reactivity index (VRx) was determined using a mobile ET. The cerebral oximetry was performed using Fore-Sight 2030 and included the detection of the SctO₂ level in the frontal lobe (FLP) region. Statistical analysis was carried out using the methods of parametric statistics. *Results.* The calculated vertical VRx was 0.781 ± 0.118 . The calculated horizontal VRx was 0.821 ± 0.107 . SctO₂ in the FLP varied within the range from 61% to 73%. The average SctO₂ values were $65.4 \pm 5.2\%$ over the left FLP and $66.2 \pm 6.3\%$ over the right FLP ($p = 0.872$). The regression analysis showed that HVRx and VVRx were correlated with SctO₂ levels in both FLPs ($p = 0.035$ and $p = 0.034$, respectively, and $p = 0.040$ and $p = 0.049$, respectively). *Conclusions.* Cerebral oxygen saturation in moderate pneumonia caused by the SARS-CoV-2 coronavirus has a significant destabilising effect on the oculomotor synergy (VVRx and HVRx) ($p < 0.05$).

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Keywords

COVID-19 pneumonia · Eye tracking ·
Cerebral oxygenation

1 Introduction

Although the pandemic, which was caused by the new Coronaviridae subspecies virus, was mainly accompanied by the development of Severe Acute Respiratory Syndrome 2 type (SARS-CoV-2) due to lung damage, it was later revealed that in a significant number of patients, other organs are also involved in the process, including the nervous system [1]. Multiple studies have shown microstructural damage to neuronal structures [2, 3], as well as oculomotor and pupillary disorders [4, 5].

Of particular interest is the analysis of oculomotor synergy disorders associated with COVID-induced lung damage, which can lead to tissue hypoxia and decreased stereopsis characteristics, which underlines the importance of visual perception studies of the COVID-19 infection.

The hypothesis was that the impairments of conjugate gaze correlate with cerebral oxygen tissue saturation in moderate COVID-19 patients.

The aim was to study the relationship between eye tracking (ET) parameters and cerebral tissue oxygen saturation (SctO₂) values in the acute period of moderate COVID-19 pneumonia (Delta variant).

2 Methods

This cohort non-randomised single-centre retrospective study was approved by the Ethics Committee and included ninety-two patients (49 men, 43 women) in the acute period of moderate severity pneumonia due to COVID-19 (ICD-10 code – J 12.82) 2–3 days after admission to the of Nizhny Novgorod Regional COVID-19 Hospital from May 2021 to November 2021 (positive results of PCR testing at admission). The mean time from admission was 1.5 ± 0.9 days. According to positive PCR testing, all patients had the Delta variant (B.1.617.2) of SARS-CoV-2 and were free of any neurodegenerative diseases, mild cognitive impairment, bipolar disorder, depression, cerebrovascular disease, traumatic brain injury history, alcohol or drug addiction disorder, and any brain lesion according to

Computed Tomography/Magnetic Resonance Imaging. The mean age was 34.7 ± 3.9 years (range 18–60 years), mean weight 72.2 ± 12.21 kg, and height 167.2 ± 9.3 cm. Ninety patients were right-handed, and two patients were left-handed. All patients scored 15 points on the Glasgow Coma Scale before ET assessment and had no symptoms of a “cytokine storm”. The diagnosis of moderate severity of COVID-19 pneumonia was based on grade CT 2 lung involvement. The study conformed to Helsinki Declaration standards, and informed consent was obtained from all patients.

According to the current guidelines for treating COVID-19 infections, patients with moderate pneumonia severity received intravenous ceftriaxone 1.0×2 times per day. Since the patients did not have any signs of a “cytokine storm”, the use of steroids and/or monoclonal antibodies to IL-1 or -6 was not recommended. The treatment was carried out with normobaric hyperoxia, Favipiravir tablets (1.8×2 times per day) and did not affect the consciousness level. There were no differences in the treatment of patients included in the study.

Eye Tracking Configuration The eye movements (the smooth pursuits and saccades) were studied in a series of visual cues at a sampling rate of 20 Hz using the iPadOS 16 tablet eye-tracking software (EyeTracker, BVG Software Group LLC). Eye tracking was carried out in the presence of a researcher (AT and KT), who monitored the correct position of the subject’s face on the tracker screen and controlled the proper execution of the study (<https://bvgsoftwaregroup.com/eyetracker-facial-recognition-application/>).

The first step of the protocol included a calibration and an eye adaptation (10 s black screen). The circle marker (diameter 0.3 cm) changed colour from white-black to red every 2 s and randomly moved across the table screen. It maintained the patient’s attention on the circle centre [6]. Post-processing analysis included identifying and removing the incorrect frames with unrecognised pupils, blinking frames, etc.) which were excluded manually using the network

refinement function. A total of 300 of the best consecutive ET frames were analysed. The total procedure duration was 30–45 s.

The vertical and horizontal Angular Velocity (AV), Left Vertical Speed (LVS), Right Vertical Speed (RVS), Left Horizontal Speed (LHS) and Right Horizontal Speed (RHS) were calculated for the left and right eye separately.

Time-averaged values of LVS vs. RVS, and LHS vs. RHS were calculated using time integration (average values of 400–450 consecutive samples) for 20–35-second intervals. Linear Pearson moving correlation coefficients were computed between 30 past consecutive 1.5-second averages of corresponding right and left eyeballs' angular velocities, designated as the vergence reactivity index (VRx). Computations were repeated with a moving window every 1.5 s. VRx provides a normalised convenient index that is suitable for comparison between eyeball movements conjugation.

Cerebral near-infrared spectroscopy (CNIRS) is appealing as it allows cerebral oxygen saturation to be measured noninvasively and continuously. CNIRS was performed using a space-resolved cerebral tissue oximeter Fore-Sight 2030 (CAS Medical Systems Inc., USA). CNIRS included the detection of SctO₂ in both frontal lobe poles (FLP). The cerebral oximeter probes were applied to the hairless scalp in both FLPs according to current guidelines. Data were obtained using a multimodal monitor providing one-by-one sec average values.

Statistical Analysis The software package Statistica 12 (TIBCO Inc., USA) was used for data analysis. Data were normally distributed and expressed as mean $\pm$ standard deviation. The statistical analysis was performed using parametric statistics and linear regression analysis methods. The level of significance was $p < 0.05$.

3 Results

The analysis of the acquired data revealed the following values of ET parameters: LVS— 0.228 ± 0.113 rad/sec; LHS— 0.231 ± 0.122 rad/

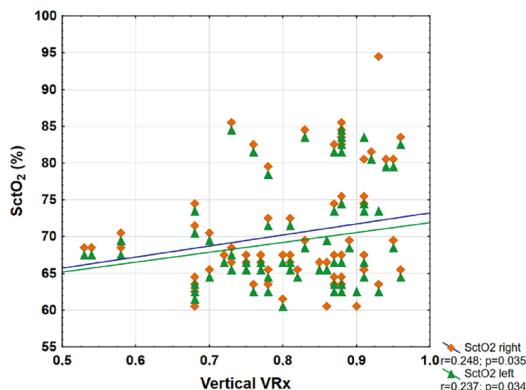


Fig. 1 Vertical version reactivity index (VVRx) plotted against SctO₂ values on the left side (sand rhombi) and right side (green triangles) in the patients with moderate COVID-19 pneumonia ($p = 0.035$; $r = 0.248$; $p = 0.0346$ $r = 0.237$, respectively)

sec; RVS— 0.237 ± 0.163 rad/sec; and RHS— 0.229 ± 0.117 rad/sec. The calculated vertical version index (VVRx) was 0.781 ± 0.118 . The calculated horizontal version index (HVRx) was 0.821 ± 0.107 .

Brain oxygen saturation varied within the range from 61% to 72%. The average SctO₂ values were $65.4 \pm 5.2\%$ over the left FLP and $66.2 \pm 6.3\%$ over the right FLP ($p = 0.872$).

The regression analysis of vertical and horizontal eye version reactivity indices and SctO₂ in FLP showed that brain oxygen saturation values had a significant effect on the indices of ocular vergence (HVRx and VVRx) ($p = 0.035$, $r = 0.248$; $p = 0.034$, $r = 0.237$ and $p = 0.040$, $r = 0.224$; $p = 0.049$, $r = 0.215$, respectively) (Figs. 1 and 2). Thus, COVID-19 infection, leading to moderate pneumonia, significantly affected oculomotor synergy ($p < 0.05$).

4 Discussion

We studied the eye-tracking changes in 92 patients with moderate COVID-19 pneumonia caused by the Delta variant. Despite the moderate lung damage severity, none of our patients have been diagnosed with cerebral hypoxia (SctO₂ > 60%). Perhaps this was due to the implementation of normobaric hyperoxia therapy in all

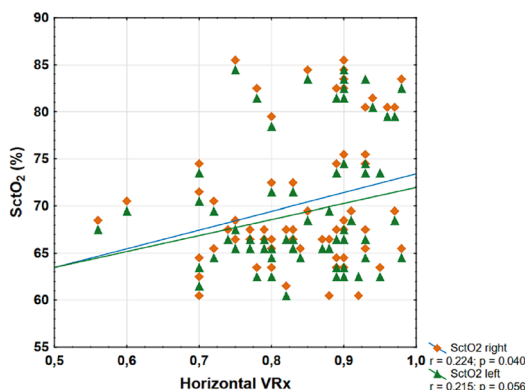


Fig. 2 Horizontal version reactivity index (HVRx) plotted against SctO₂ values on the left side (sand rhombi) and right side (green triangles) in the patients with moderate COVID-19 pneumonia ($p = 0.040$, $r = 0.224$; $p = 0.049$, $r = 0.215$, respectively)

patients of the COVID-hospital. However, we measured oxygen saturation only in the cortical-subcortical regions of FLP. On this basis, we cannot assert that oxygen saturation did not drop below the ischaemic level in other parts of the brain.

However, even against this background, significant associations between SctO₂ and eye vergence reactivity indices have been found.

Previously, Kelly et al. noted that during COVID, disturbances in the entire spectrum of oculomotor synergies, smooth pursuit, saccades, etc., were observed. Considering that they all have different anatomical representations, it can be stated that COVID-19 affects a wide range of brain structures, including the visual pathways [7].

However, it was later shown that coronavirus can not only directly affect neuronal structures and pathways in the brain but also indirectly, through regional hypoxemia, as well as owing to thrombosis of small vessels in the brain [8].

Visual impairment was also previously reported in patients with COVID-19, presumably due to retinal damage at a venous occlusion, which could potentially explain the oculomotor impairment development [9].

Yong et al. demonstrated that COVID-19-induced brain stem lesions were accompanied by oculomotor impairment, in particular by the max-

imum eye movement change. It has been suggested that the persistence of symptoms is due to the slow regeneration of damaged neurons [10].

Our study had some limitations. This study was retrospective; therefore, a certain shift in the patient population cannot be completely ruled out. Our cohort was relatively small in size. This requires the validation of the results in a larger cohort. It has been shown that the calculation error was no more than 5% at an amplitude of 20°. The angular velocity matrix analysis was carried out in the Cartesian coordinate system, neglecting the cornea curvature [11].

It should be noted that the associations between oxygen saturation and conjugate eye movement disturbances do not reflect a direct cause-and-effect relationship; however, they cannot indicate the opposite—the absence of such a relationship. A more accurate data analysis is needed, and this will be the focus of our future work.

5 Conclusion

Cerebral oxygen saturation in moderate pneumonia caused by the SARS-CoV-2 coronavirus has a significant destabilising effect on the oculomotor synergies (VVRx and HVRx) changes ($p < 0.05$).

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References

1. Henneghan AM, Lewis KA (2022) Gill E cognitive impairment in non-critical, mild-to-moderate COVID-19 survivors. *Front Psychol* 13:770459. <https://doi.org/10.3389/fpsyg.2022.770459>
2. Wu Y et al (2020) Nervous system involvement after infection with COVID-19 and other coronaviruses. *Brain Behav Immunity* 87:18–22. <https://doi.org/10.1016/j.bbi.2020.03.031>
3. Aidar MN, Gomes TM (2021) de Almeida MZH low visual acuity due to acute macular neuroretinopathy associated with COVID-19: a case report. *Am J Case Rep* 22:e931169. <https://doi.org/10.12659/AJCR.931169>
4. Yurttaser Ocak S, Ozturan SG, Bas E (2022) Pupil responses in patients with COVID-19. *Int*

- Ophthalmol 42:385–391. <https://doi.org/10.1007/s10792-021-02053-z>
5. Bitirgen G et al (2022) Abnormal quantitative pupillary light responses following COVID-19. *Int Ophthalmol* 42:2847. <https://doi.org/10.1007/s10792-022-02275-9>
6. Mucha A, Collins M, Elbin R et al (2014) A brief vestibular/ocular motor screening (VOMS) assessment to evaluate concussions. *Am J Sports Med* 42(10):2479–2486. <https://doi.org/10.1177/0363546514543775>
7. Kelly K, Anghinah R, Kullmann A et al (2022) Oculomotor, vestibular, reaction time, and cognitive tests as objective measures of neural deficits in patients post COVID-19 infection. *Front Neurol* 13:919596. <https://doi.org/10.3389/fneur.2022.919596>
8. Hanff TC, Mohareb AM, Giri J et al (2020) Thrombosis in COVID-19. *Am Hematol* 95:1578–1589. <https://doi.org/10.1002/ajh.25982>
9. Jevnikar K, Jaki Mekjavic P, Vidovic Valentincic N et al (2021) An update on COVID-19 related ophthalmic manifestations. *Ocul Immunol Inflamm* 29:684–689. <https://doi.org/10.1080/09273948.2021.1896008>
10. Yong SJ (2021) Persistent brainstem dysfunction in long-COVID: a hypothesis. *ACS Chem Neurosci* 12:573–580. <https://doi.org/10.1021/acscchemneuro.0c00793>
11. Okutin O, Okutin G (2011) Evaluation and leveling eye-tracker «noise». *Exp Psychol* 4:100–105. (In Russian)

Effect of Combined Oxygen Therapy and Use of a Surgical Mask or N95 Mask on Inspired Oxygen Fraction and Expired Carbon Dioxide Fraction

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Abstract

Combined use of a surgical mask and oxygen mask might decrease the inspired oxygen concentration and increase the risk of hypercapnia. We investigated the fraction of inspired oxygen (FiO_2) and end-tidal carbon dioxide (ETCO_2) under different combinations of masks and oxygen flows. Five healthy volunteers were administered oxygen using the following methods: oxygen mask alone (O group), oxygen mask over a surgical mask (S group), and oxygen mask over an N95 mask (N group). FiO_2 and ETCO_2 were measured at oxygen flow rates of 0, 5, and 8 L/min under each mask condition. At oxygen flow rates of 5 and 8 L/min, FiO_2 was lower in the order of N group (0.32 at 5 L/min, 0.36 at 8 L/min), S group (0.45 at 5 L/min, 0.52 at 8 L/min), and O group (0.61 at 5 L/min, 0.73 at 8 L/min). ETCO_2 was higher in the order of N, S, and O groups. In conclusion, wearing the oxygen mask over the surgical mask or N95 mask reduces FiO_2 and increases ETCO_2 in healthy

volunteers. Since patients who have emerged from general anaesthesia are more likely to have worse respiratory conditions, they need close observation to avoid hypoxemia and hypercapnia.

Keywords

COVID-19 · Oxygen mask · Surgical mask · N95 mask · Fraction of inspired oxygen · Hypoxemia

1 Introduction

During the induction of general anaesthesia, patients are administered high-flow oxygen through an oxygen mask to prevent hypoxemia due to the respiratory depression caused by administration of anaesthetic agents. Moreover, patients with infectious respiratory diseases such as influenza, pulmonary tuberculosis and coronavirus infection (COVID-19) need to wear a surgical mask or an N95 mask in the operating room to prevent the spread of infection to the medical staff. In fact, since the outbreak of COVID-19, the Japanese Society of Anaesthesiologists recommend that patients wear a surgical mask when entering the operating room, to prevent the spread of droplets and aerosols, and wear both a surgical

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mask and an oxygen mask when leaving the room after emergence from general anaesthesia [1]. This recommendation continues despite the World Health Organization declaring the end of the state of emergency on 5 May 2023 [2]. The consensus guidelines published in *Anaesthesia* also recommend that patients wear a facemask in addition to their oxygen mask or nasal cannula following tracheal extubation, whenever this is practical [3]. However, the combination of a surgical mask, an N95 mask and an oxygen mask might decrease the inspired oxygen concentration (FiO_2) and increase the risk of hypercapnia due to interference with exhalation. Therefore, we studied healthy volunteers to investigate the FiO_2 and end-tidal carbon dioxide (ETCO_2) under different combinations of masks and oxygen flows, as a simulation of oxygen therapy during induction of general anaesthesia in patients.

2 Methods

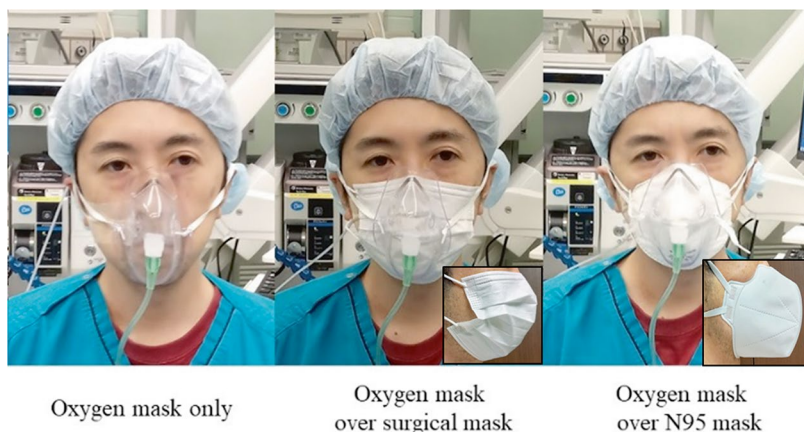
We administered oxygen to five healthy, non-smoking volunteers using the following methods: only an oxygen mask (INSPIRON™ Oxygen Masks for adults (Nihon Medical Next, Osaka, Japan)) (O group), an oxygen mask over a surgical mask equivalent to the American Society for Testing and Materials (ASTM) F2100-19 Level 2 (NS Surgical Face Mask™ (Nissho Sangyo,

Tokyo, Japan)) (S group), and an oxygen mask over an N95 mask that passed the code of Federal Regulations 42CFR Part 84 (Vilene Mask™ V-1003 N (Japan Vilene, Tokyo, Japan)) (N group) (Fig. 1). FiO_2 and ETCO_2 were measured at oxygen flow rates of 0 (for control value), 5 and 8 L/min under each mask condition. To monitor FiO_2 and ETCO_2 , the gas sampling tube that was put around the nares under each mask was connected to the multi-gas analyser (Multigas Unit GF-210R (Nihon Kohden, Tokyo, Japan)) and the data was displayed on the bedside monitoring system (Life Scope™ MU-671R (Nihon Kohden, Tokyo, Japan)). Data were recorded for five minutes on a minute-by-minute basis and a 3-minute interval was provided between each condition to avoid a carryover effect.

2.1 Data Analysis

Microsoft Excel 2013 (operated by Windows 10 Pro, Microsoft Inc.) was used for statistical analysis. For assessment of FiO_2 and ETCO_2 between each group, two-group comparisons were made using a paired *t*-test, and after application of the Bonferroni correction, cases with a risk rate of less than 1.7% were evaluated statistically as indicative of the presence of a significant difference. The numerical values were expressed as the mean level $\pm$ standard deviation and range.

Fig. 1 Three different oxygen administration methods. Left image shows O group wearing an oxygen mask only, centre image shows S group wearing an oxygen mask over surgical mask and right image shows N group wearing an oxygen mask over N95 mask. The tip of the gas sampling tube attached around the nares



3 Results

Table 1 shows the characteristics of the five volunteers. Table 2 shows the results of the FiO_2 and ETCO_2 . Figure 2 shows a boxplot of FiO_2 and Fig. 3 a boxplot of ETCO_2 . At oxygen flow rates of 5 and 8 L/min, FiO_2 was significantly lower in the N group than the O and S groups ($P < 0.0001$) and was also lower in the S group than the O group ($P < 0.0001$). ETCO_2 was significantly higher in the N group than in the O and S groups (vs. O group; $P < 0.0001$, vs. S group; $P < 0.0001$ at 0 and 5 L/min, $P = 0.012$ at 8 L/min), and was significantly higher in the S group than in the O group at 5 and 8 L/min ($P < 0.0001$).

4 Discussion

We confirmed that wearing a surgical mask or an N95 mask decreased FiO_2 and increased ETCO_2 in healthy volunteers. Oxygen masks are recommended for oxygen administration at flow rates of 5–8 L/min, which should typically pro-

vide FiO_2 of 40–45% and 55–60%, respectively. Additionally, it is noted that low-flow oxygen administration of less than 3 L/min carries a risk of rebreathing exhaled air due to its retention in the oxygen mask, leading to a risk of CO_2 narcosis. The present results showed that FiO_2 was higher than the catalogue value when the oxygen mask was used alone, whereas it did not reach the expected value with a surgical mask and oxygen flow rate of 8 L/min, and was lower at all flow conditions with an N95 mask. It also showed that ETCO_2 in the subjects wearing a surgical mask and an N95 mask was higher than that in the subjects with only an oxygen mask at oxygen flow rate of 5 and 8 L/min. Therefore, although this study was a simulation test using healthy volunteers, the findings suggest that the combined use of a surgical mask or an N95 mask and an oxygen mask might increase the risk of hypoxemia and hypercapnia during general anaesthesia induction in real patients. Further, these risks might become more serious in patients with severe respiratory disorders such as COVID-19 [4, 5]. On the other hand, it has been known that high-flow nasal cannula (HFNC), a relatively new device, can deliver a high concentrated oxygen to the peripheral lung trunk [6]. Additionally, considering that some models of HFNC can be attached under a surgical mask and an N95 mask, the application of an HFNC during general anaesthesia induction in a patient with infectious respiratory disease may be useful not just to administer sufficient oxygen to the patient but also to prevent the spread of infection to the medical staff.

Table 1 Patient characteristics

Sex	
Female <i>n</i>	2
Male <i>n</i>	3
Age (years)	31 ± 6.2 (28–42)
Weight (kg)	64.4 ± 13.7 (50–80)
Height (cm)	166.8 ± 8.3 (154–174)
BMI (kg/m ²)	22.9 ± 3.0 (18.8–24.4)

Data are presented as mean ± standard deviation (range). BMI body mass index

Table 2 Results of the FiO_2 and ETCO_2

	Group	Oxygen flow (L/min)		
		0	5	8
FiO_2 (%)	O	20.4 ± 0.5(20–21)	60.5 ± 12.7(41–88)	72.5 ± 14.3(77–94)
	S	20.1 ± 0.4(19–21)	44.9 ± 14.2(31–76)*	51.9 ± 14.2(36–86)*
	N	20.1 ± 0.4(19–21)	32.1 ± 6.6(23–46)*, ^a	36.0 ± 6.2(30–49)*, ^a
ETCO_2 (mmHg)	O	36.2 ± 6.0(23–46)	28.4 ± 7.0(16–42)	23.5 ± 7.4(16–42)
	S	36.6 ± 4.9(29–45)	35.8 ± 5.1(27–41)*	35.7 ± 5.7(24–45)*
	N	39.9 ± 6.6(30–50)*, ^a	38.7 ± 6.6(29–48)*, ^a	37.7 ± 5.6(26–46)*,**

Data are presented as average ± standard deviation (range). FiO_2 fraction of inspired oxygen, ETCO_2 endo-tidal carbon dioxide, O oxygen mask alone, S oxygen mask over surgical mask, N oxygen mask over N95 mask. * $P < 0.0001$ versus O group; ^a $P < 0.0001$ versus S group; ** $P = 0.012$ versus S group

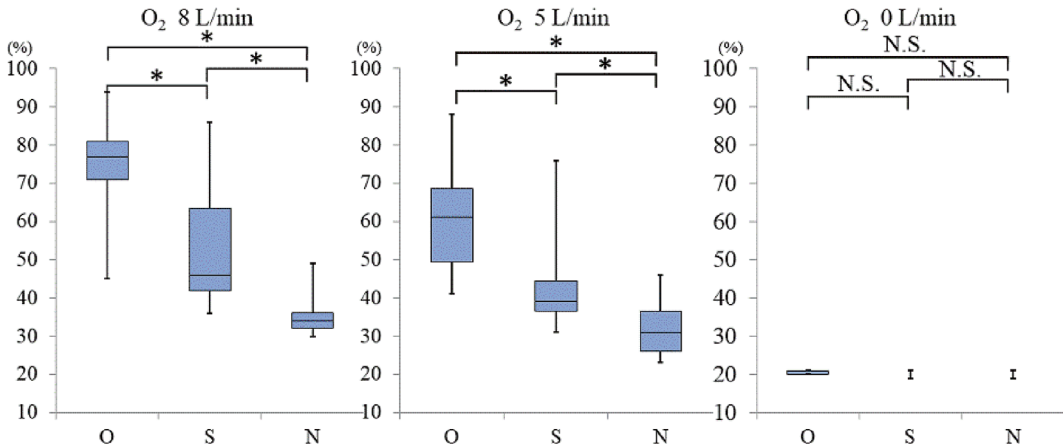


Fig. 2 Boxplot of FiO_2 at oxygen flow rates of 8 and 5 L/min. At oxygen flow rates of 5 and 8 L/min, FiO_2 was significantly lower in the N group than the O and S groups ($P < 0.0001$), and was also lower in the S group than the

O group ($P < 0.0001$). O: only an oxygen mask; S: an oxygen mask over a surgical mask; N: an oxygen mask over an N95 mask. * $P < 0.017$

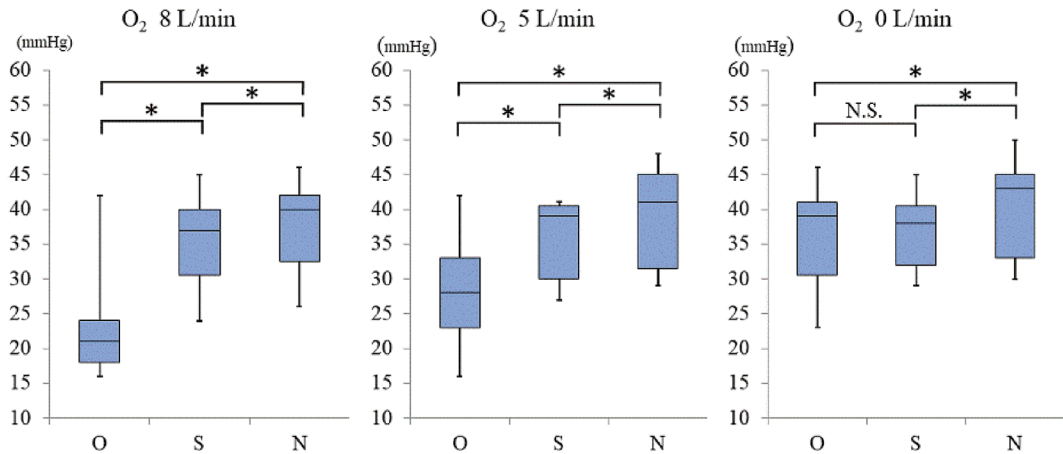


Fig. 3 Boxplot of $ETCO_2$ at oxygen flow rates of 8, 5 and 0 L/min. $ETCO_2$ was significantly higher in the N group than in the O and S groups (vs. O group; $P < 0.0001$, vs. S group; $P < 0.0001$ at 0 and 5 L/min, $P = 0.012$ at 8 L/min), and was significantly higher in the S group than in the O

group at 5 and 8 L/min ($P < 0.0001$). O: only an oxygen mask; S: an oxygen mask over a surgical mask; N: an oxygen mask over an N95 mask. * $P < 0.017$; N.S.: not significantly different

There is a limitation in this study. FiO_2 measured by the gas analyser is determined as the time phase of the minimum $ETCO_2$, while end-tidal O_2 is determined as the time phase of the maximum $ETCO_2$. This sampling method is, however, not stable compared with the measure-

ment in intubated patients under mechanical ventilation due to the inner turbulence in the oxygen mask, such as with phonation, coughing and sneezing, and due to incorporation of room air. For more precise measurements, arterial blood gas sampling must be performed.

5 Conclusion

The present study investigated the FiO_2 and ETCO_2 under different combinations of masks and oxygen flows. Wearing an oxygen mask over a surgical mask or an N95 mask significantly reduced FiO_2 and increased ETCO_2 even in healthy individuals. The combined use of a surgical mask or an N95 mask and an oxygen mask might increase the risk of hypoxemia and hypercapnia during general anaesthesia induction in patients with respiratory disorders.

References

1. Our Response to Coronavirus Disease 2019 (COVID-19). Japanese Society of Anaesthesiologists 2020. https://anesth.or.jp/img/upload/ckeditor/files/2004_07_01.pdf
2. WHO press conference on COVID-19 and other global health issues—5 May 2023. World Health Organization https://www.who.int/docs/default-source/coronaviruse/transcripts/virtual-press-conference-on-global-health-issues-5-may-2023.pdf?sfvrsn=dc963bd0_1
3. Cook TM, El-Boghdadly K, McGuire B et al (2020) Consensus guidelines for managing the airway in patients with COVID-19. *Anaesthesia* 75:785–799
4. Binks AC, Parkinson SM, Sabbouh V (2020) Oxygen: under or over a surgical facemask for COVID-19 patients? *Anaesthesia* 75:1683–1692
5. Hamada S, Tanabe N, Hirai T (2021) Effects of combined oxygen and surgical mask on inspired fraction of oxygen: relevance to COVID-19-induced respiratory failure. *Br J Anaesth* 126:215–226
6. Nishimura M (2019) High-flow nasal cannula oxygen therapy devices. *Respir Care* 64:734–742

Effects of Dual Tasks Including Gum Chewing on Prefrontal Cortex Activity

Arata Tsutsui, Tomotaka Takeda, Takahiro Sakaue, Shinji Togo, Yoshiaki Matsuda, Kazunori Nakajima, Kenichi Fukuda, and Kaoru Sakatani

Abstract

The purpose of this study was to examine the effects on prefrontal cortex (PFC) activity of listening to pleasant sounds (PS) while walking, gum chewing (GCh), or performing the dual task of walking and gum chewing at the same time (walking + GCh). A total of 11 healthy adult male volunteers participated in the study (mean age: 29.54 ± 3.37). The block design of the trial consisted of a 30-sec rest, a 60-sec task (target task or control task), and a 30-sec rest. There were three target task conditions: walking, GCh, and the dual task. All of these were performed while listening to PS. The control condition was rest (no exercise) while listening to PS. The outcomes measured and measurements used were PFC activity using two-channel near-infrared spec-

troscopy and participant self-evaluation of the pleasantness of the experience using the visual analogue scale (VAS). Compared to the control condition, there was significantly greater PFC activation during the GCh and the walking + GCh tasks. Compared to the control condition, GCh and walking + GCh showed significantly greater activation on the VAS measure. In conclusion, listening to PS while GCh or walking + GCh increases PFC activity in the lower central region and induces positive emotional change.

Keywords

Dual tasks · Gum chewing · Near-infrared spectroscopy · Prefrontal cortex · Walking

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1 Introduction

Many people in the modern society are subject to stress from diverse sources, and the recent social restrictions and reduced opportunities for exercise during the coronavirus disease 2019 pandemic have increased stress levels of the global population on both physical and mental levels.

In recent years, dual tasks including full-body exercise such as walking and running have been found to effectively increase activity in the prefrontal cortex (PFC) and relieve stress [1, 2]. In addition, many studies have been conducted on

the effects of gum chewing (GCh), a local exercise, on stress loading in dentistry and related fields [3–5]. These studies report that GCh while under stress affects stress markers such as alpha-amylase activity, cortisol levels, and immunoglobulin A secretion rate, while GCh when listening to unpleasant sounds increases right PFC activity and EEG alpha waves. During physical exercise, not only GCh but also listening to favourite music is common, with the latter associated with increased vitality, shortened Stroop interference times, and increased activation of the left-dorsolateral PFC [6].

The purpose of this study was to examine the effects of walking, GCh, and the dual task of these two exercises (walking + GCh) during pleasant sound (PS) listening on PFC activity.

2 Methods

The study participants comprised 11 healthy adult male volunteers, with a mean age of 29.54 ± 3.37 . All participants had complete natural dentition. The study was conducted in accordance with the principles of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Tokyo Dental College (ethical clearance no. 927). Written informed consent to participation and publication was obtained from all participants.

The task design and block design of this study are shown in Fig. 1. The block design of the trial consisted of a 30-sec rest, followed by a 60-sec task (target task or control task), and then another 30-sec rest. The target task consisted of three conditions: walking, GCh, and dual tasks (walking + GCh) under PS, and the control task consisted of a rest (no exercise) condition under PS. Each trial was performed two times at random, with measurement intervals at least 15 minutes apart. The trial included a control task and a GCh task, which were both performed in the sitting position; and a walking task and dual walking and GCh task, which were performed in the standing position. In this study, PS with pleasant sensitivity of 7 or higher was selected as our auditory stimuli from the International Affective

Digitized Sounds-2 (IADS) [7]. Selected sounds of approximately 5-sec length were mixed and assigned as the PS for our study conditions.

Participants were asked to refrain from food and caffeine consumption for 2 h before the test. They were also asked to keep any alcohol consumption moderate and to get a full night's sleep the night before the test. The test room was kept at 25 °C with 50% humidity. A treadmill (BW-SRM16, Barwing, Gunma, Japan) was used to ensure a uniform walking speed among participants of 4 km/h. Gum base was a prototype tasteless gum (Lotte, Saitama, Japan) of moderate hardness ($11 \times 18 \times 5$ mm, 1 g) with little change in hardness from the beginning of chewing. The chewing speed and chewing side were free. Each participant was placed with their head positioned so that the Frankfurt plane was parallel to the floor. They were then instructed to look at a cross-shaped mark in front of their eyes. Participants wore wireless earphones to eliminate any unintended auditory disturbances. White noise and PS were played through these earphones. The measures used to collect data were two-channel near-infrared spectroscopy (HOT-2000, NeU, Tokyo, Japan) (NIRS) using continuous wave, with which PFC activity was recorded, and a visual analogue scale (VAS), which was used to measure participants' self-reported pleasant experiences. The HOT-2000 consists of a light-emitting diode (LED) that emits near-infrared light at a wavelength of 810 nm on each side of the headset and two sensor units located about 1 cm and 3 cm from the LED. The device uses a method called real-time scalp signal separating to subtract the signals from the sensor 3 cm away from the LED (which captures the haemodynamics of scalp and cerebral blood flow) from those of the sensor 1 cm away from the LED (which capture the haemodynamics of scalp blood flow only). This method removes scalp-blood signals from functional near-infrared spectroscopy signals. The instrument wirelessly measures total haemoglobin at a sampling frequency of 10 Hz. A previous study found that this device can eliminate the shallow tissue effect of GCh [3].

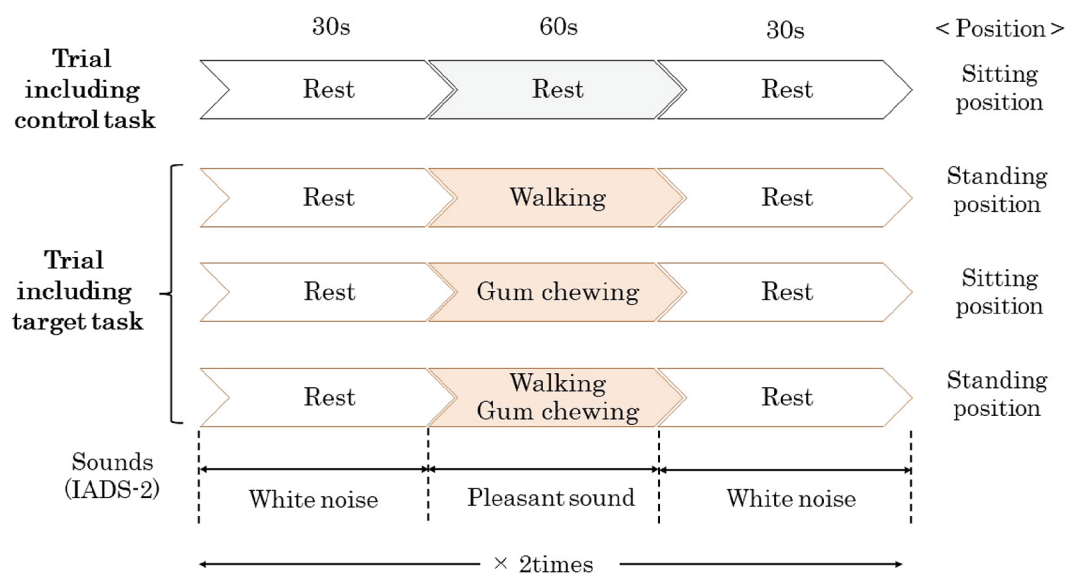


Fig. 1 The task design and block design of the trial in this study

The lower central region of the PFC, including the medial orbitofrontal cortex and the ventromedial prefrontal cortex (VMPFC), is thought to be activated during the cognitive processing of emotions and emotional experiences [8]. Based on this, the probe location was determined according to the international 10–20 system. The region of interest was placed at Fpz and total haemoglobin was measured. The VAS ratings were recorded after the completion of each task, using 0 as the reference value, with higher values up to 10 indicating a progressively more pleasant experience.

For each trial, the difference in the NIRS signal during the task compared to the signal during the rest period was calculated. Data were analysed using Excel Statistics 2016 (Bell Curve, Tokyo, Japan) software. The Shapiro–Wilk test was used to test the normality of the distribution of measured values. The Friedman test and Scheffe’s paired comparison test were used to analyse the PFC activity data obtained with the NIRS. One-way analysis of variance and Bonferroni’s multiple comparisons were used to analyse the VAS ratings. Statistical significance was set at $p < 0.05$.

3 Results

The results of our analysis of changes in total haemoglobin between resting and task performance as a measure of PFC activity are shown in Fig. 2. Compared to the control condition, there was significantly higher PFC activity during the GCh condition and the walking + GCh condition. No significant differences were found among the other parameters.

The results of our analysis of between-condition differences in the VAS ratings are shown in Fig. 3. Compared to the control condition, GCh and walking + GCh showed significantly greater activation. The VAS scores for the walking condition were also higher than for the control condition, but the difference was not significant. No significant differences were found among the other items.

4 Discussion and Conclusion

The VAS results obtained in this study suggest that the participants felt comfortable when listening to PS. Auditory information processing begins in the auditory cortex and sound recognition results from interactions between the asso-

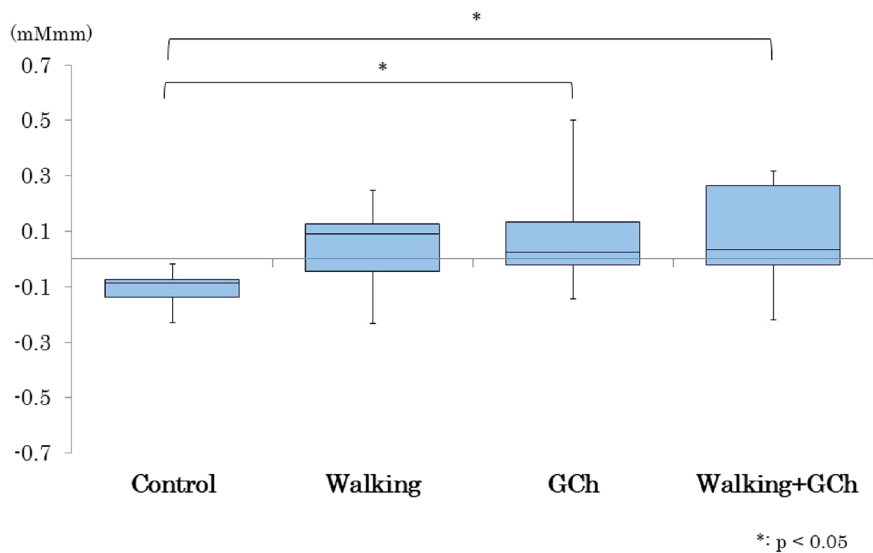


Fig. 2 Evaluation of changes in PFC activity between a resting state and a task performance state using NIRS. *GCh* gum chewing, *NIRS* near-infrared spectroscopy, *PFC* prefrontal cortex

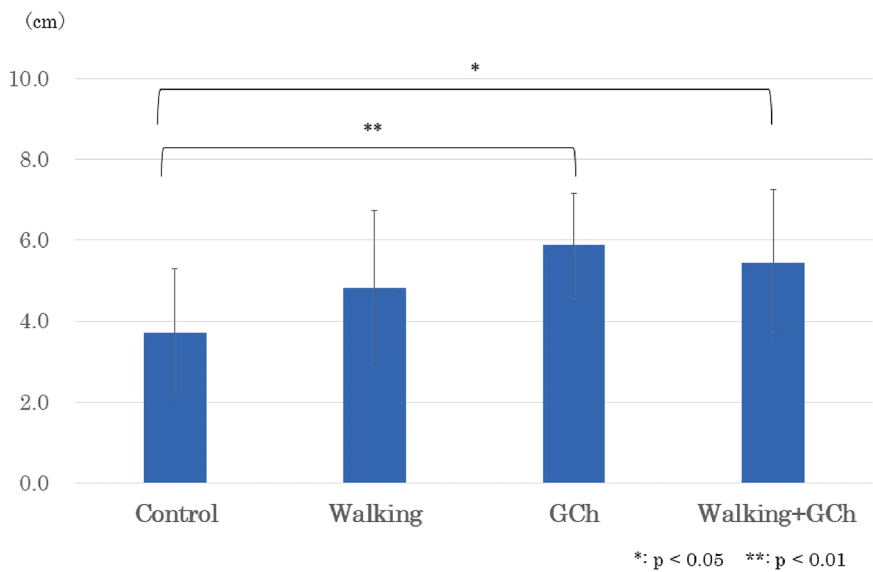


Fig. 3 Participants' VAS ratings of the pleasantness of their experience during the study tasks. *GCh* gum chewing, *VAS* visual analogue scale

ciation cortex, the parietal lobe, and the PFC. This information is then transmitted to the limbic system, eliciting an emotional response. Subsequently, various autonomic responses are triggered in the hypothalamus [9]. In particular, when feeling pleasure because of music or perceiving the music as having a positive valence,

there is diverse involvement of regions and networks, including the ventromedial and orbitofrontal cortices, the amygdala, the insula, regions of the reward circuit, the dorsomedial frontal motor areas, the hippocampus, the anterior cingulate cortex, the auditory cortices, and the temporal pole [10]. Also, the VMPFC and

orbitofrontal cortex also serve an inhibitory function in emotional responses through their output to the amygdala, the centre of emotion, including anger and fear [11, 12].

The effects of GCh on the brain identified by previous research include increased EEG alpha waves, inhibition of stress-induced catecholamines and adrenocorticotrophic hormone (ACTH), and enhanced blood flow to the PFC area due to increased heart rate and local neural activity [3, 13–15]. Considering these previous findings, the activation of the lower central region of the PFC in our study might be linked to mediation of the increased positive emotions shown in our VAS results through GCh or dual tasks. However, other research has reported that unpleasant negative valence due to occlusal discomfort and unpalatable gum induced a greater increase in activity in the frontopolar cortex and dorsolateral prefrontal cortex than did positive valence [16]. Since this study measured only PS, it is necessary to evaluate the effect of the dual task under negative sound in the future.

Regarding the influence of physical activity on PFC activity during dual tasks of cognitive and exercise, Kimura et al. [2] have shown that moderate to high-intensity exercise can effectively increase PFC activity. In the present study, the low intensity of the walking exercise may have limited its impact on PFC activity. Nevertheless, when combined, with GCh, it still appeared to have positive effects. On the other hand, there was no difference in PFC activity change among target tasks in this study. Therefore, the change in PFC activity may be influenced not only by emotional responses but also by nonspecific tasks such as walking, GCh, and the dual task. Thus, more detailed evaluation of the relationship between PFC activity and emotional change requires more multifaceted subjective emotional evaluation and clarification of the relationship with autonomic nervous system function. Furthermore, walking outdoors would likely alter the effect on PFC activity because of the additional stimuli. Future research is needed that considers factors such as exercise intensity, participants' exercise habits, and the walking environment.

The relatively low spatial resolution of NIRS limits its detection to the brain's surface, making it difficult to ensure precise regional measurements and precluding exploration of deeper regions. This was a limitation of this study. Additionally, while this study was conducted in both standing and sitting conditions, it is essential to standardise the conditions.

In conclusion, listening to PS while GCh or while walking and GCh increases lower central PFC activity and increases positive feelings.

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References

1. Weinstein AM, Voss MW, Prakash RS et al (2012) The association between aerobic fitness and executive function is mediated by prefrontal cortex volume. *Brain Behav Immun* 26:811–819
2. Kimura D, Hosokawa T, Ujikawa T et al (2022) Effects of different exercise intensities on prefrontal activity during a dual task. *Sci Rep* 12:13008
3. Konno M, Nakajima K, Takeda T et al (2020) Effect of gum chewing on PFC activity during discomfort sound stimulation. *Adv Exp Med Biol* 1232:113–119
4. Scholey A, Haskell C, Robertson B et al (2009) Chewing gum alleviates negative mood and reduces cortisol during acute laboratory psychological stress. *Physiol Behav* 97:304–312
5. Smith AP, Chaplin K, Wadsworth E (2012) Chewing gum, occupational stress, work performance and well-being. An intervention study. *Appetite* 58:1083–1086
6. Suwabe K, Hyodo K, Fukuie T et al (2021) Positive mood while exercising influences beneficial effects of exercise with music on prefrontal executive function: a functional nirs study. *Neuroscience* 454:61–71
7. Takeda T, Konno M, Kawakami Y et al (2016) Influence of pleasant and unpleasant auditory stimuli on cerebral blood flow and physiological changes in normal subjects. *Adv Exp Med Biol* 876:303–309
8. Ozawa S, Kanayama N, Hiraki K (2019) Emotion-related cerebral blood flow changes in the ventral medial prefrontal cortex: an NIRS study. *Brain Cogn* 134:21–28
9. Satoh M (2018) Cognitive and emotional processing in the brain of music. *Japan J Neuropsychol* 34:274–288. in Japanese
10. Brattico E, Bogert B, Alluri V et al (2015) It's sad but I like it: the neural dissociation between musical emotions and liking in experts and laypersons. *Front Hum Neurosci* 9:676

11. Hänsel A, von Känel R (2008) The ventro-medial prefrontal cortex: a major link between the autonomic nervous system, regulation of emotion, and stress reactivity? *BioPsychoSocial Med* 2:21
12. Pietrini P, Guazzelli M, Basso G et al (2000) Neural correlates of imaginal aggressive behavior assessed by positron emission tomography in healthy subjects. *Am J Psychiatry* 157:1772–1781
13. Hasegawa Y, Tachibana Y, Sakagami J et al (2013) Flavor-enhanced modulation of cerebral blood flow during gum chewing. *PLoS One* 8:e66313
14. Niwa M, Hiramatsu I, Nakata F et al (2005) Functional Significance of Stress-relieving Act of Chewing and its Effect on Brain Activation by Strees. *J Jap Assoc Rural Med* 54:661–666. in japanese
15. Suzuki M, Ishiyama I, Takiguchi T et al (1994) Effects of gum hardness on the response of common carotid blood flow volume, oxygen uptake, heart rate and blood pressure to gum-chewing. *J Jpn Soc Mastic Sci Health Promot* 4:51–62. in japanese
16. Hasegawa Y, Sakuramoto A, Suzuki T et al (2022) Emotional modulation of cortical activity during gum chewing: a functional near-infrared spectroscopy study. *Front Neurosci* 16:964351

Evaluation of the Relationship Between Pulmonary Perfusion Via Chest Digital Dynamic Radiography System and Diffusing Capacity Test

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Abstract

Non-invasive, visual, and quantitative in vivo evaluation of pulmonary blood flow is helpful in assessing gas exchange at the alveolar level. A chest digital dynamic radiography system (CDDR) has the potential to visually and quantitatively express pulmonary haemodynamics using temporal changes in radiographic transparency synchronised with the heartbeat. The lung-diffusing capacity

(DLCO) test involves membranous factors comprising the alveolar membrane, interstitium, and vascular walls. Additionally, DLCO includes pulmonary blood flow factors defined by pulmonary capillary blood flow and haemoglobin levels. In individuals lacking an alveolar septum, interstitial abnormalities or anaemia leading to changes in DLCO may reflect changes in the pulmonary blood flow. Here, we investigated the usefulness of CDDR in evaluating pulmonary blood flow by comparing it with DLCO.

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For pulmonary blood flow, we extracted and visualised signal changes in the lung field synchronised with heart rate during CDDR imaging and created a maximum intensity projection (MIP). The DLCO was measured using the single-breath method. Among the patients who underwent CDDR, 10 with FEV/FVC (FEV1.0%) > 70% and 16 with COPD (FEV1.0% < 70%) without noticeable interstitial changes were included, with a cut-off = 70%/predicted value. MIP was compared as a pulmonary blood flow evaluation method in the DLCO ≥ 70% group (control group: $n = 15$) and the DLCO < 70% group (decreased DLCO group: $n = 11$). The mean and median

values of MIP in the reduced group were lower than those in the control group ($p < 0.05$).

Pulmonary blood flow evaluated using CDDR may be able to assess pulmonary circulatory disturbances visually and quantitatively, as indicated by decreased pulmonary diffusion capacity in patients with negligible pulmonary interstitial changes and anaemia.

Keywords

Pulmonary blood flow · Digital X-ray imaging · Diffusion capacity · Chronic obstructive pulmonary disease · Maximum-intensity projection

1 Introduction

In vivo alterations in the pulmonary blood flow lead to impaired gas exchange in the alveoli, necessitating further evaluation. Recently, computed tomography (CT) angiography using contrast agents, magnetic resonance imaging (MRI), and pulmonary perfusion scintigraphy have been used to evaluate pulmonary arterial blood flow via right heart catheterisation and pulmonary arteriography. Nevertheless, these methods present challenges, including invasiveness, the need for expensive equipment, and difficulty in frequent performance over time; as a result, they may not be readily adaptable for routine clinical practice.

Non-invasive visual and quantitative assessments of in vivo pulmonary blood flow are helpful in evaluating gas exchange at the alveolar level. A chest digital dynamic radiography (CDDR) system has the potential to visually and quantitatively express pulmonary haemodynamics using temporal changes in radiographic transparency synchronised with the heartbeat [1, 2]. The lung diffusing capacity (DLCO) test evaluates the movement speed of a small amount of carbon monoxide (CO) gas within a certain period. It serves as a measure of the diffusing capacity of the lungs [3]. The DLCO consists of membranous factors, including alveolar mem-

branes, interstitium, and vascular walls, and pulmonary blood flow factors, defined by pulmonary capillary blood flow and haemoglobin levels [4]. In individuals without an alveolar septum, interstitial lesions, or anaemia, changes in DLCO may reflect changes in the pulmonary blood flow. In this study, we investigated the usefulness of CDDR in evaluating pulmonary blood flow by comparing it with DLCO.

2 Methods

2.1 Study Participants

CDDR testing was performed on 26 consecutive participants with normal spirometry findings or chronic obstructive pulmonary disease (COPD) without overt fibrotic lungs on high-resolution CT at our institute. The exclusion criteria were as follows: (1) two or more experienced physicians indicated that fibrotic lungs were present on high-resolution CT; (2) CDDR could not be performed because of difficulty in breathing, poor general condition, or cognitive impairment; and (3) participant consent for this study could not be obtained. None of the patients exhibited noticeable interstitial changes or fibrosis on high-resolution chest CT, and no significant differences in blood haemoglobin values were observed among the study participants. All the participants provided written informed consent before participating in the study. This study was approved by the Certified Review Board of Shiga University of Medical Science (Seta Tsukinowa-cho, Otsu, Shiga, Japan; approval number: CRB5180008) on February 12, 2019, and listed in the Japan Registry of Clinical Trials (<https://jrcr.niph.go.jp>, approval number: jRCTs052180103) on March 8, 2019.

2.2 CDDR Imaging

CDDR was performed using a flat panel detector system (Konica Minolta, Inc., Tokyo, Japan) composed of a flat panel detector (AeroDR fine; Konica Minolta, Inc.) and a pulsed X-ray genera-

tor (RAD speed Pro; Shimadzu Corporation, Kyoto, Japan) [5, 6]. The posteroanterior view was adopted in the standing position, and the anteroposterior view was adopted in the supine position. The pelvis was firmly fastened with a belt in the standing position. During CDDR imaging, participants were requested to breathe with deep inspiration and hold their breath for at least 6 s. Exposure conditions were similar to those used in a previous study. The dynamic image data captured at 15 frames/s (fps) were synchronised with the pulsed X-rays. Similar to modern fluoroscopy, the CDDR in this study utilised pulsed X-rays, thereby preventing excessive radiation exposure in the participants.

2.3 Image Analysis

Temporal changes in pixel values on sequential chest radiographs were analysed using the KINOSIS 1.00 software (Konica Minolta, Inc., Tokyo, Japan). The software was installed on an independent workstation (operating system: Windows 10 Professional, Microsoft, Redmond, WA, USA; central processing unit: Intel® Core™ i7-7500U 2.90 GHz; memory: 8 GB). The edges of the lung fields and the region of interest (ROI = 10 × 10 mm) in the left ventricle were automatically determined. Increases and decreases in the pixel values of the left ventricle were defined as diastole and systole phases, respectively. The lung fields were separated at 5-mm intervals. The cross-correlation coefficients of the alterations in each ROI within the right lung fields, employing a kernel radius of 2 cm on the side of the chest wall, were computed and compared with the left ventricle, which served as a control. Differences in pixel values between the lung fields and the left ventricle were determined throughout one cardiac cycle in each lung field block, representing the difference from the average blood volume. Measurements were performed in at least three cardiac cycles per image sequence; therefore, the breath-holding protocol was set to 6 s. This image analysis was based on the synchronisation of both ventricles.

Pulmonary perfusion was visualised using colour mapping. This visualisation method was described previously [6]. Briefly, changes in the pixel values were quantified and visualised in the form of a colour display (i.e., changes based on a colorimetric scale). Furthermore, the differences in pixel values from the baseline were sequentially calculated throughout all frames and superimposed over the original images in the form of a colour display. In the colour mapping images, an increase in blood volume is represented by a deep red colour. Colour mapping images were subsequently used to generate maximum intensity projection (MIP). In MIP, the signal change owing to blood flow is expressed using the following statistics: mean value (mean), median value (median), skewness (skew), and kurtosis (kurt).

2.4 Measurement of Lung Diffusion Capacity for Carbon Monoxide (DLCO)

DLCO was measured using the single-breath method according to prescribed guidelines [3]. Measurements were repeated at least twice by clinical laboratory technicians trained in performing function testing. The highest value of each measured item was used as the actual value.

2.5 Statistical Analysis

Quantitative variables are reported as mean values and standard deviations unless otherwise indicated. The Mann–Whitney U test was used to compare the MIP value between the control ($\geq 70\%$) and the decreased DLCO group ($< 70\%$). The predicted DLCO value was defined as normal if it was $\geq 80\%$, and a variation of $\geq 10\%$ was considered significant [7]. The cutoff value for DLCO in this study was set at 70% for the reasons mentioned above, as well as the median DLCO value of the participants in this study. All tests were two-tailed, and statistical significance was set at $p < 0.05$.

3 Results

Among the study participants who underwent CDDR at our institute, 10 with normal spirometry and forced expiratory value/forced vital capacity (FEV/FVC) (FEV 1.0% >70%) and 16 with COPD presenting with FEV 1.0% <70%), without noticeable interstitial changes, were included, with a cut-off DLCO of 70% of the predicted value. Signal changes in MIP were compared for the assessment of pulmonary blood flow between the DLCO ≥70% group (control group: *n* = 15) and the DLCO <70% group (decreased DLCO group: *n* = 11). Table 1 presents the demographic characteristics of the participants. Figure 1 and Supplementary Video 1 show pulmonary vascular imaging using chest dynamic imaging in two typical participants with normal DLCO and two participants with decreased DLCO. In participants with normal DLCO, the pulmonary blood vessels were imaged normally or densely; however, in participants with decreased DLCO, the pulmonary blood vessels were imaged sparsely.

Static figure of case representatives is placed below. Video file in the above-described URL; <https://youtu.be/VF38Y1csX9Y>, as well as in the Electronic Supplementary Materials:

As displayed in Table 2, in the quantitative image analysis, the mean and median MIP values of 0.48 ± 0.086 and 0.48, respectively, in the decreased DLCO group were lower than those in the control group with mean and median MIP values of 0.59 ± 0.133 and 0.60, respectively (*p* < 0.05). MIP skew and Kurt of MIP tended to exhibit low pulmonary blood flow signals in the decreased DLCO group, although the observed differences were not statistically significant. The mean MIP value tended to decrease as %DLCO decreased. However, this difference was not statistically significant (*r* = 0.33, *p* = 0.094).

4 Discussion

Right heart catheterisation and pulmonary angiography are the gold standards for evaluating in vivo pulmonary blood flow. However, these tests are invasive and lack versatility. Recently, non-invasive tests such as computed tomography angiography, MRI, single-photon emission CT, and pulmonary blood flow scintigraphy have been used to evaluate pulmonary blood flow. However, because these tests are expensive and

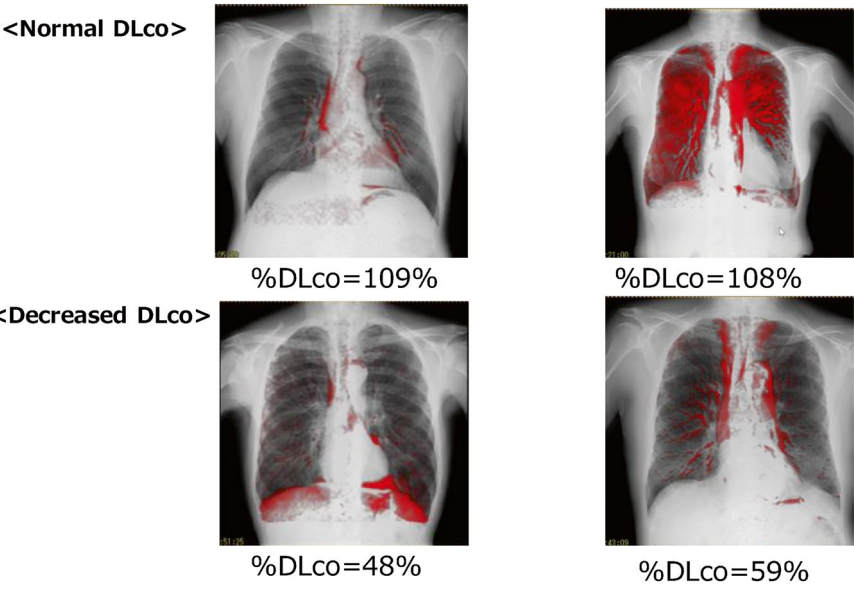


Table 1 Patient characteristics

	Normal (DLco≥70%)	Decreased (DLco<70%)	p-value
Participants (total)	15	11	
Age (years)	70±8	73±7	p = 0.392
Male subjects	13 (87%)	8 (73%)	p = 0.73
BMI	22.7±3.0	20.1±4.2	p = 0.283
FVC(L)	3.25±0.84	2.87±0.83	p = 0.151
FVC/predicted (%)	102.8±21.3	96.8±18.1	p = 0.424
FEV1(L)	2.15±0.60	1.65±0.63	p = 0.031 ^a
%FEV1/predicted(5)	93.6±21.5	80.4±27.1	p = 0.038 ^a
FEV1/FVC(%)	67.1±13.7	59.3±20.6	p = 0.038 ^a
SpO2(%) when inhaling room air	96.4±1.1	94.5±1.9	p = 0.042 ^a
DLco/predicted(%)	104.8±18.8	55.3±7.4	p<0.001 ^a
DLco/VA/predicted(%)	85.1±23.0	52.4±20.9	p<0.001 ^a

^aMann-Whitney U-test

Each value is expressed as mean ± standard deviation.

Abbreviations: *DLco* lung-diffusing capacity of carbon monoxide, *ns* statistically non-significant, *BMI* body mass index, *FVC* forced vital capacity, *FEV1* forced expiratory volume in one second, *SpO₂* Ratio of oxygen-saturated haemoglobin to total haemoglobin measured by pulse oximetry, *VA* alveolar ventilatory volume (substituted with total lung capacity)

Normal DLCO



DLCO=108%

Decreased DLCO



DLCO=59%

Fig. 1 Pulmonary vascular imaging using chest digital dynamic radiography system in one typical participant with normal lung-diffusing capacity (DLCO) and one with decreased DLCO, which demonstrates that the pulmonary blood vessels were imaged normally or densely in participants with normal DLCO; in participants with decreased DLCO, the pulmonary blood vessels are imaged sparsely. The supplementary video file can be found online: <https://youtu.be/VF38Y1csX9Y>

increase radiation exposure, repeating them frequently poses challenges.

In contrast, CDDR is inexpensive and can be used to visualise the pulmonary artery as a dynamic image with minimal radiation exposure. The MIP method was used to evaluate the density of the regional pulmonary blood flow quantitatively. We observed that the MIP was low in the decreased DLCO group. Basic research, case reports, and small-scale clinical studies have suggested that the evaluation of pulmonary blood flow using CDDR is effective [1, 2, 8, 9]. However, only a limited number of studies are available that compare the quantitative evaluation methods for pulmonary hypertension or pulmonary circulation disorders with pulmonary blood flow evaluation methods using the CDDR.

In this study, we compared the relationship between MIP and DLCO in the ROI calculated using the CDDR, mainly in patients with COPD. The study participants did not show noticeable interstitial changes or fibrosis on high-resolution chest CT. Furthermore, none of the participants had significant anaemia. Therefore, the observed decrease in DLCO in the study participants reflects a plausible reduction in peripheral pulmonary blood flow to the alveoli. CDDR is a

Table 2 Quantitative image analysis using the maximum intensity projection

	Mean MIP	Median MIP	Skew	Kurt
DLCO $\geq 70\%$	0.59 ± 0.13	0.60 ± 0.14 (0.466–0.713)	-0.33 ± 0.58	0.102 ± 0.98
DLCO $< 70\%$	0.48 ± 0.09^a	0.48 ± 0.11^a (0.384–0.562)	-0.092 ± 0.62	-0.187 ± 0.67

Each value is expressed as mean $\pm$ standard deviation. For the median MIP, first and third quartile values were added (first-third quartile value)

“Skew” means Skewness and “Kurt” means Kurtosis

^a $p < 0.05$ Mann–Whitney U test, as compared with the % DLCO $\geq 70\%$ group

simple and valuable method for visual, qualitative, and quantitative evaluation of peripheral pulmonary arterial blood flow.

This study had certain limitations. First, the cohort size was relatively small, and pulmonary arterial angiography and right heart catheterisation were not performed on all patients. Second, although the presence or absence of interstitial lung disease in the participants was determined using high-resolution CT, it was not confirmed from a histopathological perspective. Comparisons between pulmonary arterial angiography and right heart catheterisation in future studies, as well as comparative trials involving a large number of patients, will help address the limitations mentioned above. Third, this study aimed to reduce the number of tests involving high radiation exposure and expenditure. In this study, CT was performed on all study participants to exclude significant interstitial lung changes or fibrosis. For the follow-up or clinical periods, it is expected that it will be possible to conduct observations using physical findings and plain X-ray photography, including CDDR, without using CT examination. However, we admit that it is undoubtedly better to perform CT once for initial evaluation.

In conclusion, the pulmonary blood flow evaluation method using the CDDR may be able to visually and quantitatively evaluate pulmonary circulatory disturbances indicated by decreased pulmonary diffusing capacity in patients with negligible pulmonary interstitial changes and anaemia.

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Competing Interests On behalf of all the authors, the corresponding author states that there are no conflicts of interest.

Ethical Approval This study was approved by the Certified Review Board of Shiga University of Medical Science (Seta Tsukinowa-cho, Otsu, Shiga, Japan; approval number: CRB5180008) on February 12, 2019, and listed in the Japan Registry of Clinical Trials (<https://jrct.niph.go.jp>, approval number: jRCTs052180103) on 8 March 2019.

References

1. Tanaka R, Tani T, Nitta N et al (2019) Detection of pulmonary embolism based on reduced changes in radiographic lung density during cardiac beating using dynamic flat-panel detector: an animal-based study. *Acad Radiol* 26:1301–1308
2. Yamasaki Y, Abe K, Hosokawa K, Kamitani T (2020) A novel pulmonary circulation imaging using dynamic digital radiography for chronic thromboembolic pulmonary hypertension. *Eur Heart J* 41:2506
3. MacIntyre N, Crapo RO, Viegi G et al (2005) Standardization of the single-breath determination

- of carbon monoxide uptake in the lung. *Eur Respir J* 26:720–735
4. Stam H, Versprille A, Bogaard JM (1983) The components of the carbon monoxide diffusing capacity in man are dependent on alveolar volume. *Bull Eur Physiopath Respir* 19:17–22
 5. Tanaka R (2016) Dynamic chest radiography: flat-panel detector (FPD) based functional X-ray imaging. *Radiol Phys Technol* 9:139–153. <https://doi.org/10.1007/s12194-016-0361-6>
 6. Yamamoto S, Hasebe T, Tomita K et al (2020) Pulmonary perfusion by chest digital dynamic radiography: comparison between breath-holding and deep-breathing acquisition. *J Appl Clin Med Phys* 21:247–255
 7. American Thoracic Society (1991) Lung function testing: selection and reference values and interpretative strategies. *Am Rev Respir Dis* 144:1202–1218
 8. Tanaka R, Matsumoto I, Tamura M et al (2020) Comparison of dynamic flat-panel detector-based chest radiography with nuclear medicine ventilation-perfusion imaging for the evaluation of pulmonary function: a clinical validation study. *Med Phys* 47:4800–4809
 9. Yamamoto S, Sakamaki F, Takahashi G et al (2021) Novel pulmonary perfusion imaging using chest digital dynamic radiography for pulmonary artery sarcoma. *Respirol Case Rep* 9:e00737. <https://doi.org/10.1002/rcr2.737>

Preventing Fluid Retention After Subarachnoid Haemorrhage During Administration of Endothelin Receptor Antagonist

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Abstract

Prevention of delayed cerebral infarction (DCI) due to cerebral vasospasm after subarachnoid haemorrhage (SAH) has been done with intravenous Rho kinase inhibitors (ROCKI), ozagrel sodium (TXA2I), selective ROCKI infusion (ROCKI i.a.), and cerebrospinal fluid (CSF) drainage. The endothelin receptor antagonist (ERA, clazosentan) became available in 2022 and is said to be highly recommended for DCI prevention, while fluid retention such as pleural effusion and pulmonary oedema accumulation is often experienced. We investigated the relationship between patient background, fluid retention, and ERA. Ten consecutive SAH patients treated with ERA from July to December 2022 were included. We examined the results of blood sampling on admission, echocardiography, chest computed tomography (CT), with postoperative DCI, and hydrocephalus requiring cerebrospinal fluid shunt (hydro), and symptomatic fluid retention requiring albumin and furosemide (third fluid space). Two males

and eight females, mean age 63 years, mean preoperative World Federation Neurosurgical Surgeons (WFNS) grade 3.5, mean creatinine 0.94, mean brain natriuretic peptide (NT-proBNP). In 1883, two patients with Takotsubo cardiomyopathy and four patients with neurogenic pulmonary oedema are present. All patients underwent coil embolisation, and postoperative CSF drainage, ROCKI, TXA2I systemic administration, and ROCKI i.a. There were one DCI, three hydro, and five third fluid cases. Concerning the third fluid, the only significant difference was found in the age. An improvement in fluid retention after ERA discontinuation in old patients was shown. Our experience suggests that age may be the most influential factor. Based on these results, we have also found that by avoiding the use of ERA in patients older than 80 years, strictly limiting the infusion volume when using ERA, and actively using the drugs for heart failure early on, the frequency of suffering from third fluid space is reduced.

Keywords

Endothelin receptor antagonist (ERA) · Cerebral vasospasm · Subarachnoid haemorrhage · Fluid retention · Selective Rho kinase inhibitors infusion (ROCKI i.a.)

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1 Introduction

Cerebral vasospasm is a significant morbidity component after aneurysmal subarachnoid haemorrhage (aSAH). Angiographic vasospasm is observed in 70% of aSAH cases. Approximately 50% of cerebral vasospasm show delayed cerebral ischemia (DCI). Cerebral vasospasm often begins 3–4 days after aneurysm rupture, peaks 7–10 days later, and recovers spontaneously after 21 days. Treatment of DCI due to cerebral vasospasm, fluid retention, and secondary lung injury after aSAH is conflicting [1]. In particular, endothelin receptor antagonists (ERA, clazosentan) are set to be highly recommended for DCI prevention in Japanese stroke care guidelines, increasing their priority, while fluid retention such as pleural effusion and pulmonary oedema accumulation that cannot be overlooked is often experienced [2]. This study first investigates the initial use experience efficacy of ERA. Then, we determine the risk factor for fluid retention under ERA.

2 Methods

2.1 Patient Preparation

Our institutional review board approved this retrospective study. All patients provided informed consent for us to use their data. Ten consecutive aSAH cases with ERA between July 2022 and December 2022 were included. All patients underwent microsurgical clipping or coil embolisation within 24 h of onset, and clipping cases were excluded. Concerning the aSAH themselves, we collected the World Federation of Neurosurgical Societies (WFNS) grade and Fisher group and modified Rankin Scale (mRS) score at 30 days after onset. Postoperative therapy usually consisted of intravenous Rho kinase inhibitors (ROCKI) [3], ozagrel sodium (TXA2I), oral antiplatelets, statins, antiepileptic agents, and so on. Intravenous ERA was usually continued from the postoperative day (POD) 1 to POD 14. As a rule, cerebrospinal fluid (CSF) drainage using an indwelling spinal or ventricle catheter

was continued. Additionally, central venous pressure (CVP) was measured using a central venous catheter in all patients during the acute phase of intensive care [4]. If fluid retention requiring albumin and furosemide (third fluid) was sufficient to increase oxygen demand despite the use of albumin and furosemide, ERA was discontinued. ERA was also discontinued if hypotension did not improve with the administration of a pressure-raising drug. All patients underwent digital subtraction angiography (DSA) between POD 7 and POD 10, except for those who were too critically ill to be weaned off the ventilator. In cases with signs of cerebral vasospasm on DSA, aggressive selective ROCKI infusion (ROCKI i.a.) was performed [5]. Cerebral magnetic resonance imaging (MRI) was performed at least once and chest computed tomography (CT) multiple times by POD14. We examined the blood sampling results on admission, echocardiography, chest CT, postoperative MRI, hydrocephalus requiring cerebrospinal fluid shunt (hydro), and symptomatic fluid retention requiring albumin and furosemide (third fluid).

2.2 Statistical Analysis

We report the data as means. We used Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. All statistical analyses were conducted using JMP (version 10.1 software: SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically significant.

3 Results

3.1 Overall

Overall, patients, two males and eight females, had a mean age of 63 years, mean WFNS grade of 3.5, mean Fisher group of 2.6, mean creatinine of 0.94, mean eGFR of 83, and mean NT-proBNP of 1883. Table 1 shows the characteristics of the third fluid group and non-third fluid group. Each group included five cases. There were no signifi-

Table 1 The characteristics of the third fluid group and the non-third fluid group with endothelin receptor antagonist

	Third fluid (n = 5)	Non-third fluid (n = 5)	P value
Age (years)	82	44	0.011
Male	20%	20%	1
WFNS grade	3.7	4.3	0.15
Fisher group	3.0	2.8	0.42
Creatinine	1.33	0.55	0.074
eGFR	52.4	114	0.059
NT-proBNP	3059	707.7	0.53
Takotsubo	20%	20%	1
NPE	40%	40%	1
CSF shunt	20%	40%	1
Symptomatic DCI	0%	20%	1
mRS at 30 days	4.0	1.2	0.054

Third fluid symptomatic fluid retention requiring albumin and furosemide, *WFNS* World Federation of Neurosurgical Societies, *Takotsubo* Takotsubo cardiomyopathy, *NPE* neurogenic pulmonary oedema, *CSF* cerebrospinal fluid, *DCI* delayed cerebral ischemia, *mRS* modified Rankin

cant differences in sex, WFNS grade, or Fisher group. No significant differences were found in creatinine or eGFR, possibly due to the limited number of cases, nor in NT-proBNP, Takotsubo cardiomyopathy, neurogenic pulmonary oedema, cerebrospinal fluid shunt procedure, or symptomatic DCI. The mRS at 30 days also showed no significant differences, although the limited number of cases may have influenced this. On the other hand, despite the small number of cases, there was a significant age difference. In particular, all patients over the age of 80 years had third fluid (Table 1). Furthermore, after discontinuation of ERA use, fluid retention improved promptly in all patients except one who died four weeks later due to progressive worsening of renal and cardiac failure, which had been present before aSAH (Fig. 1).

3.2 A Case Avoiding Fluid Retention

A 34-year-old male was intubated at the emergency room with impaired consciousness. Head

CT showed massive SAH due to a ruptured right vertebral artery dissection aneurysm. He was classified as WFNS grade 5 and Fisher group 3. Chest CT showed mild pleural effusion, and an internal trap with neuroendovascular therapy was performed (Fig. 2a). The next day, he was extubated and treated with ERA, TXA2I, ROCKI, antiplatelet agents, statins, and anticonvulsants. CVP was measured up to POD 14, and CSF was drained by an indwelling spinal catheter mRS:3 and slightly drowsy at POD 7. Cerebral vasospasm was observed on DSA, so ROCKI i.a. was performed via the left distal radial artery access (Fig. 2b). At this time, the heart failure agents called the fantastic four, including angiotensin receptor/neprilysin inhibitors (ARNIs), beta-blockers (BBs), mineralocorticoid receptor antagonists (MRAs), and sodium–glucose co-transporter 2 inhibitors (SGLT2Is), were introduced. The head MRI did not detect any DCI, and the patient was discharged on his own with no neurological deficit at POD21 and returned to work at POD60.

4 Discussion

Fluid retention requiring albumin and furosemide administration occurred in 50% of cases; previous clinical studies on ERA have reported respiratory complications in 10–25% of patients [2]. In our study, 20% of cases led to discontinuation of ERA. Fluid retention was particularly prominent in patients older than 80 years. In the period under study, perioperative aSAH therapeutic management aimed to achieve a positive fluid balance, as in the past. However, pulmonary complications may occur with ERA. Furthermore, ERAs are peripheral vasodilators and may also increase capillary permeability. Therefore, inevitably, excessive postoperative infusions may cause pulmonary oedema [6, 7]. Furthermore, several reports have shown that a positive fluid balance in the early postoperative period is associated with DCI and poor outcomes [8]; further research will be needed on perioperative infusion management in patients using ERAs.

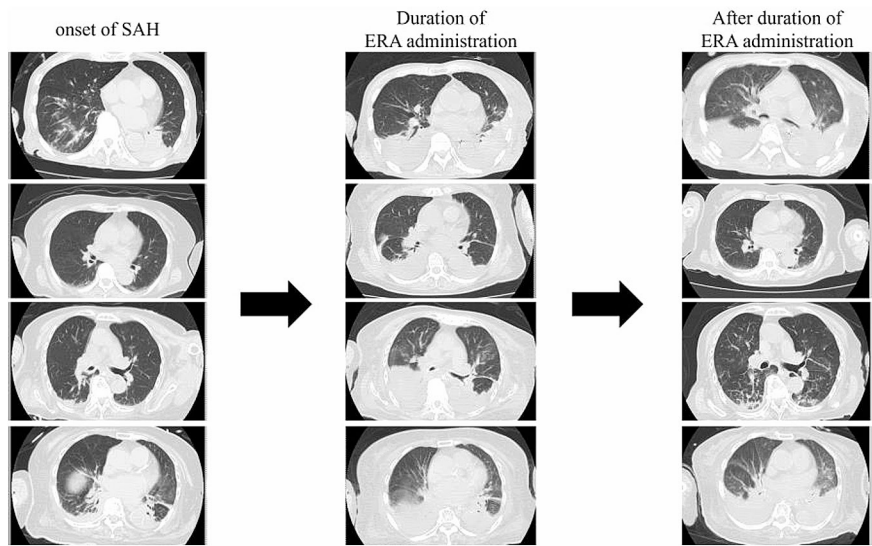


Fig. 1 All over 80-year-old cases showed fluid retention worsening on endothelin receptor antagonists

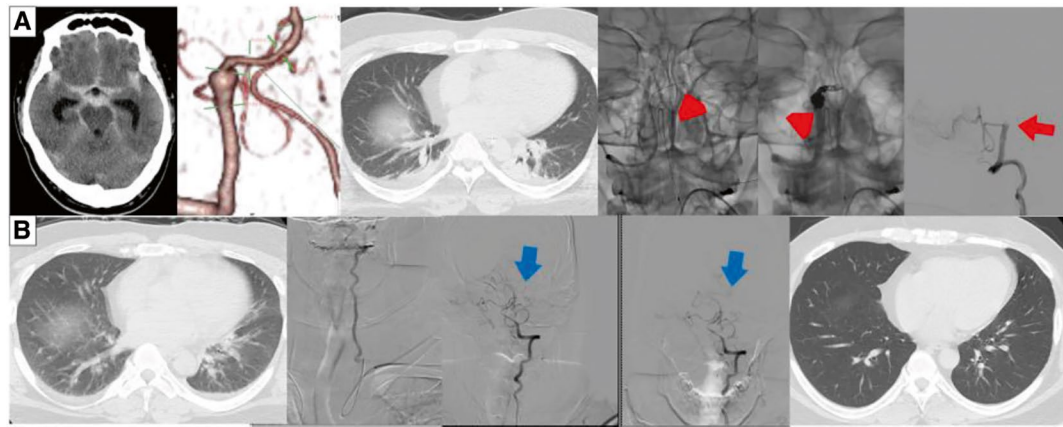


Fig. 2 (a) An internal trap with neuroendovascular therapy was performed. During endothelin receptor antagonist intravenous continuous infusion, (b) Rho kinase inhibitors selective infusion (ROCK i.a.) was performed via the left distal radial artery access without progressive fluid retention. The red arrowhead showed the tip of the

catheter before coiling and coil mass after internal trapping. The red arrow showed preservation of the right posterior cerebellar artery. The blue arrow showed defecting left posterior cerebral artery (PCA) flow before ROCK i.a. and recovering PCA flow after ROCK i.a

Cardiopulmonary dysfunction can occur after aSAH, which is associated with catecholamine release and sympathetic overstimulation; patients with higher WFNS grades of aSAH and more invasive procedures such as craniotomy clipping are more likely to suffer cardiopulmonary complications. To reduce confounding factors, we excluded clipping cases in this study.

Echocardiography may demonstrate abnormal wall motion, one of the most typical findings being takotsubo cardiomyopathy and neurogenic pulmonary oedema [4]. Furthermore, ERAs are vasodilatory and can cause hypotension, worsening cardiac output and cerebral perfusion. These post-aSAH mechanisms have led us to the aggressive use of heart failure agents, called the

Fantastic Four, including ARNIs, MRAs, SGLT2Is, and BBs [9].

As mentioned above, catecholamine release and sympathetic overstimulation are dependent on highly invasive treatments [4], so minimally invasiveness is recommended even for invasive procedures. Therefore, we use either the left or right distal radial artery approach for ROCKI i.a., which is not only minimally invasive intraoperatively, but also reduces postoperative haemostatic stress and allows early resumption of rehabilitation.

Based on these results, from the beginning of 2023, we revised the protocol for preventing DCI. In elderly patients over 80, ERA was avoided, the Fantastic Four was administered after aSAH, and the infusion volume was controlled strictly. Since then, third fluid after ERA administration, including clipping surgery cases, has been avoided in all cases. Generally speaking, DCI occurred in about 30% of cases before ERA came [10]. Although decreasing, DCI has partially occurred even after ERA came. However, 10% DCI occurred during the initial use of ERA, and 10% DCI occurred during the period of appropriate ERA with the revised protocol. So, the next step in treatment methods is still awaited.

There are several limitations to this study. First, because of the large number of medications used and age as a possible confounding factor, it is impossible to say that fluid retention is solely attributable to ERAs. Second, this is a small, retrospective study of ERAs in aSAH undergoing coil embolisation. A more extensive prospective cohort study would be needed to assess this more accurately.

5 Conclusions

We evaluated our initial experience with the use of ERA after aneurysmal coil embolisation for aSAH in 10 consecutive patients, and half of the cases had fluid retention requiring treatment; fluid retention on ERA was particularly likely in the elderly and might warrant careful consideration of its use in patients over 80 years of age.

Perioperative fluid management and treatment of heart failure may be necessary. ROCKI i.a. via the distal radial artery access may have a high affinity for ERA. Despite our best efforts, the next step in unavoidable DCI cases is awaited.

References

- Obata Y, Takeda J, Sato Y, Ishikura H, Matsui T, Isotani E (2016) A multicenter prospective cohort study of volume management after subarachnoid hemorrhage: circulatory characteristics of pulmonary edema after subarachnoid hemorrhage. *J Neurosurg* 125(2):254–263. <https://doi.org/10.3171/2015.6.Jns1519>
- Endo H, Hagihara Y, Kimura N et al (2022) Effects of clazosentan on cerebral vasospasm-related morbidity and all-cause mortality after aneurysmal subarachnoid hemorrhage: two randomized phase 3 trials in Japanese patients. *J Neurosurg* 137(6):1707–1717. <https://doi.org/10.3171/2022.2.Jns212914>
- Suzuki Y, Shibuya M, Satoh S, Sugimoto Y, Takakura K (2007) A postmarketing surveillance study of fasudil treatment after aneurysmal subarachnoid hemorrhage. *Surg Neurol* 68(2):126–131.; Discussion 131–122. <https://doi.org/10.1016/j.surneu.2006.10.037>
- Bruder N, Rabinstein A (2011) Cardiovascular and pulmonary complications of aneurysmal subarachnoid hemorrhage. *Neurocrit Care* 15(2):257–269. <https://doi.org/10.1007/s12028-011-9598-4>
- Kuwano A, Ishiguro T, Nomura S et al (2023) Predictive factors for improvement of symptomatic cerebral vasospasm following subarachnoid hemorrhage by selective intra-arterial administration of fasudil hydrochloride. *Interv Neuroradiol*:15910199231155037. <https://doi.org/10.1177/15910199231155037>
- Macdonald RL, Kassell NF, Mayer S et al (2008) Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. *Stroke* 39(11):3015–3021. <https://doi.org/10.1161/strokeaha.108.519942>
- Macdonald RL, Higashida RT, Keller E et al (2012) Randomized trial of clazosentan in patients with aneurysmal subarachnoid hemorrhage undergoing endovascular coiling. *Stroke* 43(6):1463–1469. <https://doi.org/10.1161/strokeaha.111.648980>
- Vergouw LJM, Egal M, Bergmans B et al (2020) High early fluid input after aneurysmal subarachnoid hemorrhage: combined report of association with delayed cerebral ischemia and feasibility of cardiac output-guided fluid restriction. *J Intensive Care Med* 35(2):161–169. <https://doi.org/10.1177/0885066617732747>

9. Bauersachs J (2021) Heart failure drug treatment: the fantastic four. *Eur Heart J* 42(6):681–683. <https://doi.org/10.1093/eurheartj/ehaa1012>
10. Connolly ES Jr, Rabinstein AA, Carhuapoma JR et al (2012) Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/american Stroke Association. *Stroke* 43(6):1711–1737. <https://doi.org/10.1161/STR.0b013e3182587839>

Hyperoxia-Induced Secondary Respiratory Failure in a Systemic Ischaemia-Reperfusion Injury

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Abstract

Recent studies revealed that excessive supplemental oxygen, such as inhaled 100% O₂, damages various organ functions in post-cardiac arrest (CA) patients. Optimal indicators of supplemental oxygen are therefore important to prevent hyperoxic organ injuries. In this study, we evaluated a hyperoxic pulmonary injury and assessed the association between alveolar-arterial oxygen difference (AaDO₂) and a degree of lung oedema. In this study, we focused on the hyperoxia-induced lung injury and its association with changes of gas-exchange parameters in post-CA rats. Rats were resuscitated from 10 min of asphyxial CA and stratified into two groups: those with inhaled 100% O₂

(CA-FiO₂ 1.0) and those with 30% O₂ (CA-FiO₂ 0.3). We prepared a sham surgery group for comparison (sham-FiO₂ 0.3). After 2 h, animals were sacrificed, and the lung wet-to-dry (W/D) weight ratio was measured. We collected blood gas results and measured the ratio of partial pressure arterial oxygen and fraction of inspired oxygen (p/f ratio), and calculated AaDO₂. The lung W/D ratio in the CA-FiO₂ 1.0 group (5.8 ± 0.26) was higher than in the CA-FiO₂ 0.3 (4.6 ± 0.42) and sham-FiO₂ 0.3 groups (4.6 ± 0.38 , $p < 0.01$). There was a significant difference in AaDO₂ between CA-FiO₂ 1.0 (215 ± 49.3) and, CA-FiO₂ 0.3 (36.8 ± 32.3), and sham-FiO₂ 0.3 groups (49.0 ± 20.5 , $p < 0.01$). There were also significant changes in pH and blood lactate levels in the early phase among the three groups. AaDO₂ showed the strongest correlation with W/D ratio ($r = 0.9415$, $p < 0.0001$), followed by pH ($r = -0.5131$, $p = 0.0294$) and p/f ratio ($r = -0.3861$, $p = 0.1135$). Hyperoxic injury might cause the pulmonary oedema after CA. Measuring respiratory quotient (RQ) in rodents enabled an accurate calculation for AaDO₂ at a variety level of inhaled O₂. Given that AaDO₂ measurement is non-invasive, we therefore consider AaDO₂ to be a potentially optimal indicator of post-CA hyperoxic pulmonary injury.

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Keywords

Alveolar-arterial oxygen difference (AaDO₂) · Respiratory quotient (RQ) · Hyperoxia · Secondary respiratory failure · Ischemia-reperfusion injury

1 Introduction

Recent studies revealed that excessive supplemental oxygen, such as inhaled 100% O₂, endangers various organ functions in post-cardiac arrest (CA) [1]. We have already made presentations about post-cardiac arrest hyperoxic whole body injury by respiratory quotient (RQ)² and brain injury in oxyhaemoglobin (oxy-Hb) and rSO₂ by near-infrared spectroscopy (NIRS)³. In this study, we observed a hyperoxic lung injury after CA. Successful monitoring of hyperoxic lung injury would have a huge impact on respiratory and critical care medicine.

2 Methods

2.1 Animal Preparation

The Institutional Animal Care and Use Committees of the Feinstein Institutes for Medical Research approved the study protocol. The details of the methods for a rat asphyxia CA model have been described previously [4]. Briefly, adult male Sprague-Dawley rats were anaesthetised with 4% isoflurane and intubated with a 14-gage angio-catheter. Anesthesia was maintained with isoflurane 2% at a fraction of inspired O₂ (FiO₂) of 0.3 under mechanical ventilation. The left femoral artery was cannulated for continuous arterial pressure monitoring. The oesophageal temperature was maintained at 36.5 ± 1.0 °C during the surgical procedure. The left femoral vein was cannulated with a polyethylene-50 catheter, which was advanced into the inferior vena cava for drug infusion. Data averaged every 20 s were used. After instrumenta-

tion setup, the neuro-muscular blockade was achieved by slow intravenous administration of 2 mg/kg of vecuronium bromide (Hospira, USA). Asphyxia was induced in the rats by switching off the ventilator, and CA normally occurred 3–4 min after asphyxia started. We defined CA as a MAP below 20 mmHg; CA was completely untreated for 10 min after asphyxia. Mechanical ventilation was re-started at a FiO₂ of 1.0, and manual cardiopulmonary resuscitation (CPR) was delivered to the CA animals. Chest compressions were performed at a rate of 240–300 per min. At 30 s after the beginning of CPR, a 20 µg/kg bolus of adrenaline was given to the animals through the venous catheter. Following the return of spontaneous circulation (ROSC), defined as a systolic blood pressure >60 mmHg, CPR was discontinued. At 10 min after CPR, the FiO₂ was switched back to 0.3 in the normoxic therapy group while it was kept at 1.0 in the hyperoxia group. Blood gas analysis was performed at 120 min after starting CPR. At 120 min after CPR, animals were sacrificed from the sham-normoxia, CA-normoxia, and CA-hyperoxia groups. In order to evaluate secondary pulmonary oedema, we used the wet-to-dry lung weight as a lung wet/dry ratio (W/D ratio). The right lower lobe of each animal was weighed immediately after collection and then placed into a 60 °C oven to dry. After three days, the tissue was weighed to determine the W/D ratio [1].

2.2 Calculation Method

We measured the ratio in mechanically ventilated, anaesthetised, rats. We were able to calculate respiratory quotient (RQ) using the mass ratio in conjunction with the conventional measurement of FiO₂, FeO₂, and FeCO₂. We calculated VO₂ and VCO₂ by the following equations:

$$R = \frac{\text{Volume of inspired gas (VI)}}{\text{Volume of expired gas (VE)}}$$

$$\begin{aligned} \text{Oxygen consumption (VO}_2\text{)} = \\ \text{VI} \times \text{Fraction of inspired oxygen (FiO}_2\text{)} \\ - \text{VE} \times \text{Fraction of expired oxygen (FeO}_2\text{)} \end{aligned}$$

$$\begin{aligned} \text{Carbon dioxide generation (VCO}_2\text{)} = \\ \text{VE} \times \text{Fraction of expired carbon dioxide (FeCO}_2\text{)} \\ - \text{VI} \times \text{Fraction of inspired carbon dioxide (FiCO}_2\text{)} \end{aligned}$$

$$\text{RQ} = \text{VCO}_2 / \text{VO}_2$$

FiCO₂ was assumed to be zero since the inhaled gas did not contain CO₂. We kept the room temperature and the atmospheric pressure almost equal in each experiment. We used R, so we were able to calculate, regardless if VI equalled VE or not. The RQ, VO₂, and VCO₂ were calculated by the following equation:

$$\text{VO}_2 = (\text{R} \times \text{FiO}_2 - \text{FeO}_2) \times \text{VE}$$

$$\text{VCO}_2 = \text{FeCO}_2 \times \text{VE}$$

$$\text{RQ} = \text{FeCO}_2 / (\text{R} \times \text{FiO}_2 - \text{FeO}_2)$$

When VE was measured conventionally, the mass ratio allowed for the calculation of VO₂ and VCO₂. All these measurements were capable and reliable regardless of the level of FiO₂. We next calculated PaO₂/FiO₂ ratio (p/f ratio) and arterial-alveolar oxygen tension difference (AaDO₂) by the following equations. Since our system could calculate RQ over time, we also calculated a more accurate AaDO₂ using the RQ at that time instead of the AaDO₂ conventionally calculated by 0.8, and compared the two [2, 5]:

$$\text{p/f ratio} = \frac{\text{Arterial oxygen partial pressure (PaO}_2\text{)}}{\text{FiO}_2}$$

$$\text{AaDO}_2 = \text{Alveolar oxygen partial pressure (PAO}_2\text{)} - \text{PaO}_2$$

$$\approx (\text{Atmospheric pressure (Patm)} - \text{Partial pressure of water (PH}_2\text{O)}) \text{ FiO}_2 - \text{Arterial carbon dioxide partial pressure (PaCO}_2\text{)}/0.8 - \text{PaO}_2 \text{ (> as conventional formula)}$$

$$= (\text{Patm} - \text{PH}_2\text{O}) \text{ FiO}_2 - \text{PaCO}_2/\text{RQ} - \text{PaO}_2 \text{ (> as precise formula)}$$

2.3 Statistical Analysis

Data are presented as mean and standard deviation. Values were compared between the groups by 1-way analysis of variance (ANOVA) with post-hoc analysis of the Tukey test. In multiple parameter's comparisons, correlation coefficient (r) values were collected. All statistical analyses were conducted using JMP (version 10.1 software: SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically significant (Fig. 1).

3 Results

ROSC was achieved in all animals. The lung W/D ratio, the CA-FiO₂ 1.0 group (5.8 ± 0.26) was higher than in the CA-FiO₂ 0.3 (4.6 ± 0.42) and sham-FiO₂ 0.3 groups (4.6 ± 0.38 , $p < 0.01$). There was a significant difference in AaDO₂ between CA-FiO₂ 1.0 (215 ± 49.3) and, CA-FiO₂ 0.3 (36.8 ± 32.3), and sham-FiO₂ 0.3 groups (49.0 ± 20.5 , $p < 0.01$). There were also significant changes in pH, and blood lactate levels in the early phase among the three groups. AaDO₂ showed the strongest correlation with W/D ratio ($r = 0.9415$, $p < 0.0001$), followed by pH ($r = -0.5131$, $p = 0.0294$) and p/f ratio ($r = -0.3861$, $p = 0.1135$). The exact AaDO₂ using RQ ($r = 0.9415$, $p < 0.0001$) in the formula showed a stronger correlation with the lung W/D ratio than the AaDO₂ using the approximate value of 0.8 ($r = 0.9f97$, $p < 0.0001$) (Table 1).

4 Discussion

This experiment reported here demonstrates that the AaDO₂ was associated with the lung W/D ratio. The exact AaDO₂ using RQ in the formula showed a stronger correlation with the lung W/D ratio than the AaDO₂ using the approximate value of 0.8. This was the first study showing AaDO₂ using RQ to be a potentially optimal indicator of post-CA hyperoxic pulmonary injury.

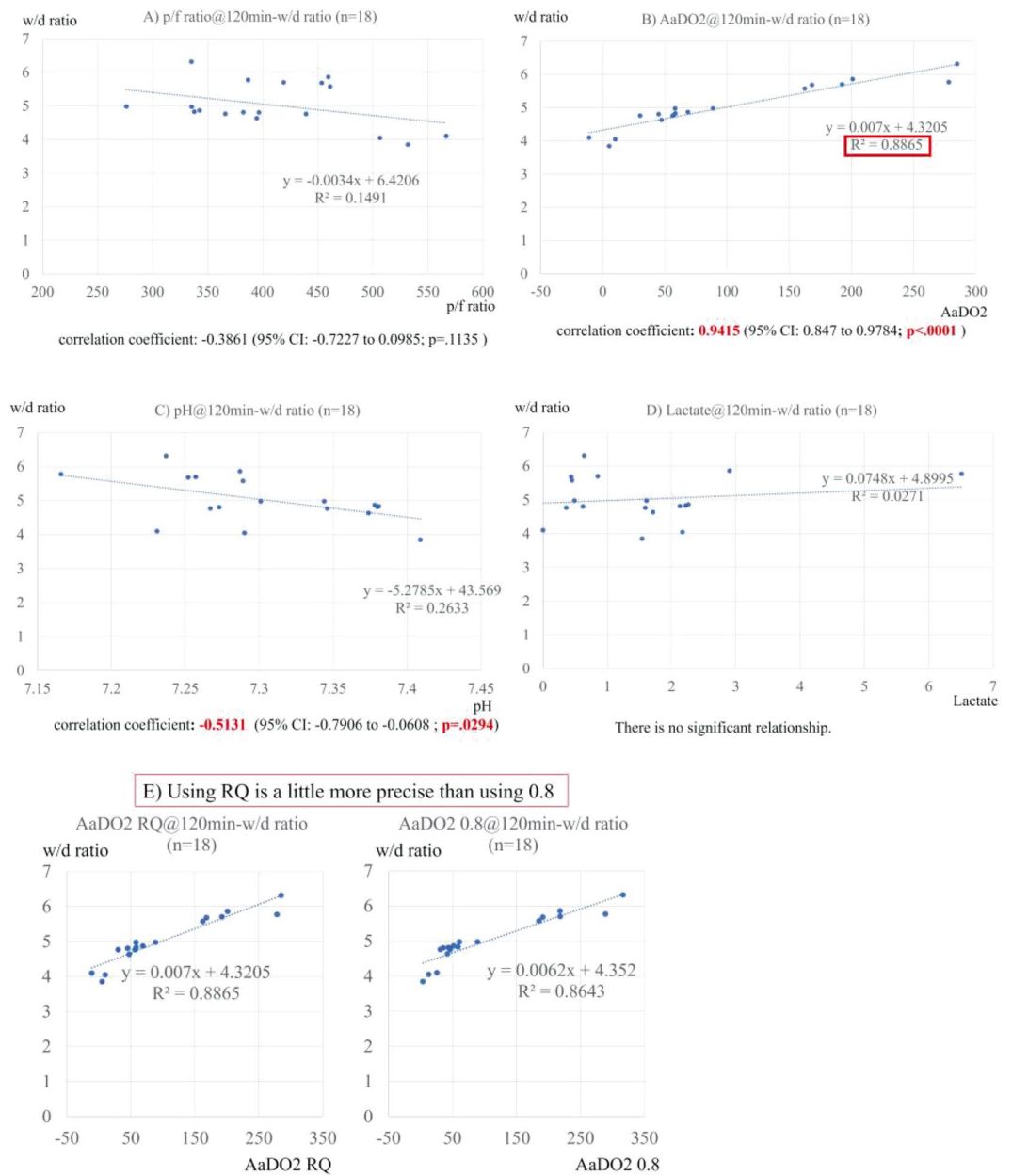


Fig. 1 Each parameter correlation with W/D ratio at 120 min after cardiopulmonary resuscitation. W/D ratio lung wet-to-dry weight ratio, p/f ratio PaO₂/FiO₂ ratio,

AaDO₂ alveolar-arterial oxygen difference, AaDO₂ RQ AaDO₂ using respiratory quotient, AaDO₂ 0.8 AaDO₂ using 0.8 as approximate respiratory quotient

Generally speaking, supplemental oxygen is one of the most important components of the critical care including cardiac arrest, acute ischaemic or haemorrhagic stroke, and trauma in order to compensate the imbalance between oxygen supply and requirements. However,

aggressive treatment against hypoxia might lead to patients' exposure to high levels of FiO₂, even higher than the values strictly necessary to maintain physiological values of PaO₂. The effects of hyperoxia might have a detrimental effect on outcome mediated by several patho-

Table 1 sham-FiO₂ 0.3: sham surgery with inhaled 30% O₂ for 120 min; CA-FiO₂ 0.3: post-cardiac arrest with inhaled 30% O₂ for 120 min; CA-FiO₂ 1.0: post-cardiac arrest with inhaled 100% O₂ for 120 min

	Sham-FiO ₂ 0.3 (n = 6)	CA-FiO ₂ 0.3 (n = 6)	CA-FiO ₂ 1.0 (n = 6)
Lung W/D ratio	4.6 ± 0.38	4.6 ± 0.42	5.8 ± 0.26 ## **
AaDO ₂	49.0 ± 20.5	36.8 ± 32.3	215 ± 49.3 ## **

Lung W/D ratio lung wet-to-dry weight ratio, *AaDO₂* alveolar-arterial oxygen difference; ##: $p < 0.01$ compared with CA-FiO₂ 0.3; **: $p < 0.01$ compared with sham-FiO₂ 0.3

physiological mechanisms. Hyperoxia may produce mitochondrial damage, lipid peroxidation of neuronal cells and an increase in reactive oxygen species from high tissue oxygen tension and induce lung injury. Hyperoxia following prolonged breathing of very high FiO₂ has shown to be able to cause a direct damage to pulmonary capillary endothelium with consequent altered permeability pulmonary oedema and may exacerbate alveolar inflammation causing severe hyperoxic acute lung injury, whose severity is directly proportional to PaO₂. Moreover, hyperoxic reperfusion may lead to neurological complications and brain injury. Several retrospective studies suggest that even short-term hyperoxia is associated with increased mortality and morbidity, especially in patients after cardiac arrest, stroke, and traumatic brain injury. Recently, O₂ has been recognised as “friend and foe”. This is particularly pronounced during ischaemia/reperfusion and/or hypoxia/re-oxygenation. Consequently, O₂ toxicity, especially pulmonary, is a matter of concern, and optimal dosing remains unclear, and the monitoring method has been sought in critical care [1, 6, 7].

Previously, we reported that oxy-Hb, measured using NIRS may be used to evaluate the brain mitochondrial dysfunction and oxy-Hb may be a useful parameter for ischaemia/reperfusion injury [3]. Like oxy-Hb for brain, we recognised the possibility that accurate AaDO₂ could be a useful parameter for minimally invasive lung hyperoxic injury.

The present study has several limitations. The AaDO₂ and lung W/D ratio should be compared not only at one-time points but also at additional time points, which may be realised in future studies. The extent to which ischaemic and reperfu-

sion injuries are involved also needs to be evaluated.

5 Conclusions

Given that accurate AaDO₂ measurement is non-invasive, we, therefore, consider AaDO₂ to be a potentially optimal indicator of post-CA hyperoxic pulmonary injury.

References

- Okuma Y, Becker LB, Hayashida K et al (2021) Effects of post-resuscitation normoxic therapy on oxygen-sensitive oxidative stress in a rat model of cardiac arrest. *J Am Heart Assoc* 10:e018773. <https://doi.org/10.1161/jaha.120.018773>
- Shinozaki K, Okuma Y, Saeki K et al (2021) A method for measuring the molecular ratio of inhalation to exhalation and effect of inspired oxygen levels on oxygen consumption. *Sci Rep* 11(1):12815. <https://doi.org/10.1038/s41598-021-91246-8>
- Okuma Y, Shinozaki K, Yagi T et al (2022) Oxyhaemoglobin level measured using near-infrared spectrometer is associated with brain mitochondrial dysfunction after cardiac arrest in rats. *Adv Exp Med Biol* 1395:385–390. https://doi.org/10.1007/978-3-031-14190-4_63
- Okuma Y, Shinozaki K, Yagi T et al (2019) Combination of cardiac and thoracic pump theories in rodent cardiopulmonary resuscitation: a new method of three-side chest compression. *Intensive Care Med Exp* 7(1):62. <https://doi.org/10.1186/s40635-019-0275-9>
- Wood LD, Bryan AC, Bau SK, Weng TR, Levison H (1976) Effect of increased gas density on pulmonary gas exchange in man. *J Appl Physiol* 41(2):206–210. <https://doi.org/10.1152/jappl.1976.41.2.206>
- Kallet RH, Matthay MA (2013) Hyperoxic acute lung injury. *Respir Care* 58(1):123–141. <https://doi.org/10.4187/respcare.01963>
- Lius EE, Syafaah I (2022) Hyperoxia in the management of respiratory failure: a literature review. *Ann Med Surg (Lond)* 81:104393. <https://doi.org/10.1016/j.amsu.2022.104393>

Prefrontal Cortex Oxygenation During Walking in an Acute Ischaemic Stroke Patient with Severe Internal Carotid Artery Stenosis: A Case Report

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Abstract

This study investigated the oxygenation in the prefrontal cortex (PFC) during walking in a 75-year-old male patient in the acute phase of a left frontal lobe cerebral infarction complicated by severe left internal carotid artery stenosis. The patient regained independent ambulation on the fifth day after the onset of symptoms, and the study was conducted on the eighth day after the onset of symptoms. The

patient rested for 10 s before walking for 70 s. Then he rested for 60 s. The levels of oxyhaemoglobin (O₂Hb), deoxyhaemoglobin (HHb), and total haemoglobin (THb) in the PFC were quantified during the rest and walking phases using a wearable near-infrared spectroscopy device. Comparative analysis with baseline values during rest revealed decreases in the O₂Hb, HHb, and THb in both hemispheres of the PFC during walking (O₂Hb: $-19.76/-14.88$ $\mu\text{mol/L}$, HHb: $-1.18/-2.00$ $\mu\text{mol/L}$, THb: $-20.96/-16.88$ $\mu\text{mol/L}$, right/left, respectively). The recovery of the O₂Hb was delayed by 15.8 seconds in the affected hemisphere. These findings support the effectiveness of wearable NIRS for the evaluation of cerebral oxygenation during rehabilitation in patients with acute stroke to facilitate evaluations and individualise patient care.

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Keywords

Internal carotid artery stenosis · Prefrontal
cortex · Oxyhaemoglobin · Walking · Acute
stroke · Near-infrared spectroscopy

1 Introduction

Near-infrared spectroscopy (NIRS) is used to assess variations in blood flow and oxygen metabolism in biological tissues [1]. A wearable NIRS device has been used to evaluate cerebral oxygenation and deoxygenation during activity in clinical settings. A previous study reported that the concentration of oxyhaemoglobin (O_2Hb) in the prefrontal cortex (PFC), measured using NIRS, increased during walking in healthy individuals and patients with chronic stroke [2]. However, studies regarding patients with acute stroke are limited.

A previous study reported that 76% of patients with acute ischaemic stroke have angiographic evidence of complete occlusion [3]. Atherosclerotic plaques can rupture and cause thrombosis or embolism, while carotid artery involvement increases the risk of stroke [4]. PFC oxygenation measured using NIRS is reduced in patients with carotid artery stenosis [5]; however, few studies have reported the oxygenation of the PFC during activity. Therefore, an understanding of the cerebral oxygenation during activity in patients with acute stroke and internal carotid artery stenosis is useful to determine the unique responses and their significance in this patient population.

In this study, the oxygenation of the PFC during walking was monitored in a patient with acute stroke and severe internal carotid artery stenosis using a wearable NIRS device to detect changes in cerebral oxygenation during walking in this patient population.

2 Methods

The patient provided informed consent for the publication of this report. All privacy protection measures regarding the potential future disclosure of the data in academic conferences or research publications were taken.

2.1 History of Present Illness

A 75-year-old male with chronic kidney disease and hypertension experienced an acute cerebral infarction in the left frontal lobe (National Institutes of Health Stroke Scale score: 1) (Fig. 1a). Magnetic resonance imaging also revealed severe left internal carotid artery stenosis (peak systolic velocity = 600 cm/s) (Fig. 1b). Additionally, single-photon emission computed tomography imaging revealed mild hypoperfusion in the left cerebral hemisphere compared to the right hemisphere (Fig. 1c). The patient was admitted for the treatment and prevention of recurrent stroke. The patient-initiated walking exercises with activity restrictions five days after the onset of symptoms. A walking assessment was conducted seven days after the onset of symptoms, and the oxygenation was measured eight days after the onset of symptoms. The patient was discharged nine days after the onset of symptoms and later underwent left carotid artery stent placement.

2.2 Experimental Procedure

The patient rested for approximately 10 s before walking at a comfortable pace for 70 s then resting for 60 s. The walking speed (m/s), cadence (steps/s), and stride length (m) were used to assess the walking performance. The systolic and diastolic blood pressures (mmHg), pulse (bpm), percutaneous oxygen saturation (%), and rate of perceived fatigue were assessed using the Borg scale.

Measurements of O_2Hb ($\mu\text{mol/L}$), deoxyhaemoglobin (HHb, $\mu\text{mol/L}$), and total haemoglobin (THb, $\mu\text{mol/L}$) in the PFC during walking and rest were obtained using a wearable continuous wave-NIRS device (Brain Activity Monitor Hb133, Astem, Kawasaki City, Japan). The NIRS optode locations were determined using the international 10–20 system at Fp1 and Fp2. The inter-channel distances were 30 and 35 mm, corresponding to the distance between the source and detector. The near-infrared light had wave-

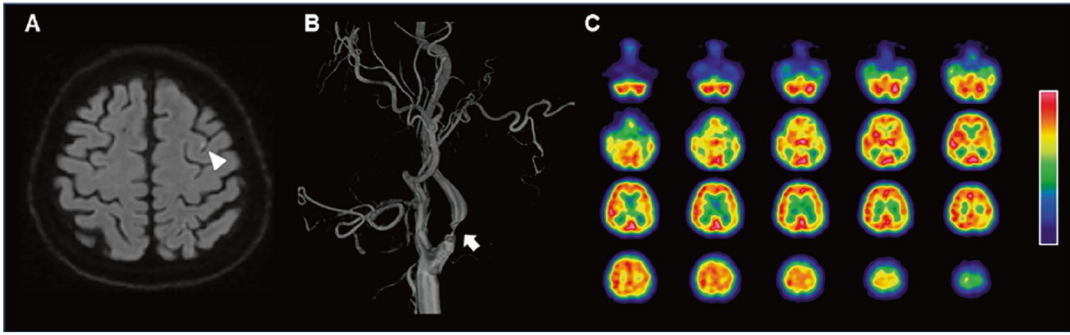


Fig. 1 Diffusion-weighted imaging (a), 3D rotational angiography (b) and Single photon emission computed tomography imaging (c) on the day of admission are shown. The triangle in panel A represents subtle acute infarction in the left superior frontal gyrus, while the

arrow in panel B indicates severe stenosis of the left internal carotid artery. In addition, on panel C, decreased blood flow in the left cerebral hemisphere compared to the right hemisphere can be observed

lengths of 760 and 850 nm, and the sampling rate was set at 1.0 Hz.

2.3 Analysis of Haemoglobin Levels

The haemoglobin values during walking were computed as the difference between the mean baseline values at rest before walking and the mean values during 6–67 s of walking.

Changes in the haemoglobin values during walking were determined as the difference between the mean values between 7–37 s of walking (early period) and from 37–67 s of walking (late period).

The time required for the O_2Hb to return to baseline from the point of maximum deviation was also determined.

3 Results

The patient's walking speed was 1.60 m/s, cadence was 2.17 steps/s, and stride length was 0.74 m. Before the walking task, systolic blood pressure was 154 mmHg, diastolic blood pressure was 92 mmHg, and pulse rate was 65 bpm. However, after the walking task, they were recorded as 164 mmHg, 92 mmHg, and 69 bpm, respectively.

The O_2Hb , HHb, and THb levels were reduced in both PFC regions after the initiation of walking compared to baseline levels (Figs. 2 and 3a). Specifically, O_2Hb gradually decreased upon initiation of walking and continued to decline until the end of the activity. During the late period of the walking task, the O_2Hb and THb levels continued to decrease in both PFC regions and the HHb levels were relatively unchanged (Fig. 3b). The time required for the O_2Hb to return to baseline values differed between the left (28.9 s) and right PFC (13.1 s) (Fig. 3c).

4 Discussion

This study assesses the cerebral oxygenation during walking in a patient with acute stroke and severe internal carotid artery stenosis using a wearable NIRS device. The oxygenation of both PFC regions decreased as the patient began walking, and the recovery of the decreased values was delayed, especially in the hemisphere affected by internal carotid artery stenosis.

Previous studies targeting older adults [6] have reported that individuals with lower walking abilities show an increase in O_2Hb in the left PFC with increasing walking speed, whereas those with higher walking abilities exhibit no significant association between walking speed and PFC O_2Hb . Additionally, a study involving stroke

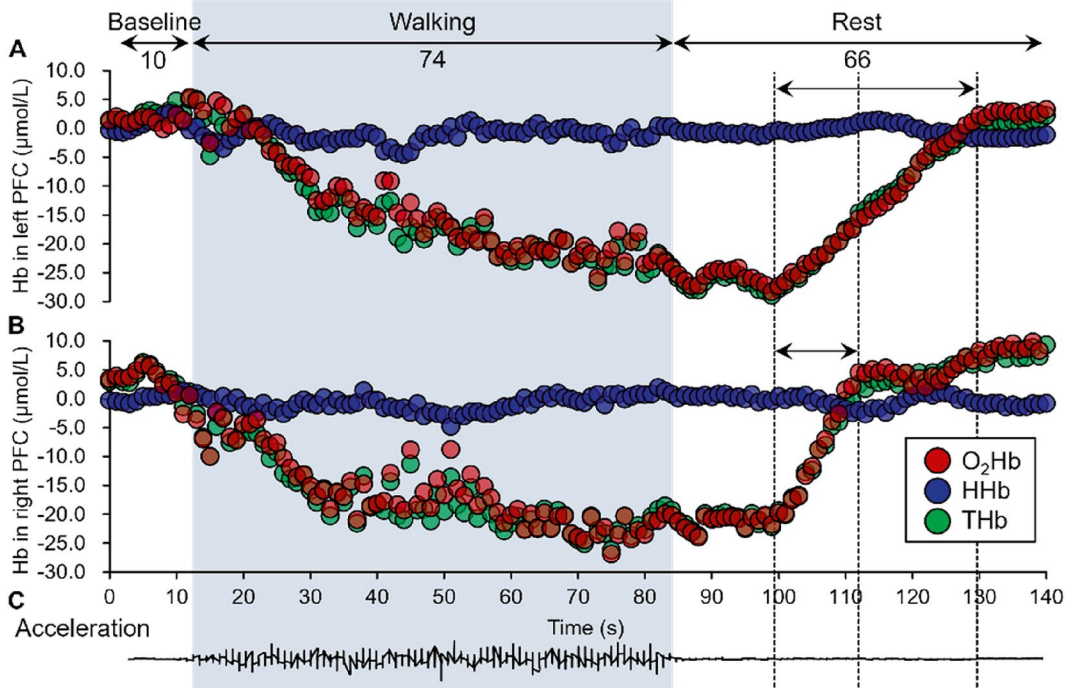


Fig. 2 Changes in haemoglobin concentration in the prefrontal cortex (PFC) (a: left, b: right) measured using near-infrared spectroscopy are shown. (c) The patient's acceleration during the walking task is shown. Abbreviations: *O₂Hb* oxyhaemoglobin, *HHb* deoxyhaemoglobin, *THb* total haemoglobin

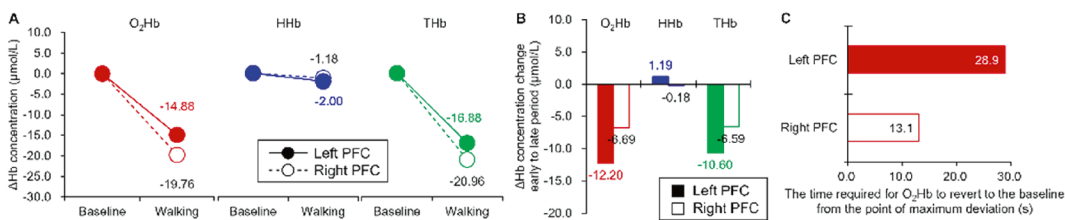


Fig. 3 Summary of changes in haemoglobin concentration during walking: The haemoglobin concentration during walking is compared to that at baseline (a). The haemoglobin concentrations of the early and late walking periods are compared (b). The time required to recover the oxyhaemoglobin concentration is shown (c). Abbreviations: *O₂Hb* oxyhaemoglobin, *HHb* deoxyhaemoglobin, *THb* total haemoglobin

patients [7] measured PFC O₂Hb during comfortable walking, slow walking, and fast walking, finding no significant differences in O₂Hb based on walking speed. The participant in our study had a comfortable walking speed of 1.60 m/s, indicating high walking ability, and no decrease in blood pressure was observed after walking. Therefore, considering the results of our study and the aforementioned previous studies, it is likely that the walking speed used in our study

has minimal impact on blood pressure and PFC O₂Hb.

In previous studies involving rats [8], it has been demonstrated that reducing cerebral blood flow results in a decrease in O₂Hb. In previous studies that included healthy adults [9, 10], the O₂Hb decreased immediately after the initiation of bicycle ergometer exercises and then gradually increased, suggesting a temporary reduction in cerebral blood flow immediately after exercise initiation, which may be due to the redirection of

blood flow to the active muscles. Clinical studies [11–13] targeting patients with internal carotid artery stenosis without stroke or transient ischaemic attacks have reported reduced O₂Hb levels in the hemisphere affected by stenosis during rest, breath-holding tasks to induce vasodilation, and Stroop tasks. The results of the current and previous studies suggest that internal carotid artery stenosis may affect the recovery of the O₂Hb levels after exercise.

This study had several limitations. First, this single-case study limits the generalisation of the results to patients with acute stroke. Second, the use of NIRS in this study focused only on the PFC, and the oxygenation variables of motor areas that are considered relevant when walking were not assessed. Additionally, the influence of potential collateral circulation in the hemisphere with carotid artery stenosis cannot be ruled out; however, addressing this specific factor is challenging within the scope of the current study. Finally, the wearable NIRS used in this study may have been influenced by cutaneous blood flow, which is also difficult to avoid.

In conclusion, the O₂Hb and THb of the PFC decreased during walking in a patient with acute stroke, and the restoration of the reduced O₂Hb in the PFC was delayed, especially in the affected hemisphere. Wearable NIRS devices are valuable for novel assessments and risk management as they allow the visualisation of cerebral oxygenation during the rehabilitation of patients with acute stroke.

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References

- Herold F, Wiegel P, Scholkmann F, Müller NG (2018) Applications of functional near-infrared spectroscopy (fNIRS) neuroimaging in exercise-cognition science: a systematic, methodology-focused review. *J Clin Med* 22(7):466. <https://doi.org/10.3390/jcm7120466>
- Bishnoi A, Holtzer R, Hernandez ME (2021) Brain activation changes while walking in adults with and without neurological disease: systematic review and meta-analysis of functional near-infrared spectroscopy studies. *Brain Sci* 26(11):291. <https://doi.org/10.3390/brainsci11030291>
- Fieschi C, Argentino C, Lenzi GL, Sacchetti ML, Toni D, Bozzao L (1989) Clinical and instrumental evaluation of patients with ischemic stroke within the first six hours. *J Neurol Sci* 91:311–321. [https://doi.org/10.1016/0022-510x\(89\)90060-9](https://doi.org/10.1016/0022-510x(89)90060-9)
- Eliasziw M, Streifler JY, Fox AJ, Hachinski VC, Ferguson GG, Barnett HJ (1994) Significance of plaque ulceration in symptomatic patients with high-grade carotid stenosis. North American symptomatic carotid endarterectomy trial. *Stroke* 25:304–308. <https://doi.org/10.1161/01.str.25.2.304>
- Mishra RK, Salihundam DC, Surve RM (2023) Normalization of low frontal regional cerebral oxygenation after evacuation of chronic subdural hematoma in a patient with bilateral internal carotid artery stenosis. *J Clin Monit Comput* 37:925–928. <https://doi.org/10.1007/s10877-022-00943-w>
- Harada T, Miyai I, Suzuki M, Kubota K (2009) Gait capacity affects cortical activation patterns related to speed control in the elderly. *Exp Brain Res* 193(3):445–454. <https://doi.org/10.1007/s00221-008-1643-y>
- Lim SB, Peters S, Yang CL, Boyd LA, Liu-Ambrose T, Eng JJ (2023) Premotor and posterior parietal cortex activity is increased for slow, as well as fast walking poststroke: an fNIRS study. *Neural Plast* 2023(13):2403175. <https://doi.org/10.1155/2023/2403175>
- Hoshi Y, Kobayashi N, Tamura M. Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *J Appl Physiol* (1985). 2001;90(5):1657–1662. <https://doi.org/10.1152/jappl.2001.90.5.1657>
- Tsubaki A, Takai H, Kojima S, Miyaguchi S, Sugawara K, Sato D et al (2016) Changes in cortical oxyhaemoglobin signal during low-intensity cycle ergometer activity: a near-infrared spectroscopy study. *Adv Exp Med Biol* 876:79–85. https://doi.org/10.1007/978-1-4939-3023-4_10
- Tsubaki A, Takehara N, Sato D, Morishita S, Tokunaga Y, Sugawara K et al (2017) Cortical oxyhemoglobin elevation persists after moderate-intensity cycling exercise: a near-infrared spectroscopy study. *Adv Exp Med Biol* 977:261–268. https://doi.org/10.1007/978-3-319-55231-6_36
- Hiura M, Nariai T, Takahashi K, Muta A, Sakata M, Ishibashi K et al (2018) Dynamic exercise elicits dissociated changes between tissue oxygenation and cerebral blood flow in the prefrontal cortex: a study using NIRS and PET. *Adv Exp Med Biol* 1072:269–274. https://doi.org/10.1007/978-3-319-91287-5_43

12. Forero J, Novi L, Avelar M, Anjos A, Menko G, Forti RM et al (2017) Use of near-infrared spectroscopy to probe occlusion severity in patients diagnosed with carotid atherosclerotic disease. *Med Res Arch* 5(6)
13. Mason SA, Al Saikhan L, Jones S, James SN, Murray-Smith H, Rapala A et al (2022) Association between carotid atherosclerosis and brain activation patterns during the Stroop task in older adults: an fNIRS investigation. *NeuroImage* 15(257):119302. <https://doi.org/10.1016/j.neuroimage.2022.119302>

Relationship Between Regional Cerebral Oxygen Saturation and Percutaneous Oxygen Saturation at Initial Mobilisation in Patients with Acute Heart Failure

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Abstract

In patients with acute heart failure, early mobilisation is performed according to physical condition, biometric information, and haemodynamic stability. Regional cerebral oxygen saturation (rSO₂) is useful in monitoring patients during assisted circulation and cardiac arrest, but the measurement of rSO₂ during early mobilisation is not standardised, and its relationship with percutaneous oxygen saturation (SpO₂) has not been investigated.

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This study aimed to investigate the relationship between rSO₂ and SpO₂ during initial mobilisation in patients with acute heart failure. Patients with acute heart failure who presented to our emergency centre and were referred for physical therapy were included in this study. Data from 21 of the 24 patients who decided to start mobilisation based on the criteria of the Japanese Society of Intensive Care Medicine were analysed. The resting rSO₂ was 58.6%, and it significantly changed ($p < 0.001$), with a decrease in the first half of sitting on the edge of the bed (SEB) and an increase from the second half to the end of the SEB. The resting SpO₂ was 96.0%, with a trend towards an increase in the latter half of the SEB ($p = 0.05608$). Changes in rSO₂ during initial mobilisation in patients with acute heart failure differed from those in SpO₂.

Keywords

Acute heart failure · Regional cerebral oxygen saturation · Percutaneous oxygen saturation · Rehabilitation · Early mobilisation

1 Introduction

In the acute phase of heart failure, treatments such as oxygenation and drug therapy are selected according to the pathological condition, using blood pressure and percutaneous oxygen saturation (SpO_2) as indicators to improve the pathological condition. In early mobilisation, the criteria for discontinuation and progression are established and monitored according to respiratory status, haemodynamics, consciousness, and subjective symptoms [1–4]. However, information in the brain is evaluated based on the level of consciousness and not according to objective indices.

The regional cerebral oxygen saturation (rSO_2) measured by near-infrared spectroscopy (NIRS) is the oxygen saturation in a specific brain region. It is known to be useful in patients using assisted circulation in the perioperative period [5] and in cardiac arrest patients whose SpO_2 is difficult to measure [6]. The rSO_2 in patients with compensated heart failure correlates with well-known prognostic factors, suggesting its potential prognostic value [7].

To date, rSO_2 has not been evaluated as an indicator of cerebral circulation during mobilisation, and its relationship with common vital signs, such as SpO_2 and mean arterial pressure (MAP), has not been investigated. This study aimed to investigate the relationship between rSO_2 and SpO_2 during initial mobilisation in patients with acute heart failure.

2 Methods

2.1 Participants

Patients with acute heart failure who presented to our emergency centre between June 2020 and January 2023 and were referred for physical therapy were included in this study. Of the 24 patients who decided to start mobilisation based on the criteria of the Japanese Society of Intensive Care Medicine [4], we excluded one patient with an incompatible NIRS probe and two patients who were displaced during bed release; finally, 21

patients who could be analysed were included. Basic patient information was collected from medical records. Data were collected after obtaining informed consent from patients or their families. Ethical approval was obtained from the Ethics Committee of Uonuma Kikan Hospital (approval no. 02-006).

2.2 Measurements

In this study, “mobilisation” was defined as rehabilitation at sitting on the edge of the bed (SEB) or higher [8]. The sequence of the procedure was as follows: back-lying position, elevation of the head of the bed (EHOB) at 45° , SEB, and back-lying position, with EHOB at 45° and SEB, each lasting approximately 3 min. Patients relaxed in bed for 5 min before the start of the exercise, and the head and trunk sway was minimised during the exercise.

Wearable brain NIRS (Brain Activity Monitor Hb133; Astem, Kanagawa, Japan) was used to measure rSO_2 . A dedicated head mount was worn during the initial mobilisation, attached such that the probes were in close contact with FP1 and FP2 using the international 10–20 method, and we measured rSO_2 continuously throughout the experiments. The sampling rate was 0.1 seconds. The measured data of FP1 and FP2 were averaged, and the average values of 1 min before and after EHOB 45° , 1 min before and after SEB, and 1 min before and after the end of SEB were used. At the same time, SpO_2 and MAP were measured using a biometric monitor (IntelliVue X2; Philips, Amsterdam, the Netherlands) and recorded at six time points within 1 minute before and after the postural change.

2.3 Statistical Analysis

Temporal changes in rSO_2 , SpO_2 , and MAP during mobilisation were compared using a two-way repeated-measures analysis of variance and using Shaffer’s modified sequentially rejective Bonferroni procedure as a post-hoc test. Correlations between SpO_2 and rSO_2 and between

MAP and rSO₂ at six time points during mobilisation were investigated using Pearson’s correlation coefficient or Spearman’s rank correlation coefficient. All statistical analyses were performed using EZR in R Commander version 1.54 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $p < 0.05$.

3 Results

Patient backgrounds are shown in Table 1. The most common initial pathogenesis of acute heart failure on admission was clinical scenario (CS)1 in 14 patients (67%), CS2 in five patients (24%), and CS3 in two patients (9%). Valvular heart disease was the most common primary disease in nine patients (43%), followed by ischaemic heart disease in five patients (24%), hypertension in two patients (9%), congenital heart disease in one patient (5%), arrhythmia in three patients (14%), and other disease in one patient (5%).

The changes in rSO₂ and SpO₂ during initial mobilisation showed a significant decrease in rSO₂ from rest to the first half of SEB ($p < 0.05$; Fig. 1a), an increase from the first half to the end

of SEB ($p < 0.05$; Fig. 1a), while SpO₂ showed no significant change ($p = 0.056$; Fig. 1b). The MAP did not change significantly with postural changes (Fig. 2). The SpO₂ and rSO₂ values and MAP and rSO₂ values showed no significant correlation at any stage of bed rest (Tables 2 and 3).

4 Discussion

In this study, we compared the rSO₂ measured using NIRS during initial mobilisation in patients with acute heart failure with SpO₂, a representative index for determining oxygenation status in clinical practice. The rSO₂ at the time of initial mobilisation was different from the change in SpO₂, did not correlate with the phase, and differed from the change in MAP.

Regarding the different changes in SpO₂ and rSO₂, Zhou et al. stated that in cases of severe brain injury or under relatively low haemoglobin concentrations, SpO₂ was normal (>90%), whereas rSO₂ was significantly lower. They also reported that SpO₂ and rSO₂ recovery did not occur in the same manner when the disease was relieved [9]. In addition, a report on a patient on one-lung ventilation during thoracic surgery who underwent an intervention to maintain peripheral oxygen saturation >90% showed no significant correlation with cerebral tissue oxygen saturation [10]. Wood et al. reported that blood pressure was not associated with rSO₂ as a determinant of rSO₂ in critically ill patients who required shock or ventilator management [11].

The rSO₂ in the acute phase of the disease may fluctuate independently of interventions to maintain organ blood flow if the MAP and SpO₂ are preserved. In acute heart failure, patients are treated similarly to maintain proper circulatory and respiratory status, and their general vital signs are under proper control when they are deemed capable of mobilisation. Therefore, rSO₂ is considered to show changes that are different from those of general vital signs such as SpO₂ and MAP. The variation in rSO₂ may be related to the patient’s condition and treatment status, but further investigation is needed to determine the effects of this variation in rSO₂.

Table 1 Patient characteristics (n = 21)

Age (years)	83.9 ± 7.0
Sex (female)	8 (38)
BMI (kg/m ²)	22.0 ± 3.5
Days to initial mobilisation (days)	1.9 ± 1.8
ICU stay (days)	4.4 ± 4.2
Hb (g/dL)	11.1 ± 2.0
APACHE II at ICU admission	12.1 ± 3.1
Oxygenation	10 (48)
FiO ₂	0.25 ± 0.06
Intubation history	4 (19)
Catecholamine use history	4 (19)
Usage of vasodilating agents	17(81)
LVEF (%)	50.6 ± 13.9
BNP (pg/mL)	935.1 ± 727.5

Values are presented as mean ± standard deviation or n (%). BMI body mass index, ICU intensive care unit, Hb haemoglobin, APACHEII Acute Physiology and Chronic Health Disease Classification System II, FiO₂ fraction of inspiratory oxygen, LVEF left ventricular ejection fraction, BNP brain natriuretic hormone

Fig. 1 Comparison of the temporal changes in rSO₂ and SpO₂. **(a)** The rSO₂ at initial mobilisation showed a significant change ($p < 0.001$), decreasing in the first half of SEB ($p < 0.05$) and increasing from the first half of SEB to the end of SEB ($p < 0.05$). **(b)** SpO₂ showed no significant change in postural change. rSO₂ regional cerebral oxygen saturation, SpO₂ percutaneous oxygen saturation, MAP mean arterial pressure, EHOB45° elevation of the head of the bed 45°, * $p < 0.05$ post-hoc test

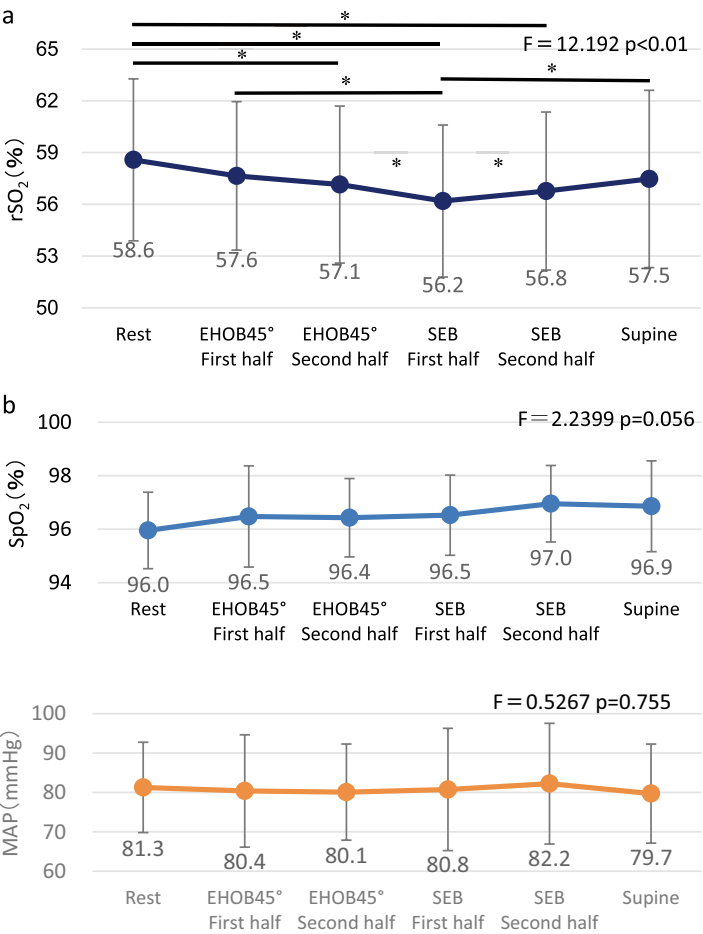


Fig. 2 Temporal changes in MAP. MAP showed no significant change in postural change. MAP mean arterial pressure, EHOB45° elevation of the head of the bed 45°

Table 2 Correlation between rSO₂ and SpO₂

	<i>r</i>	<i>p</i> value
Rest (supine)	0.00906	0.969
First half of EHOB45°	−0.311	0.171
Second half of EHOB45°	−0.266	0.244
First half of SEB	0.0937	0.686
Second half of SEB	−0.0912	0.694
Supine	−0.0355	0.879

EHOB45° elevation of the head of the bed 45°, SEB sitting on the edge of the bed

Table 3 Correlation between rSO₂ and MAP

	<i>r</i>	<i>p</i> value
Rest (supine)	0.277	0.224
First half of EHOB45°	0.34	0.132
Second half of EHOB45°	0.358	0.111
First half of SEB	0.241	0.292
Second half of SEB	0.222	0.332
Supine	0.207	0.368

EHOB45° elevation of the head of the bed 45°, SEB sitting on the edge of the bed

A limitation of this study is that the NIRS signals were greatly affected by extracranial skin blood flow [12], and it was difficult to exclude these effects. In addition, owing to the

nature of mobilisation in a clinical setting, it was difficult to avoid talking to the patient, and body movements could not be completely controlled.

5 Conclusion

The rSO₂ changes during mobilisation in patients with acute heart failure were different from those in SpO₂, which is commonly used for monitoring. rSO₂ may be useful as an independent vital sign and a simple index for monitoring cerebral circulation during bed release. Future studies are needed to investigate the effects of rSO₂ changes during mobilisation.

References

1. Adler J, Malone D (2012) Early mobilization in the intensive care unit: a systematic review. *Cardiopulm Phys Ther J* 23:5–13
2. Hodgson CL, Stiller K, Needham DM et al (2014) Expert consensus and recommendations on safety criteria for active mobilization of mechanically ventilated critically ill adults. *Crit Care* 18:658
3. Pohlman MC, Schweickert WD, Pohlman AS et al (2010) Feasibility of physical and occupational therapy beginning from initiation of mechanical ventilation. *Crit Care Med* 38:2089–2094
4. Ad Hoc Committee for Early Rehabilitation, The Japanese Society of Intensive Care Medicine (2017) The Japanese Society of Intensive Care Medicine: evidence-based expert consensus for early rehabilitation in the intensive care unit. *J JPN Soc Intensive Care Med* 24:255–303
5. Yu Y, Zhang K, Zhang L, Zong H, Meng L, Han R (2018) Cerebral near-infrared spectroscopy (NIRS) for perioperative monitoring of brain oxygenation in children and adults. *Cochrane Database Syst Rev* 1:CD010947
6. Wik L (2016) Near-infrared spectroscopy during cardiopulmonary resuscitation and after restoration of spontaneous circulation: a valid technology? *Curr Opin Crit Care* 22:191–198
7. Chen Y-J, Wang J-S, Hsu C-C, Lin P-J, Tsai F-C, Wen M-S, Kuo C-T, Huang S-C (2018) Cerebral desaturation in heart failure: potential prognostic value and physiologic basis. *PLoS One* 13:e0196299
8. Liu K, Shibata J, Fukuchi K, Takahashi K, Sonoo T, Ogura T, Goto T (2022) Optimal timing of introducing mobilization therapy for ICU patients with sepsis. *J Intensive Care* 10:22
9. Zhou C-L, Liu Y-F, Zhang J-J et al (2009) Measurement of brain regional oxygen saturation in neonates in China: a multicenter randomized clinical trial. *Zhonghua Er Ke Za Zhi* 47:517–522
10. Hemmerling TM, Bluteau MC, Kazan R, Bracco D (2008) Significant decrease of cerebral oxygen saturation during single-lung ventilation measured using absolute oximetry. *Br J Anaesth* 101:870–875
11. Wood MD, Jacobson JA, Maslove DM, Muscedere JG, Boyd JG, Cerebral Oxygenation and Neurological Outcomes Following Critical Illness (CONFOCAL) Research Group (2019) The physiological determinants of near-infrared spectroscopy-derived regional cerebral oxygenation in critically ill adults. *Intensive Care Med Exp* 7:23
12. Takahashi T, Takikawa Y, Kawagoe R, Shibuya S, Iwano T, Kitazawa S (2011) Influence of skin blood flow on near-infrared spectroscopy signals measured on the forehead during a verbal fluency task. *NeuroImage* 57:991–1002

Relevance Between Reduction of SpO₂ and Parasympathetic Nervous Activity During Sleep

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Abstract

Sleep apnea syndrome (SAS) is associated with cardiopulmonary and dental nasopharyngeal diseases. The blood oxygen saturation (SpO₂) detected using pulse oximetry is a diagnostic screening method for SAS. SAS severity is assessed using the oxygen desaturation index (ODI), which measures the number of times the SpO₂ decreases by more than 3% for longer than 10 s. This study investigated the association between reduced SpO₂ and parasympathetic nervous system activity (PSNA) during night sleep in young adults ($n = 63$; aged 20–32 years). Changes in PSNA were measured every minute for 24 h during a free-moving day using an ActiveTracer accelerometer. Pulse oximetry was performed simultaneously during sleep. All participants

had significantly lower PSNA when the SpO₂ decreased by 3% or more than when it did not. There were no significant differences in PSNA when an ODI cutoff of 5 events/h was used. However, participants with an ODI >5 events/h had significantly lower PSNA during normal SpO₂ than those with an ODI <5 events/h, suggesting an association between SpO₂ desaturation and PSNA decline during sleep. A high ODI may indicate reduced PSNA levels during sleep, affecting sleep efficiency. Treatment aimed at reducing the ODI may improve sleep quality, even in young adults.

Keywords

Blood oxygen saturation · Oxygen desaturation index · Parasympathetic nervous activity · Sleep apnea syndrome · Sleep

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1 Introduction

Sleep apnea syndrome (SAS) is a major sleep-related breathing disorder causing generalised hypoxemia. When not treated properly, SAS negatively affects daytime functioning and increases the risk of motor accidents and cardiovascular events. SAS is typically diagnosed using the apnoea-hypopnea index, which is the average number of apnoea-hypopnea events per hour during sleep, categorised as mild (5–15 events/h),

moderate (15–30 events/h), or severe (30 or more events/h) [1]. Blood oxygen saturation (SpO_2) during sleep, measured using pulse oximetry, is a screening test for SAS. A drop in SpO_2 begins approximately 10–30 s after the onset of an apnoeic episode and recovers shortly after the hypoventilation ends [2]. The oxygen desaturation index (ODI) is the average number of times the blood oxygen level decreases by at least 3% for more than 10 s during 1 h of night sleep and is comparable to the apnea-hypopnea index [3].

Sleep efficiency is associated with autonomic nervous system activity, especially parasympathetic nervous system activity (PSNA). Several studies have reported that the autonomic nervous system (ANS) activity and PSNA levels change during sleep [4–6]. Heart rate variability (HRV) is a non-invasive parameter used to assess beat-to-beat intervals and the ANS activity. The PSNA is calculated using the high- and low-frequency ranges of the HRV spectrum: normalised high frequency (HFnu) = $\text{high}/(\text{high} + \text{low})$ frequency [7]. The HFnu increases during sleep and decreases after awakening.

SAS is an age-related disease. The prevalence of SAS increases with age and plateaus at approximately 60 years [1]. A few studies regarding SAS in young adults have been reported.

A previous study reported that the lowest oxygen desaturation events are drivers of increased sympathetic modulation and decreased PSNA in patients with SAS [8]. Apnea-hypopnea events almost always cause arousal events on electroencephalograms. However, few studies regarding the relationship between PSNA and night sleep quality in younger participants have been reported. Therefore, this study investigates the association between reduced SpO_2 and PSNA during nocturnal sleep in young adults without any specific diseases.

2 Methods

2.1 Participants

Sixty-three young adults (age range: 20–32 years; mean age: 21.7 ± 1.6 years old; 35 males and 28 females) with no specific diseases volunteered

for this study. The participants were asked not to drink alcohol on the day of the study.

2.2 Protocol

The participants arrived at the laboratory at approximately 13:00 and underwent electrocardiography. A portable accelerometer monitor (ActiveTracer AC301; GMS Inc., Tokyo, Japan) was used to record the physical acceleration and R-R intervals for 24 h. During monitoring, the participants were instructed to avoid bathing but to continue otherwise with their daily routines. They were fitted with a portable pulse oximetry device (WEC-7201; Nihon Kohden Co. Ltd., Tokyo) before night sleep that was removed after awakening at home. The times at which the participants fell asleep and woke up were estimated based on the changes in body position recorded by the ActiveTracer.

2.3 Oxygen Desaturation Index

SpO_2 was recorded via pulse oximetry. SpO_2 data were obtained every second. The ODI was calculated as the average number of desaturation episodes $\geq 3\%$ that lasted for more than 10 s during 1 h of night sleep. ODI was used to categorise SAS severity as mild (5–15 events/h), moderate (15–30 events/h), or severe (30 or more events/h) [1, 3]. SpO_2 desaturation was defined as a 3% or more decrease in SpO_2 . Decreases of SpO_2 that were $< 3\%$ were considered normal.

2.4 HRV Analysis

An ActiveTracer equipped with a triaxial accelerometer (72 g) was used to measure HRV [9, 10]. An accelerometer was placed at the anterior midline of the waist above the umbilicus to avoid disturbances in sleep or free movement. The spectral analysis of the HRV was performed at 1-min intervals using maximal entropy combined with the least squares method (MEMCalc System; Suwa Trust Co., Ltd., Tokyo, Japan), and the data were then separated into high- and low-frequency

ranges for power analysis [4]. Low frequency (0.04–0.15 Hz) and high frequency (0.15–0.40 Hz) bands were analysed as HRV parameters [4]. In this study, PSNA was defined as a normalised high frequency (HFnu) = high frequency/ (low frequency + high frequency) [7]. Although a 1-min analysis of HRV has less reliability than the 5-min analysis, the 1-min HRV analysis was used in this study based on the mobile settings described in a previous study [11]. HRV and SpO₂ data were collected simultaneously.

2.5 Statistical Analysis

Data are expressed as mean $\pm$ standard deviation. The data were compared using Student's t-test. Statistical significance was set at $p < 0.05$.

2.6 Ethics

This study was approved by the ethics committee of Nagahama Institute of Bio-Science and Technology (approval number: 006). All participants provided written informed consent prior to their participation in the study.

3 Results

Eleven participants had an ODI >5 events/h, and 52 participants had an ODI <5 events/h (Table 1). Participant age, sleeping hours, nocturnal awakening times, heart rate, and physical acceleration during sleep were not significantly different between the high- and low-ODI groups. However, the high-ODI group had significantly higher body mass index than the low-ODI group ($p < 0.05$).

The HFnu was significantly lower in all participants when the SpO₂ was decreased $\geq 3\%$ than when it was not. There were no significant differences between the high- and low-ODI groups when the SpO₂ decreased by $\geq 3\%$. However, when the SpO₂ was normal, the high-ODI group had a significantly lower HFnu than the low-ODI group ($p < 0.05$) (Fig. 1).

Table 1 Participant characteristics

	<5 events/h (low)	>5 events/h (high)
ODI		
N	52	11
Age (years)	21.9 $\pm$ 2.1	21.9 $\pm$ 1.1
Body mass index (kg/m ²)	21.0 $\pm$ 2.2	23.3 $\pm$ 2.9*
Sleeping time (min)	361 $\pm$ 70	358 $\pm$ 89
Nocturnal awakening times (n)	1.0 $\pm$ 0.6	1.3 $\pm$ 1.3
Heart rate during sleep (bpm)	58.4 $\pm$ 7.3	59.9 $\pm$ 6.3
Physical acceleration during sleep (mG)	1.7 $\pm$ 1.1	2.5 $\pm$ 1.4

* $p < 0.05$ high ODI vs low ODI

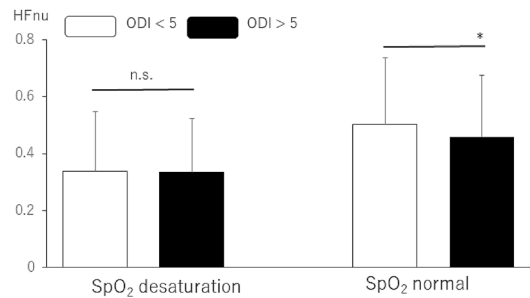


Fig. 1 HFnu with or without SpO₂ desaturation. Abbreviations: ODI oxygen desaturation index, SpO₂ normal SpO₂ did not decrease by 3%, SpO₂ desaturation SpO₂ decreased by $\geq 3\%$. * $p < 0.05$, high ODI vs. low ODI; n.s. $p > 0.05$, high ODI vs. low ODI

4 Discussion

This study determined the association between SpO₂ and PSNA in young adults. Participants with a high ODI had significantly lower HFnu during night sleep when the SpO₂ was normal than participants with a low ODI.

Obesity is the strongest risk factor for SAS as it is most likely related to upper airway narrowing caused by adipose tissue [12]. In this study of young adults, participants with a high ODI had a significantly higher body mass index. Therefore, decreasing the BMI may help prevent SAS and improve sleep efficiency even in young adults.

A previous study showed that the ODI was strongly correlated with decreased PSNA in an ultrashort-term HRV analysis [13]. Another study reported that arousal events caused by apnea-hypopnea were significantly correlated with

HFnu [14]. In this study, the high-ODI group tended to have lower PSNA levels during sleep, and PSNA was significantly lower in the high-ODI group than in the low-ODI group when the SpO₂ was not reduced $\geq 3\%$. This association between PSNA and SpO₂ desaturation was present in all patients, regardless of ODI. The PSNA did not recover when the SpO₂ was normal in patients with a low ODI.

SAS is associated with increased catecholamine levels [15]. Catecholamines play fundamental roles in the regulation of physiological processes. Norepinephrine is a neuromodulator of the peripheral sympathetic nervous system [15]. This relationship may help explain the results of the current study, though more research is necessary to clarify these relationships.

This study is not without limitations. First, the degree of oxygen desaturation was not significantly correlated with the HRV parameters. This may be due to the fact that no patients with severe SAS and few patients with moderate SAS were included in this study and that the degree of oxygen desaturation was approximately 3–5%. However, more studies are needed to clarify this relationship. Second, the exact times at which the participants fell asleep and woke up could not be determined without the use of electroencephalography.

This study identified a significant association between desaturation of SpO₂ and decreased PSNA during sleep. Even in young adults, high ODI scores decrease the HFnu and may decrease sleep efficiency. Treatments aimed at reducing the ODI may improve sleep quality in young adults.

References

1. American Academy of Sleep Medicine (2014) International classification of sleep disorders, 3rd edn. American Academy of Sleep Medicine; Darien, IL
2. Berry RB, Budhiraja R, Gotlib DJ et al (2012) Rules for scoring respiratory events in sleep: update of the 2007 AASM manual for the scoring of sleep and associated events. Deliberation of the sleep apnea definitions task force of the American academy of sleep medicine. *J Clin Sleep Med* 8:597–619
3. Peppard PE, Young T, Barnett JH, Palta M, Hagen EW, Hla KM (2013) Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol* 177:1006–1014
4. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 17:1053–1065
5. Taniguchi K, Shimouchi A, Jinno N, Okumura N, Seiyama A (2021) Parasympathetic nervous activity associated with discoordination between physical acceleration and heart rate variability in patients with sleep apnea. *Adv Exp Med Biol* 1269:229–234
6. Taniguchi K, Shimouchi A, Jinno N, Seiyama A (2021) Coordination between heart rate variability and physical activity may be diminished by fatigability in non-older women in the hour before sleep. *Physiol Rep* 9:e15126
7. Kleiger RE, Bigger JT, Bosner MS et al (1991) Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol* 68:626–630
8. Sukegawa M, Noda A, Sugiura T et al (2005) Assessment of continuous positive airway pressure treatment in obstructive sleep apnea syndrome using 24-hour urinary catecholamines. *Clin Cardiol* 28:519–522
9. Iwashita S, Takeno Y, Okazaki K et al (2003) Triaxial accelerometry to evaluate walking efficiency in older subjects. *Med Sci Sports Exerc* 35:1766–1772
10. Taniguchi K, Shimouchi A, Seki J et al (2015) Factors affecting coordination between heart rate variability and physical acceleration in daily lives of free-moving adults. *Adv Biomed Eng* 4:35–41
11. Salahuddin L, Cho J, Jeong MG et al (2007) Ultra short term analysis of heart rate variability for monitoring mental stress in mobile settings. *Annu Int Conf IEEE Eng Med Biol Soc* 2007:4656–4659
12. Lévy P, Kohler M, McNicholas WT et al (2015) Obstructive sleep apnoea syndrome. *Nat Rev Dis Primers* 1:15015
13. Seung SH, Dong KK (2022) Diagnostic efficacy of ultra-short term HRV analysis in obstructive sleep apnea. *J Pers Med* 12:1494
14. Xuehao G, Leidan H, Liu X et al (2021) Correlation analysis between polysomnography diagnostic indices and heart rate variability parameters among patients with obstructive sleep apnea hypopnea syndrome. *PLoS One* 11:e0156628
15. Bratell T, Wennlund A, Carlstrom K (1999) Pituitary reactivity, androgens and catecholamines in obstructive sleep apnoea. Effects of continuous positive airway pressure treatment (CPAP). *Respir Med* 93:1–7

Therapeutic Potentials of Near-Infrared II Photobiomodulation to Treat Cerebrovascular Diseases via Nitric Oxide Signalling

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Abstract

Endothelial dysfunction featuring insufficient endothelial nitric oxide synthase (eNOS) and accompanying nitric oxide (NO) deficiency is implicated in the pathogenesis of cardiovascular diseases. Restoring endothelial NO repre-

sents a promising approach to treating cerebrovascular diseases, including stroke. Low-power near-infrared (NIR) light shows diverse beneficial effects, broadly defined as photobiomodulation (PBM). The literature reports that PBM increases bioavailable NO. These lines of evidence indicate that PBM could be used to treat cerebrovascular diseases. Recent investigations revealed that PBM improved stroke outcomes in animal models via augmenting NO signalling and other pathways. However, clinical trials of PBM using NIR light in the NIR-I window (630–900 nm) have yet to demonstrate the beneficial effect of PBM on ischaemic stroke. Since NIR light in the NIR-II window (1000–1700 nm) with the largest penetration depth into tissues compared to NIR I has also been reported to augment NO bioavailability and cerebral blood flow ameliorating stroke injury, PBM using NIR-II light may be suitable for therapeutic use. This new non-pharmacological modality using a physical parameter of NIR-II laser could provide a new avenue for therapeutic strategies for cerebrovascular diseases. Since impaired NO production has been associated with neurological

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abnormalities, this novel therapeutic approach could be broadly explored to treat various disease conditions such as traumatic brain injury, stroke, and Alzheimer's disease. This review summarises recent findings on PBM in treating stroke and discusses its potential to treat other neurological diseases.

Keywords

Photobiomodulation · Endothelial nitric oxide synthase · Near-infrared II · Stroke · Meningeal lymphatics

1 Introduction

Endothelial dysfunction featuring insufficient endothelial nitric oxide synthase (eNOS) and accompanying nitric oxide (NO) deficiency is implicated in the pathogenesis of cardiovascular diseases [1]. NO maintains vascular homeostasis as a vasodilator and antithrombotic factor to induce disaggregation of pre-aggregated platelets [2]. Thus, restoring endothelial NO represents a promising approach to counter endothelial dysfunction in cerebrovascular diseases, including stroke. Low-power near-infrared (NIR) light shows diverse beneficial effects, broadly defined as photobiomodulation (PBM) [3]. The literature reports that PBM augmented NO bioavailability in situ via multiple mechanisms, including photo-dissociation of NO from cytochrome c oxidase (CoO), modulation of CoO by binding to heme a₃, and activation of NO-mediated signalling, NO release from intracellular stores, and eNOS phosphorylation at serine 1177 (S1177) in humans (S1176 in mouse sequence), which is a clinically important mechanism for regulating eNOS enzyme activity [4]. These lines of evidence indicate that PBM increases the release and production of NO and could treat cerebrovascular diseases. In this review, we summarise recent findings on PBM to treat stroke and discuss its potential to treat other neurological diseases.

2 Recent Progress on PBM to Treat Ischaemic Stroke

Stroke is a leading cause of morbidity and mortality worldwide [5]. The current standard management is fibrinolysis and endovascular therapies to establish reperfusion [5, 6]. However, these interventions are quite limited because they are only effective within 4.5–6 h of stroke onset [6]. New treatment modalities to overcome these limitations have been sought but with little success [6]. A novel strategy to significantly improve stroke outcomes is therefore highly desired. As a part of such efforts, PBM using NIR light in the NIR window I (630–900 nm) has been explored in animal models with encouraging results. In a rat stroke model of the middle cerebral artery occlusion (MCAO), PBM with 808 nm laser 24 h post-stroke improved neurological function [7, 8]. Remarkably, the improvements lasted for 4 weeks [8]. Since the NO signalling pathway is critical to preventing vascular constriction and platelet aggregation and protecting against stroke injury [9], the protective effect of PBM is linked to the NO signalling [10]. PBM with 808 nm laser post-hypoxic ischaemia or preconditioning before ischaemia could also prevent brain injuries by maintaining the mitochondrial function, decreasing oxidative stress, and inhibiting neuronal apoptosis in a model of neonatal hypoxic-ischaemic encephalopathy in rats [11, 12], suggesting other pathways than NO signalling are involved in the beneficial effect of PBM. Following these results, clinical trials to determine the therapeutic effect of PBM on stroke patients using the 810 nm NIR-I laser “Neurothera Effectiveness and Safety Trials” (NEST-1) [13], NEST-2 [14], and NEST-3 were initiated [15]. Although NEST-1 and NEST-2 found an improved outcome even when the treatment was performed within 24 h of stroke onset [13–15], a large-scale clinical trial of NEST-3 was terminated for futility [16], presumably due to the shallow light penetration and insufficient energy delivery into stroke regions, the too-long interval between stroke onset and treatment, insufficient treatment frequency, and not taking into account

the specific anatomical location of the stroke [10]. These clinical trial results represent room for optimisation of the PBM in stroke therapy. Amongst NIR wavelengths, NIR light in the NIR window II (1000–1700 nm) with reduced scattering and minimal tissue absorption shows the largest penetration depth into tissues compared to NIR I (630–900 nm) [17]. NIR-II light could be absorbed by the putative photoreceptor CoO and activated mitochondrial retrograde signalling [18], indicating its suitability for therapeutic use to treat cerebrovascular diseases, including stroke, with its optical properties to pass through scalp and skull tissues and reach brain tissue. Transcranial PBM (tPBM) using 1060–1080 nm light showed beneficial effects on neurocognitive functions in subjects with dementia [19] and cerebral blood flow (CBF) in older adults [20].

Recently, our group demonstrated that the optimal dose of low-power NIR-II laser also increased NO generation (Fig. 1) [21]. In this study, the 1064 or 1270 nm laser was more efficient in inducing NO than the conventional 808 nm NIR-I laser in cultured endothelial cells. The study further elucidated that NIR-II laser

activated mitochondrial retrograde signalling and enhanced the phosphorylation of eNOS at S1177 and Akt at S473, leading to augmented NO generation. We further demonstrated that the preconditioning with NIR-II laser ameliorated reperfusion and stroke outcome in the filament MCAO model of ischaemic stroke in mice (Fig. 2) [22]. Following preconditioning with a low-power 1064 nm NIR-II laser at 50 mW/cm² for 5 min once a day for 4 consecutive days, we challenged mice with MCAO by filament for 30 min with subsequent reperfusion for 48 h.

The NIR-II laser-pretreated wild-type (WT) mice demonstrated an increased CBF at reperfusion, decreased infarct volume, and improved neurologic scoring. Interestingly, the NIR-II pretreatment did not decrease the infarct volume and neurological deficit in eNOS knock-in mice containing an alanine in place of the serine (S1176A mice), so it cannot be phosphorylated [23]. These results suggest that the beneficial effects are mediated by S1176 eNOS phosphorylation. Restoring the function of lymphatic clearance has also been suggested to improve the prognosis of stroke [24]. Indeed, a low-power 1267 nm

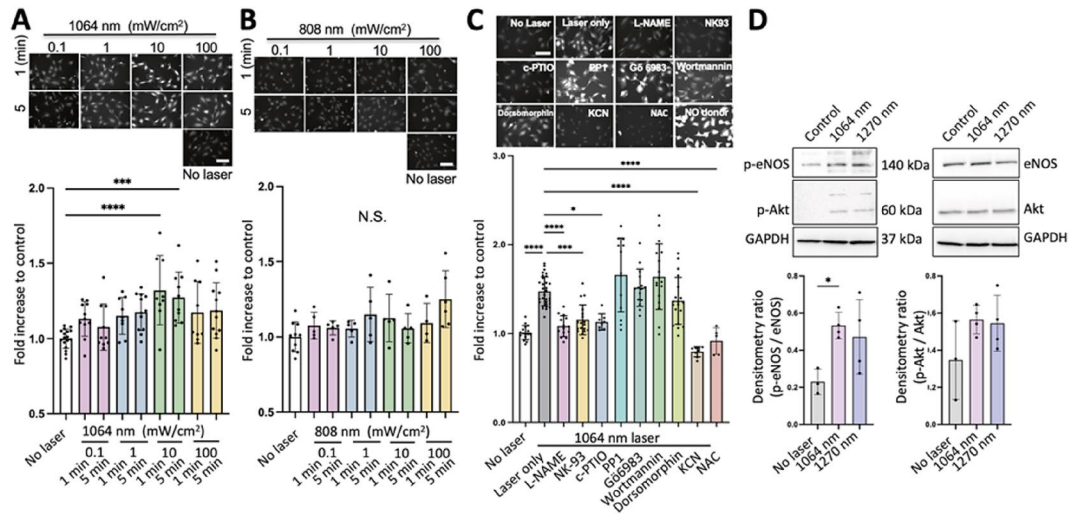


Fig. 1 Effect of NIR-II laser on NO generation and eNOS phosphorylation. (a, b) Cultured endothelial cells were exposed to 1064 (a) or 808 nm (b) laser at 0.1–100 mW/cm² for 5 min. NO generation was determined using diaminofluorescein-2 diacetate (DAF-2 DA). Scale bar = 100 μ m. (c) Cells were pre-treated with an inhibitor and then a 1064 nm laser at 10 mW/cm² for 5 min. (d)

Immunoblot analysis of cultured cells treated with 1064 or 1270 nm laser at 10 mW/cm² for 5 min. The membrane was then probed with anti-phospho eNOS or anti-phospho Akt, anti-total eNOS, or anti-total Akt antibody. (a–d) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.0001$. N.S. not significant. (Modified from Yokomizo et al. 2022 [21] with permission from John Wiley and Sons)

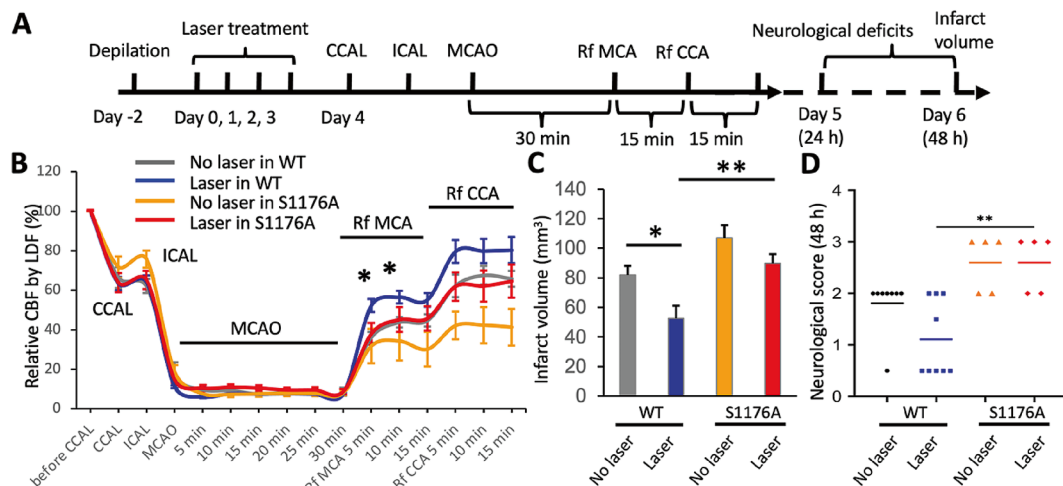


Fig. 2 Effect of NIR-II laser on CBF and infarct volume upon MCAO in WT and S1176A mice. (a) The timeline of the experiment. (b) cerebral blood flow (CBF) measured by laser Doppler flowmetry during 30 min of MCAO with subsequent 15 min of the MCA reperfusion and 15 min of CCA reperfusion; * $P < 0.05$ between non-treated and NIR-II laser-pretreated wild-type (WT) mice for each time point. CCAL, common carotid artery ligation; ICAL, internal carotid artery ligation; Rf MCA, reperfusion of

MCA; Rf CCA, reperfusion of CCA; S1176A, S1176A eNOS knock-in mice. (c) Infarct volume in mice subjected to MCAO for 30 min followed by 48 h of reperfusion. * $P < 0.05$, ** $P < 0.01$. (d) Neurological deficits upon MCAO. ** $P < 0.01$. (b–d), $n = 8, 9, 5, 5$ for no laser in WT, laser pretreated in WT, no laser in S1176A, and laser pretreated in S1176A group, respectively. (Modified from Yokomizo et al. 2024 [22] with permission from Wolters Kluwer Health, Inc.)

NIR-II laser activated meningeal lymphatic clearance via NO signalling and provided fast recovery in a rat model of neonatal intraventricular haemorrhage [25], suggesting that NIR-II might improve stroke outcome via augmenting lymphatic clearance. Since PBM with 808 nm NIR-I [26] and 1267 nm NIR-II [27] proved to potentiate lymphatic clearance of amyloid β (A β) and improve cognitive functions in a mouse model of Alzheimer's disease, PBM offers a potential strategy to treat cerebrovascular, cardiovascular, and neurodegenerative diseases.

3 Discussion

Non-invasive PBM ameliorates stroke injury and can be considered for therapy against stroke. The beneficial effects of PBM are partially, if not entirely, mediated by NO signalling, but further investigation is warranted to elucidate the mechanisms of action. All the current pharmacological approaches have risks of undesirable drug-drug interactions, which limit the choice of therapy

and negatively impact the efficacy and safety of the therapy. Still, a laser-based approach can be broadly combined with other therapies. Laser is a physical parameter and, with the correct range, poses no risk of side effects. Thus, PBM offers a viable option for prophylactic and therapeutic purposes and can be applied to patients of all ages with various clinical conditions.

4 Conclusion

PBM augments NO bioavailability and improves stroke outcomes by increasing cerebrovascular perfusion and lymphatic clearance. This therapeutic modality could be broadly explored to treat various diseases, such as Alzheimer's disease.

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References

1. Atochin DN, Huang PL (2010) Endothelial nitric oxide synthase transgenic models of endothelial dysfunction. *Pflugers Arch* 460:965–974
2. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C (2012) The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol* 10:4–18
3. Huang YY, Sharma SK, Carroll J, Hamblin MR (2011) Biphasic dose response in low level light therapy—an update. *Dose Response* 9:602–618
4. Kashiwagi S, Morita A, Yokomizo S, Ogawa E, Komai E, Huang PL, Bragin DE, Atochin DN (2023) Photobiomodulation and nitric oxide signaling. *Nitric Oxide* 130:58–68
5. Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, Donnan GA (2019) Ischaemic stroke. *Nat Rev Dis Primers* 5:70
6. Phipps MS, Cronin CA (2020) Management of acute ischemic stroke. *BMJ* 368:l6983
7. Oron A, Oron U, Chen J, Eilam A, Zhang C, Sadeh M, Lampl Y, Streeter J, DeTaboada L, Chopp M (2006) Low-level laser therapy applied transcranially to rats after induction of stroke significantly reduces long-term neurological deficits. *Stroke* 37:2620–2624
8. Detaboada L, Ilic S, Leichter-Martha S, Oron U, Oron A, Streeter J (2006) Transcranial application of low-energy laser irradiation improves neurological deficits in rats following acute stroke. *Lasers Surg Med* 38:70–73
9. Shvedova M, Litvak MM, Roberts JD Jr, Fukumura D, Suzuki T, Sencan I, Li G, Reventun P, Buys ES, Kim HH, Sakadzic S, Ayata C, Huang PL, Feil R, Atochin DN (2019) cGMP-dependent protein kinase I in vascular smooth muscle cells improves ischemic stroke outcome in mice. *J Cereb Blood Flow Metab* 39:2379–2391
10. Hamblin MR (2016) Shining light on the head: photobiomodulation for brain disorders. *BBA Clin* 6:113–124
11. Tucker LD, Lu Y, Dong Y, Yang L, Li Y, Zhao N, Zhang Q (2018) Photobiomodulation therapy attenuates hypoxic-ischemic injury in a neonatal rat model. *J Mol Neurosci* 65:514–526
12. Yang L, Dong Y, Wu C, Li Y, Guo Y, Yang B, Zong X, Hamblin MR, Liu TC, Zhang Q (2019) Photobiomodulation preconditioning prevents cognitive impairment in a neonatal rat model of hypoxia-ischemia. *J Biophotonics* 12:e201800359
13. Lampl Y, Zivin JA, Fisher M, Lew R, Welin L, Dahlof B, Borenstein P, Andersson B, Perez J, Caparo C, Ilic S, Oron U (2007) Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the NeuroThera effectiveness and safety Trial-1 (NEST-1). *Stroke* 38:1843–1849
14. Huisa BN, Stemer AB, Walker MG, Rapp K, Meyer BC, Zivin JA (2013) Nest, investigators, transcranial laser therapy for acute ischemic stroke: a pooled analysis of NEST-1 and NEST-2. *Int J Stroke* 8:315–320
15. Zivin JA, Sehra R, Shoshoo A, Albers GW, Bornstein NM, Dahlof B, Kasner SE, Howard G, Shuaib A, Streeter J, Richieri SP, Hacke W, N.—investigators (2014) NeuroThera® efficacy and safety Trial-3 (NEST-3): a double-blind, randomized, sham-controlled, parallel group, multicenter, pivotal study to assess the safety and efficacy of transcranial laser therapy with the NeuroThera(R) laser system for the treatment of acute ischemic stroke within 24 h of stroke onset. *Int J Stroke* 9:950–955
16. Lapchak PA, Boitano PD (2016) Transcranial near-infrared laser therapy for stroke: how to recover from futility in the NEST-3 clinical trial. *Acta Neurochir Suppl* 121:7–12
17. Golovynskyi S, Golovynska I, Stepanova LI, Datsenko OI, Liu L, Qu J, Ohulchanskyy TY (2018) Optical windows for head tissues in near-infrared and short-wave infrared regions: approaching transcranial light applications. *J Biophotonics* 11:e201800141
18. Katagiri W, Lee G, Tanushi A, Tsukada K, Choi HS, Kashiwagi S (2020) High-throughput single-cell live imaging of photobiomodulation with multispectral near-infrared lasers in cultured T cells. *J Biomed Opt* 25:1–18
19. Berman MH, Halper JP, Nichols TW, Jarrett H, Lundy A, Huang JH (2017) Photobiomodulation with near infrared light helmet in a pilot, placebo controlled clinical trial in dementia patients testing memory and cognition. *J Neurol Neurosci* 8
20. Vargas E, Barrett DW, Saucedo CL, Huang LD, Abraham JA, Tanaka H, Haley AP, Gonzalez-Lima F (2017) Beneficial neurocognitive effects of transcranial laser in older adults. *Lasers Med Sci* 32:1153–1162
21. Yokomizo S, Roessing M, Morita A, Kopp T, Ogawa E, Katagiri W, Feil S, Huang PL, Atochin DN, Kashiwagi S (2022) Near-infrared II photobiomodulation augments nitric oxide bioavailability via phosphorylation of endothelial nitric oxide synthase. *FASEB J* 36:e22490
22. Yokomizo S, Kopp T, Roessing M, Morita A, Lee S, Cho S, Ogawa E, Komai E, Inoue K, Fukushi M, Feil S, Kim HH, Bragin DE, Gerashchenko D, Huang PL, Kashiwagi S, Atochin DN (2024) Near-infrared II photobiomodulation preconditioning ameliorates stroke injury via phosphorylation of eNOS. *Stroke* 55:1641
23. Kashiwagi S, Atochin DN, Li Q, Schleicher M, Pong T, Sessa WC, Huang PL (2013) eNOS phosphorylation on serine 1176 affects insulin sensitivity and adiposity. *Biochem Biophys Res Commun* 431:284–290
24. Wang YJ, Sun YR, Pei YH, Ma HW, Mu YK, Qin LH, Yan JH (2023) The lymphatic drainage systems in the brain: a novel target for ischemic stroke? *Neural Regen Res* 18:485–491
25. Li D, Liu S, Yu T, Liu Z, Sun S, Bragin D, Shirokov A, Navolokin N, Bragina O, Hu Z, Kurths J, Fedosov

- I, Blokhina I, Dubrovski A, Khorovodov A, Terskov A, Tzoy M, Semyachkina-Glushkovskaya O, Zhu D (2023) Photostimulation of brain lymphatics in male newborn and adult rodents for therapy of intraventricular hemorrhage. *Nat Commun* 14:6104
26. Wang M, Yan C, Li X, Yang T, Wu S, Liu Q, Luo Q, Zhou F (2024) Non-invasive modulation of meningeal lymphatics ameliorates ageing and Alzheimer's disease-associated pathology and cognition in mice. *Nat Commun* 15:1453
27. Semyachkina-Glushkovskaya O, Klimova M, Iskra T, Bragin D, Abdurashitov A, Dubrovsky A, Khorovodov A, Terskov A, Blokhina I, Lezhnev N, Vinnik V, Agranovich I, Mamedova A, Shirokov A, Navolokin N, Khlebsov B, Tuchin V, Kurths J (2021) Transcranial photobiomodulation of clearance of Beta-amyloid from the mouse brain: effects on the meningeal lymphatic drainage and blood oxygen saturation of the brain. *Adv Exp Med Biol* 1269:57–61

Part IV

Methods and Techniques

Alleviation of Post-sepsis Ischaemia by Drag-Reducing Polymers

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Abstract

Sepsis, leading to septic shock and multiple organ dysfunction syndrome, is characterised by inflammation, coagulopathy, and microvascular dysfunction, the primary cause of in-hospital mortality. Novel approaches are needed to prevent the consequences of sepsis. We showed that nanomolar concentrations of intravascular blood-soluble drag-reducing polymers (DRPs) significantly improve micro-

vascular perfusion and tissue oxygenation and protect neurons in rat brains after traumatic brain injury and haemorrhagic shock. The aim of this work was to determine whether DRPs-enhanced perfusion could alleviate sepsis-associated microvascular dysregulation in a mouse model of lipopolysaccharide (LPS)-induced sepsis. LPS (*Salmonella* Typhosa, 10 mg/kg, i.v.) was administered intravenously to induce acute sepsis in C57BL/6 J mice. DRPs (final concentration 5 ppm in the blood) or saline was injected i.v. (10 mice/group) 1 h after LPS injection to evaluate the efficacy of haemorheological modulation of microvascular dysregulation. In-vivo two-photon laser scanning microscopy was used to monitor cerebral (parietal cortex) and peripheral (ear) microcirculation (i.v. fluorescein isothiocyanate dextran) and tissue oxygen supply (nicotinamide adenine dinucleotide autofluorescence) at a baseline and during 4 h after septic shock induction. Differences between groups were determined using a two-way analysis of variance for multiple comparisons with post hoc testing. The statistical significance was set at $p < 0.05$. LPS-induced sepsis led to microvascular dysfunction and tissue hypoxia in the brain and peripheral tissue (ear). DRPs alleviated microthrombosis formation, microvascular dysfunction, and tissue hypoxia in the brain and peripheral tissue compared to the saline control group

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($p < 0.05$). Therefore, haemorheological modulation of blood flow by DRPs effectively improves systemic and peripheral circulation, reducing microthrombosis formation, microvascular dysfunction, and tissue hypoxia that can alleviate sepsis, shock, and multiple organ dysfunction syndrome.

Keywords

Sepsis · Ischaemia · Drag-reducing polymers

1 Introduction

Sepsis, leading to septic shock and multiple organ dysfunction syndrome (MODS), is characterised by microvascular dysfunction, coagulopathy, and inflammation, the primary cause of in-hospital mortality [1]. Sepsis causes short- and long-term cerebral dysfunction via hypoperfusion and neuroinflammation, leading to amyloid β and tau protein accumulation. Novel, efficient approaches are needed to prevent the consequences of sepsis and MODS. We previously showed that nanomolar concentrations of intravascular blood-soluble drag-reducing polymers (DRPs) significantly improved microvascular perfusion and tissue oxygenation and protected neurons in a rat brain suffering traumatic brain injury and haemorrhagic shock [2, 3]. DRPs improved blood chemistry, haemodynamic, and survival in different animal models of haemorrhagic shock [4]. We hypothesised that DRPs alleviate panvascular dysregulation of microvascular blood flow in sepsis and MODS and tested the hypothesis in a mouse model of lipopolysaccharide (LPS)-induced sepsis.

2 Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Lovelace Biomedical Research Institute. During the study, laboratory-acclimated male wild-type C57BL/6 J mice (25–30 g) were maintained on isoflurane (1%), nitrous oxide (69%), and oxygen (30%) face-mask anaesthesia. In-vivo

two-photon laser scanning microscopy was used to monitor cerebral (parietal cortex) and peripheral (ear) microcirculation and blood-brain barrier permeability (i.v. fluorescein isothiocyanate dextran) and tissue oxygen supply (nicotinamide adenine dinucleotide autofluorescence, NADH) at a baseline and during 4 h after septic shock induction. After baseline imaging, LPS (*Salmonella* Typhosa, 10 mg/kg, i.v.) was administered intravenously to induce acute sepsis. DRPs (final concentration 5 ppm in the blood) or saline was injected i.v. (10 mice/group) 1 h after sepsis induction (second) imaging session to evaluate the efficacy of haemorheological modulation of microvascular dysregulation.

DRPs preparation. Polyethylene oxide (PEO, MW ~4000 kDa) was dissolved in saline to 0.1% (1000 ppm), dialysed against saline using a 50 kD cutoff membrane, diluted in saline to 50 ppm, slow rocked for ~2 h, and then sterilised using a 0.22 μ m filter [2]. The volume of DRP solution (ml) to be injected was determined by: [(mouse weight (grams) $\times$ 0.07]/50 ppm) $\times$ 2 [2].

Two-photon laser scanning microscopy. The number of perfused capillaries, microcirculation, and tissue oxygen supply were visualised using Olympus BX 51WI upright microscope and water-immersion XLUMPlan FI 20x/0.95 W objective as previously described [2]. Excitation (740 nm) was provided by a Prairie View Ultima multiphoton laser scan unit powered by a Millennia Prime 10 W diode laser source pumping a Tsunami Ti: sapphire laser (Spectra-Physics, Mountain View, CA). Blood plasma was labelled by i.v. injection of tetramethylrhodamine isothiocyanate dextran (155 kDa) in physiological saline (5% wt/vol). Red blood cell flow velocity was measured in microvessels ranging from 3–50 μ m diameter up to 500 μ m below the surface of the parietal cortex and ear skin. NADH autofluorescence measurement was used to evaluate mitochondrial activity (metabolic status) and tissue oxygenation [5]. Tetramethylrhodamine fluorescence was band-pass filtered at 560–600 nm, and NADH autofluorescence was band-pass filtered at 425–475 nm. In offline analyses using NIH ImageJ software, three-dimensional anatomy of the vasculature in areas of interest was reconstructed from two-dimensional (planar) scans of

the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack).

Optical clearing. For skull optical clearing, Solution 1 (saturated supernatant solution of 75% (ethanol and urea) was applied to the exposed skull for about 10 min to allow the skull to turn transparent [6]. Then, Solution 1 was removed, and Solution 2 (sodium dodecyl benzenesulfonate) was added to the same area for further clearing within 5 min. For skin imaging, the ear was shaved and treated with a skin-clearing solution (PEG-400 + Thiazone + Sucrose) before each imaging [7].

Statistical analyses were done using GraphPad Prism software (La Jolla, CA, USA) by independent Student's *t*-tests or Kolmogorov-Smirnov tests where appropriate. Differences between groups and time were determined using a two-way repeated measures ANOVA analysis for multiple comparisons and post hoc testing. Variables are expressed as mean $\pm$ SEM. Statistical significance level was set at $p < 0.05$.

3 Results

LPS-induced sepsis substantially impaired cerebral and peripheral microcirculation without difference between groups. During septic coagulopathy and microthrombosis (1 h after LPS injection), the number of functioning capillaries decreased from 1009 ± 51 and 955 ± 48 per mm^3 at a baseline to 627 ± 49 and 638 ± 52 per mm^3 in the brain cortex and ear skin, respectively (Fig. 1, $p < 0.05$). Capillary red blood cell flow velocity fell from 1.39 ± 0.18 and 1.21 ± 0.19 mm/s to 0.81 ± 0.19 and 0.86 ± 0.2 mm/s in the brain cortex and ear skin, respectively (Fig. 2, $p < 0.05$). The microcirculatory impairment reduced mitochondrial respiration and tissue oxygen supply inversely reflected by an increase in NADH autofluorescence (1.13 ± 0.04 and 1.16 ± 0.05 normalised units in the brain cortex and ear skin, respectively, Fig. 3, $p < 0.05$). Unlike saline, DRPs alleviated microthrombosis formation, microvascular dysfunction, and tissue hypoxia in the brain and peripheral tissue ($p < 0.05$). By the end of the monitoring period (4 h after LPS injection), the number of

functioning capillaries further decreased to 431 ± 41 and 439 ± 44 per mm^3 in the saline group and increased to 782 ± 68 and 699 ± 52 per mm^3 in the DRPs group in the brain and skin, respectively (Fig. 1, $p < 0.05$). Similarly, capillary red blood cell flow velocity fell further to 0.57 ± 0.18 and 0.62 ± 0.19 mm/s in the saline group and increased to 1.01 ± 0.21 and 0.96 ± 0.19 mm/s in the DRPs group in the brain and skin, respectively (Fig. 2, $p < 0.05$). In the saline group, further degradation of microcirculation led to an increase in tissue hypoxia (1.22 ± 0.06 and 1.28 ± 0.07 normalised units in the brain cortex and ear skin, respectively, $p < 0.05$). In the DRPs group, in contrast, microcirculation improvement partially restored tissue oxygen supply (1.05 ± 0.03 and 1.1 ± 0.04 normalised units in the brain cortex and ear skin, respectively, Fig. 3, $p < 0.05$).

4 Discussion

Haemorheological blood flow modulation by DRPs effectively improved cerebral and peripheral microcirculation, reducing microthrombosis formation, microvascular dysfunction, and tissue hypoxia in a mouse model of LPS-induced sepsis. The mechanisms of DRP action on blood rheology include reduction of pressure loss in arterioles by increasing near-wall blood flow velocity, diminishing flow separations at bifurcations, and, thus, increasing precapillary pressure [2, 8]. This increases blood flow volume perfused through arterioles, promoting an increase in the density of functioning capillaries and eliminating capillary stasis. The net effect is improved microcirculation and increased red blood cell (RBC) traffic in microvessels due to decreased plasma skimming, which improves oxygen delivery to the brain and peripheral tissue [2, 9, 10].

Pharmacological studies showed that high-MW PEO mechanically degrades in the vascular system into shorter low-molecular-weight fragments, which are easily excreted by the urinary system [11]. It should also be noted that low-molecular-weight PEO (also known as polyethylene glycol) is FDA-approved for i.v. administration at significantly higher doses than

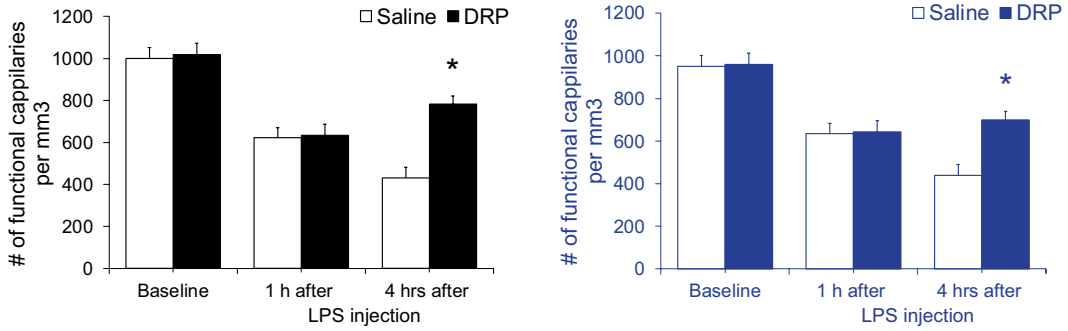


Fig. 1 Number of functional capillaries per mm³ of tissue. Left (black): Brain; Right (blue): Ear skin. $N = 10$ mice per group ($*p < 0.05$). DRP or saline was injected 1 h post LPS imaging

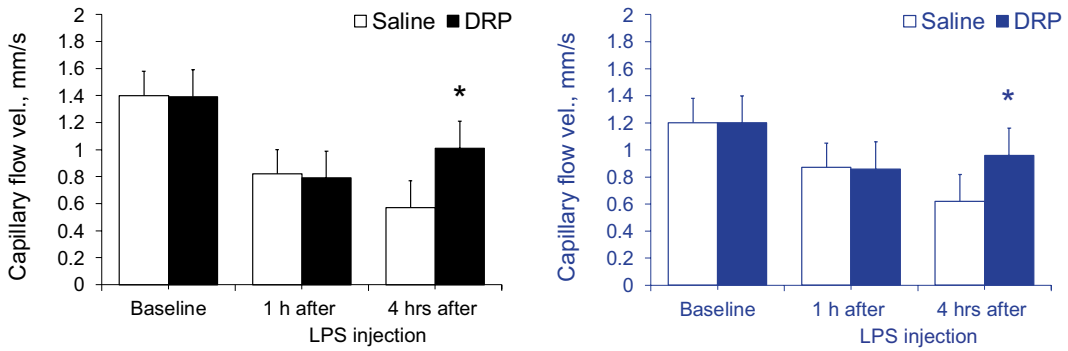


Fig. 2 Capillary flow velocity (mm/s) Left (black): Brain; Right (blue): Ear skin. $N = 10$ mice per group ($*P < 0.05$). DRP or saline was injected 1 h post LPS imaging

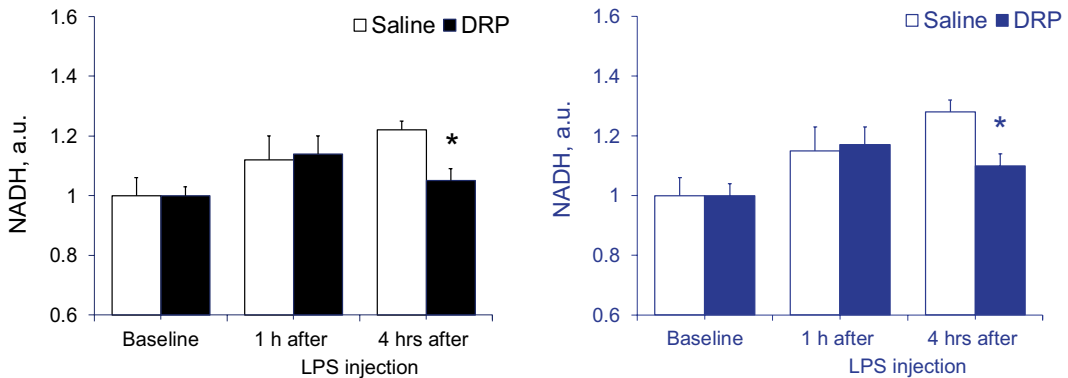


Fig. 3 NADH autofluorescence (inversely reflecting tissue oxygen supply). Left (black): Brain; Right (blue): Ear skin. $N = 10$ mice per group ($*P < 0.05$). DRP or saline was injected 1 h post LPS imaging

we used here and recognised as a biologically inert substance [12]. Since the effects of DRPs are based on physical principles, not pharmacological, the efficiency of DPRs does not depend on receptors or tissue conditions, which explains

the universality of DRP application in multiple pathologies tested thus far. However, future broad pharmacological and pharmacokinetics studies are necessary before this approach can be translated to clinical research.

5 Conclusion

DRPs effectively improved but did not restore baseline cerebral and peripheral microcirculation, thereby reducing microthrombosis, microvascular dysfunction, and tissue hypoxia in a mouse model of LPS-induced sepsis.

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References

1. Singer et al (2016) The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 315(8):801–810
2. Bragin et al (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. *J Cereb Blood Flow Metab* 37(3):762–775
3. Bragin et al (2020) Resuscitation with drag reducing polymers after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress. *Adv Exp Med Biol* 1232:39–45
4. Kameneva et al (2004) Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments. *Biorheology* 41(1):53–64
5. Chance B, Cohen P, Jobsis F et al (1962) Intracellular oxidation-reduction states in vivo. *Science* 137(499–508):8
6. Zhang et al (2018) A large, switchable optical clearing skull window for cerebrovascular imaging. *Theranostics* 8(10):2696–2708
7. Shi R, Guo L, Zhang C, Feng W, Li P, Ding Z, Zhu D (2017 Jun) A useful way to develop effective in vivo skin optical clearing agents. *J Biophotonics* 10(6–7):887–895. <https://doi.org/10.1002/jbio.201600221>. Epub 2016 Dec 23. PMID: 28009130.6
8. Marhefka et al (2009) Drag-reducing polymers improve tissue perfusion via modification of the RBC traffic in microvessels. *Biorheology* 46(4):281–292
9. Pacella et al (2012) Modulation of pre-capillary arteriolar pressure with drag-reducing polymers: a novel method for enhancing microvascular perfusion. *Microcirculation* 19(7):580–585
10. Zhao et al (2010) Drag-reducing polymers diminish the near-wall concentration of platelets in microchannel blood flow. *Biorheology* 47(3–4):193–203
11. Chernysheva et al (2006) Pharmacokinetics of high molecular weight polyethylene oxide. *Pharm Chem J* 40(9):467–468
12. <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm?event=BasicSearch.page>

Assessing Near-Infrared Optical Tomography's Depth Capability in Imaging Brain Vessels: An Experimental Study

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Abstract

Advanced brain vessel imaging is crucial for diagnosing and treating brain-related conditions such as lesions and strokes, ultimately enhancing brain health. Among the range of cerebrovascular imaging modalities, near-infrared optical tomography (NIROT) stands out for its cost-effectiveness and brain oxygenation quantification. The objective of this project, as a continuation of our prior simulation study, is to evaluate in vitro the Pioneer system for imaging blood vessels. An experimental study was performed on a silicon phantom with a tube inclusion mimicking the superficial blood vessels at a depth of 5 mm. The experiment employed a time domain (TD) NIROT called Pioneer system. Image reconstruction was performed using the obtained TD data. We used root mean square error (RMSE) to evaluate the accuracy of the reconstructed images. We were able to detect the location and structure of the tube with a RMSE of 0.0285. This experimental study showed

that the TD NIROT Pioneer system can detect vessel-like inclusion at the depth of 5 mm.

Keywords

Brain vessels · Medical imaging · Near infrared light · Optical properties · Silicone phantom

1 Introduction

Imaging brain vessels is essential for diagnosing neurological conditions, offering detailed insight into vascular anatomy, which is important for understanding blood supply to different brain regions [1] and detecting abnormalities such as lesions, and strokes [2]. Near-infrared optical tomography (NIROT) is an emerging non-invasive technique that offers valuable insights into the brain's vascular system.

A groundbreaking development in this field is the handheld time-domain reflectance near-infrared optical tomography (TD NIROT) system "Pioneer", developed by the Biomedical Optics Research Laboratory (BORL) in Zurich [3–5]. This innovative system, utilising a high-sensitivity SPAD camera with a photon detection probability of 12% at 800 nm, holds immense potential for imaging brain vessels. The SPAD camera consists of 1024 pixels, which is 32 higher than any previous NIROT instrument.

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With its capacity to accommodate the unprecedented number of 11,000 source-detector pairs, this Pioneer instrument obtains more information about the tissue which will enable higher image quality. This offers exciting opportunities for non-invasive neuroimaging, providing a pathway to enhance our understanding of the anatomy of cerebral blood vessels and their role in brain health and disease.

Our previous study has demonstrated its outstanding performance in visualising complex letter-shape structures in diffusive media and its high sensitivity to detecting deep anomalies [6]. In our previous simulation study aimed at detecting cerebral vessels, we simulated both a simple shape tissue model and a realistic head model that included all segmented vessels from MRI images [7]. In the simulation, the image reconstruction was performed utilising both a model-based approach and a hybrid model-U-Net method. The results demonstrated the ability to visualise the superficial vessels with NIROT. In this paper, as a next step, we aim to evaluate the Pioneer system in vitro for imaging blood vessels by conducting an experimental study on a silicon phantom with a tube inclusion at a depth of 5 mm, mimicking a blood vessel. This choice of depth replicates superficial blood vessels, allowing a controlled initial assessment of the Pioneer system's performance before progressing to deeper brain vessel imaging. This approach ensures method validation, iterative refinement, and consideration of ethical questions before attempting to image the deeper cerebral vasculature.

2 Methods

2.1 Phantom Creation

Based on the literature [7–11], and beginning with a model of superficial blood vessels positioned at a depth of 5 mm, we used 300 g of each SILPURAN 2420 component silicone A and B (Wacker, Germany) to create a cylindrical phan-

tom of 120 mm in diameter and 45 mm in thickness with a cylindrical inclusion of 3 mm in diameter and 120 mm in length at a depth of 5 mm. To simulate the optical properties of the brain tissue for 689 nm, we mixed 667 μl of silicone-compatible white ink ELASTOSIL RAL 9010 (Wacker, Germany) to obtain a specific scattering coefficient (μ_s') of 1 mm^{-1} . An absorption coefficient (μ_a) of 0.01 mm^{-1} was adjusted by adding 800 μl of carbon black powder (Wacker, Germany). After the mixture was prepared, it was degassed in a vacuum chamber, placed in a mould with a 3 mm diameter PVC tube inside at a depth of 5 mm from the centre of the tube, and slightly shifted by 0.6 cm to the left from the phantom centre, then cured at 65°C in the oven (Fig. 1). To validate the optical properties of the phantom, we used the commercial device Imagent (ISS Inc., Champaign, IL, USA).

In order to obtain the optical properties of blood, we prepared a solution by adding 15 ml of intralipid to 0.5 l of distilled water, resulting in a scattering coefficient (μ_s') of 5.22 mm^{-1} . Additionally, we added 32.5 ml of diluted Indian Ink (1:20 Indian Ink to water) to achieve an absorption coefficient (μ_a) of 0.235 mm^{-1} [12]. During the experimentation, we repeatedly injected a portion of this liquid phantom into the tube. The repetition of this process ensured consistency and reliability in our measurements.



Fig. 1 Silicone phantom with a tube inclusion at the depth of 5 mm. The phantom is a cylindrical phantom of 120 mm in diameter and 45 mm in thickness with a cylindrical inclusion of 3 mm in diameter and 120 mm in length at a depth of 5 mm

2.2 Measurement with Pioneer System

The Pioneer is a new-generation TD NIROT instrument based on a time-of-flight 32×32 SPAD camera with a high photon detection probability in the NIR range [3–5]. Using an open space arrangement enables us to interrogate a relatively large field of view (FoV) of 25 mm diameter. In this experiment, we used 80% of the laser power for an exposure time of 10 ms, a photon number threshold of 100 Photons/S and a pixel size of 1.08 mm. Time-of-flight (ToF) information from all 11 sources at a wavelength of 689 nm was recorded by each pixel of the image sensor. These ToF of photons were accumulated in histograms. The dark photon counts were removed to correct the raw histogram data and deconvoluted with system response function. The corrected TD data were used for image reconstruction.

2.3 Image Reconstruction

Light-tissue interaction can be approximately described with diffusion equations (DEs) [3]. Finite element techniques (FEM) are commonly utilised to numerically solve DEs for complex geometries. To model the forward and inverse problem, we used NIRFASTER, an open-source FEM software that supports GPUs [13–15]. We selected 286 detectors within the field of view and transformed the corresponding histogram into frequency domain with a discrete Fourier transform [16]. First, we reconstructed global optical properties, μ_a and μ_s' from the measured data using a multi-distance method [3]. Then, we used the frequency-domain (FD) method at ≈ 100 MHz to accomplish a quick (minutes) 3D image reconstruction and to reduce memory utilisation. To focus the simulation process on this region of interest (ROI) and facilitate the computation, a fine mesh (a cylinder of 60 mm in diameter and 35 mm in height) of 99,932 nodes was used to simulate the forward results. For the inverse problem, we employed a coarser mesh of 10,021 nodes to alleviate the computa-

tional burden. A least-squares minimisation optimisation was used to reconstruct the 3D image [17]. The ground truth was implemented as a simple homogeneous mesh, where a cylindrical tube with the optical properties of blood was placed inside at a depth of 5 mm. The previously created phantom, which included a tube filled with the liquid mixture mimicking blood, was measured by placing the probe in the centre of the phantom (Fig. 2), the tube was positioned with an 8-degree angular offset relative to both Source 1 and Source 7 (Fig. 3). The total number of photons acquired by the SPAD camera for the 11 sources was 1.5425×10^8 photons for an exposure time of 10 s.

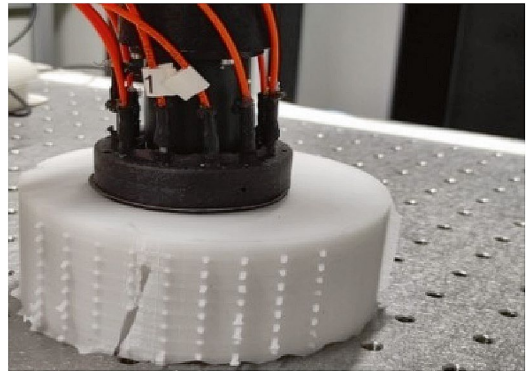


Fig. 2 Phantom measurement using the Pioneer system

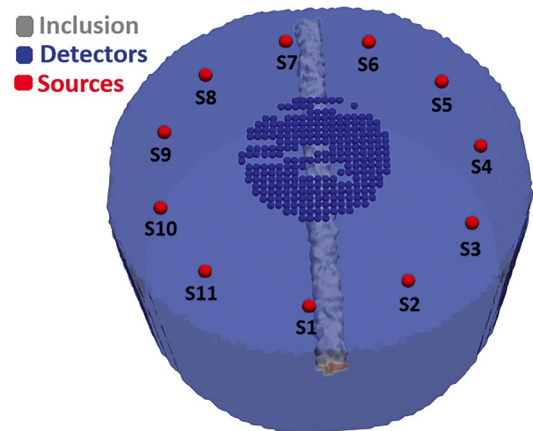


Fig. 3 Sources and detectors positions

3 Results and Discussion

Root mean squared error (RMSE) was used for evaluating the reconstructed image, specifically focusing on the tube region. It quantifies the average deviation between the reconstructed image (y') and the ground truth (y).

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - y'_i)^2}$$

where y_i represents the absorption coefficient at the i -th node in the mesh and n represents the total number of nodes within the tube region. Compared to the ground truth (Fig. 4), the image reconstruction (Fig. 5) was able to reveal the approximate shape and position of the embedded tube with a RMSE of 0.0285, which is satisfactory with a reconstructed tube of 3.64 mm in diameter, a rotational error of 2.32 degrees and a lateral shift of 1.627 mm to the left, due to the rotation error, we could not see the full tube shape in the YZ cross section. The image reconstruction process was optimised to prioritise the discovery of correct shapes rather than solely focusing on reconstructing the correct μ_a . This approach successfully yielded approximate results for a vessel shape located at a depth of 5 mm. However, the presence of noise and measurement errors in the experiment leads to a reduction in contrast and positional accuracy compared to the ground truth (Figs. 4 and 5). Despite these challenges, this is a promising first step and the positional discrepancy can be mitigated with an improved experimental setup and optimised algorithm designed to be more sensitive to blood vessels. Additionally, the image quality depends on the signal-to-noise ratio, which can be improved by collecting more photons, e.g., by acquiring for a longer period of time. It is important to note that the study's limitation is the placement of these vessels at a shallower depth (5 mm) in the phantom, whereas brain blood vessels typically reside much deeper within the tissue.

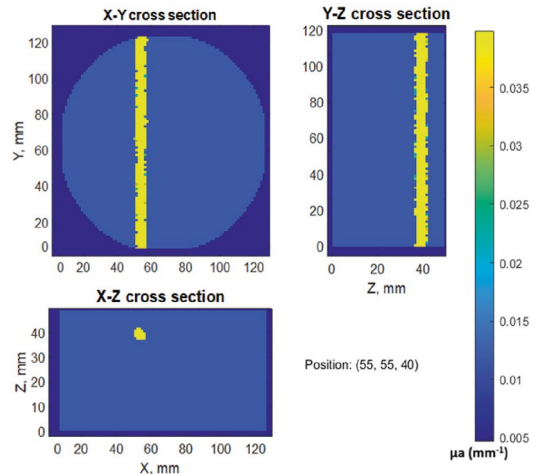


Fig. 4 Ground-truth cross-sectional image at a depth of 5 mm. The colormap represents the absorption coefficient. The mesh is a cylindrical mesh of 120 mm in diameter and 45 mm in thickness with a cylindrical inclusion of 3 mm in diameter and 120 mm in length at a depth of 5 mm

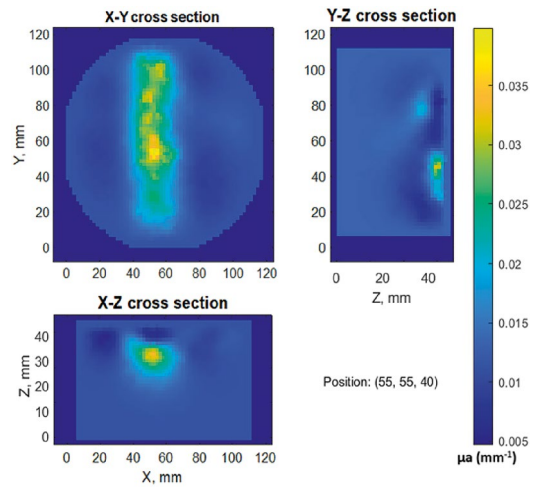


Fig. 5 Reconstruction image at a depth of 5 mm. The colormap represents the absorption coefficient

4 Conclusion

An experimental study using the Pioneer system showed promise in vessels structures detection at the depth of 5 mm. However, the system's reliability in measuring deep brain vessels remains a

challenge. To enhance its capabilities for cerebrovascular imaging, further improvements are imperative.

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Conflicts of Interest Martin Wolf declares that he is the president of the board and co-founder of OxyPrem AG and Alexander Kalyanov works partially for this company.

References

1. Duvernoy HM et al (2005) The human brain: surface, blood supply, and three-dimensional sectional anatomy. Springer
2. Wardlaw JM, Smith EE, Biessels GJ et al (2013) Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *The Lancet Neurol* 13(1):133–146. <https://doi.org/10.1364/BOE.441061>. PMID: 35154859; PMCID: PMC8803024
3. Jiang J, Di Costanzo Mata A, Lindner S et al (2021) 2.5 Hz sample rate time-domain near-infrared optical tomography based on SPAD-camera image tissue hemodynamics. *Biomed Opt Express* 13(1):133–146. <https://doi.org/10.1364/BOE.441061>. PMID: 35154859; PMCID: PMC8803024
4. Jiang J, Di Costanzo Mata A, Lindner S et al (2020) Dynamic time domain near-infrared optical tomography based on a spad camera. *Biomed Opt Express* 11(10):5470–5477. PMID: vol. 3314
5. Jiang J, Di Costanzo Mata A, Lindner S et al (2020) Image reconstruction for novel time domain near infrared optical tomography: towards clinical applications. *Biomed Opt Express* 11(8):4723–4734. PMID: vol. 3292
6. Jiang J, Ackermann M, Russomanno E et al (2022) Resolution and penetration depth of reflection-mode time-domain near infrared optical tomography using a ToF SPAD camera. *Biomed Opt Express* 13(12):6711–6723
7. Yacheur D, Ackermann M, Li T et al (2023) Imaging cerebral blood vessels using near-infrared optical tomography: a simulation study. *Adv Exp Med Biol* 1438:203–207. https://doi.org/10.1007/978-3-031-42003-0_32. PMID: 37845462.6
8. Camasão DB, Mantovani D (2021) The mechanical characterization of blood vessels and their substitutes in the continuous quest for physiological-relevant performances. A critical review. *Materials Today Bio* 10:100106. <https://doi.org/10.1016/j.mtbio.2021.100106>
9. Chen Z, Li H, Wu M et al (2020) Caliber of intracranial arteries as a marker for cerebral small vessel disease. *Front Neurol* 11. <https://doi.org/10.3389/fneur.2020.558858>
10. Garn MS, Selby S, Young R (1954) Scalp thickness and fat loss theory of balding. *Arch Dermatol Syphilo* 70:601–608
11. Light AE (1949) Histologic study of human scalps exhibiting various degrees of non-specific baldness. *J Invest Dermatol* 13:53
12. Kalyanov A, Jiang J, Russomanno E et al (2022) Development and validation of robust and cost-effective liquid heterogeneous phantom for time domain near-infrared optical tomography. In: Scholkmann F, LaManna J, Wolf U (eds) *Oxygen transport to tissue XLIII. Advances in experimental medicine and biology*, vol 1395. Springer, Cham. https://doi.org/10.1007/978-3-031-14190-4_33
13. [Online]. Available: <https://github.com/nirfaster/NIRFASTER>
14. Doulgerakis-Kontoudis M, Eggebrecht A, Wojtkiewicz S et al (2017) Toward real-time diffuse optical tomography: accelerating light propagation modeling employing parallel computing on gpu and cpu. *J Biomed Opt* 22(12):125001
15. Dehghani H, Eames ME, Yalavarthy PK et al (2009) Near infrared optical tomography using nirfast: Algorithm for numerical model and image reconstruction. *Commun Numer Meth Engng* 25(6):711–732
16. Jiang J, Wolf M, Sánchez Majos M (2017) Fast reconstruction of optical properties for complex segmentations in near infrared imaging. *J Mod Opt* 64(7):732–742
17. Yalavarthy PK, Pogue BW, Dehghani H et al (2007) Weight-matrix structured regularization provides optimal generalized least-squares estimate in diffuse optical tomography. *Med Phys* 34(6):2085–2098

Augmenting Blood Test and Periodontal Examination Data with Generative Adversarial Networks for Enhanced Dementia Risk Prediction

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Abstract

This study investigates the effectiveness of data augmentation to improve dementia risk prediction using deep neural networks (DNNs). Previous research has shown that basic blood test data were cost-effective and crucial in predicting cognitive function, as

indicated by mini-mental state examination (MMSE) scores. However, creating models that can accommodate various conditions is a significant challenge due to constraints related to blood test and MMSE results, such as high costs, limited sample size, and missing data from specific tests not conducted in certain facilities. Periodontal examinations have also emerged as a cost-effective tool for mass screening. To address these issues, this study explores the use of generative adversarial networks (GANs) for generating synthesised data from blood test and periodontal examination results. We used DNNs with four hidden layers to compare prediction accuracy between real and GAN-synthesised data from 108 participants at Nihon University Itabashi Hospital. The GAN-synthesised DNNs achieved a mean absolute error (MAE) of 1.91 ± 0.30 compared to 2.04 ± 0.37 for real data, indicating improved accuracy with synthesised data. Importantly, synthesised data showcased enhanced robustness against missing important variables including age information, and better managed data imbalances. Considering the difficulties in amassing extensive medical data, the augmentation approach is promising in refining dementia risk prediction.

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Keywords

Deep neural networks (DNNs) · Mini-mental state examination (MMSE) · Cognitive screening · Cognitive function · Cognitive assessment · Mild cognitive impairment (MCI)

1 Introduction

The rapidly increasing elderly population has led to an increase in the prevalence of dementia, including Alzheimer's disease (AD). Projections indicate that by 2050, the number of AD patients could exceed 100 million [1]. Accurate diagnosis is complex due to the subtleties of mental status assessment and the similarity of AD to other dementia types. Identification of mild cognitive impairment (MCI), a precursor to AD, is widely recognised as vital for early detection and intervention.

Blood tests have emerged as a promising tool for cognitive assessment, specifically as measured by the mini-mental state examination (MMSE) scores [2, 3]. Recent studies also suggest that there is a close relationship between cognitive function, oral health, and systemic metabolic function in seniors, with the number of healthy teeth being a significant predictor [4]. However, the development of predictive models that can accurately forecast using diverse patient data faces several obstacles, including high costs, limited sample sizes, and missing data from tests not conducted.

In response to the challenges in medical data collection, our study employs generative adversarial networks (GANs) to generate synthetic data from blood test and periodontal examination results. GANs leverage a pair of neural networks: the generator and the discriminator, which are trained concurrently through a competitive process. The Generator creates synthetic data from random noise, learning to make it indistinguishable from real data. Conversely, the discriminator learns to accurately distinguish whether the data presented is generated or real [5]. This augmenta-

tion approach not only aims to mitigate the issues of data scarcity and inconsistency but also has the potential to enhance the robustness and accuracy of dementia risk prediction models.

2 Methods

2.1 Subjects

We studied 108 subjects [age 69.4 ± 9.7 (mean $\pm$ std) range 33–85] who visited Nihon University Itabashi Hospital (Tokyo, Japan) for oral health assessments. A dentist conducted the periodontal examinations, recording variables including the number of remaining teeth, average probing depth (AvePD), average clinical attachment level (AveCAL), bleeding on probing (BOP), periodontal inflammatory surface area (PISA), and periodontal epithelial surface area (PESA). Each participant's cognitive function was evaluated using MMSE to systematically assess mental status. We also investigated demographic and blood test data as shown in Table 1. Gender was not included as a variable in our model due to preliminary analysis showing no significant impact on predictive accuracy [2, 3]. Specifically, age, height, blood urea nitrogen (BUN), number of remaining teeth, and PESA were identified as significantly correlated ($p < 0.1$) with the MMSE score in this dataset.

The mean MMSE score was 27.5 ± 2.4 (range 21–30). These measured MMSE scores served as the response variable to calculate the error from the scores estimated by DNNs, which factored in age, blood test, and periodontal examinations.

2.2 GAN-Synthesised DNN for Dementia Risk Prediction

Our GAN model was deployed on the Tensorflow 2 platform. The generator was designed with three hidden layers containing 256, 128, and 64 units, respectively. Each layer, starting from the input layer, employed the rectified activation function followed by batch normalisation.

Table 1 Measured data ($N = 108$): Each participant's cognitive function was systematically assessed using the MMSE as response variable for the regression models. For each item, the mean value along with the standard deviation (SD) is presented

Item		Mean $\pm$ SD	Spearman's correlation with MMSE score
Response	MMSE	27.5 $\pm$ 2.4	–
Demographic data	Age	69.4 $\pm$ 9.7	–0.36
	Height	164.3 $\pm$ 0.1	0.19
	Weight	65.2 $\pm$ 11.2	0.11
Blood test	WBC count	5519 $\pm$ 1634.2	–0.05
	Haemoglobin (Hb)	13.9 $\pm$ 1.4	0.10
	Platelet count (Plt)	24.6 $\pm$ 25.1	–0.06
	AST	24.8 $\pm$ 8.8	–0.05
	T-Cho	168.5 $\pm$ 36.1	0.07
	HDL cholesterol	56.3 $\pm$ 17.5	–0.03
	LDL cholesterol	85.2 $\pm$ 26.8	0.10
	TG	143.2 $\pm$ 115.9	0.06
	BUN	17.1 $\pm$ 5.9	–0.20
	UA	5.7 $\pm$ 2.2	0.06
Periodontal examination	Number of remaining teeth	21.4 $\pm$ 7.9	0.15
	Ave. PD	2.6 $\pm$ 0.7	–0.07
	Ave. CAL	3.7 $\pm$ 1.3	–0.15
	BOP	15.3 $\pm$ 12.9	0.04
	PISA	213 $\pm$ 215.9	0.10
	PESA	1066.6 $\pm$ 422.1	0.18

WBC white blood cell, AST aspartate aminotransferase, T-Cho total cholesterol, TG triglyceride, BUN blood urea nitrogen, UA uric acid, Ave. PD average probing depth, Ave. CAL average clinical attachment level, BOP bleeding on probing, PISA periodontal inflammatory surface area, PESA periodontal epithelial surface area

Similarly, the discriminator was structured with three hidden layers, with 128, 64, and 32 units, respectively. The model was configured with a batch size of 16 and a learning rate of 0.0001. The training was conducted for 500 epochs, which was based on the observation that the loss functions of the Generator and Discriminator were closely aligned at this point, suggesting a better balance between the two networks. Using this selected model, we generated 5000 synthesised data entries to enhance the robustness and generalisability of the dementia risk prediction.

Subsequently, deep neural networks (DNNs) were implemented to estimate MMSE scores from real and synthesised datasets, aiming to compare predictive accuracy. This task was carried out using the machine learning library on the Tensorflow 2 platform, which facilitates the configuration of multilayer feedforward neural networks. The input to the DNNs included all 19 items as listed in Table 1. In line with the GAN

model, the DNNs featured four hidden layers, with each layer having 256, 128, 64, and 32 units, respectively, and each was followed by the rectified activation function and batch normalisation.

3 Results

In our five-fold cross-validation process, each fold involved augmenting the training dataset with 5000 GAN-synthesised data points prior to model training, followed by evaluation on the corresponding test dataset. We employed the mean absolute error (MAE) for evaluation metric, defined as the average of the absolute differences between predicted and actual MMSE scores for each test dataset. The average MAE across all folds was calculated to determine overall model performance. The DNN trained on real data to predict MMSE scores exhibited an MAE of 2.04 ± 0.37 . In comparison, the DNN model

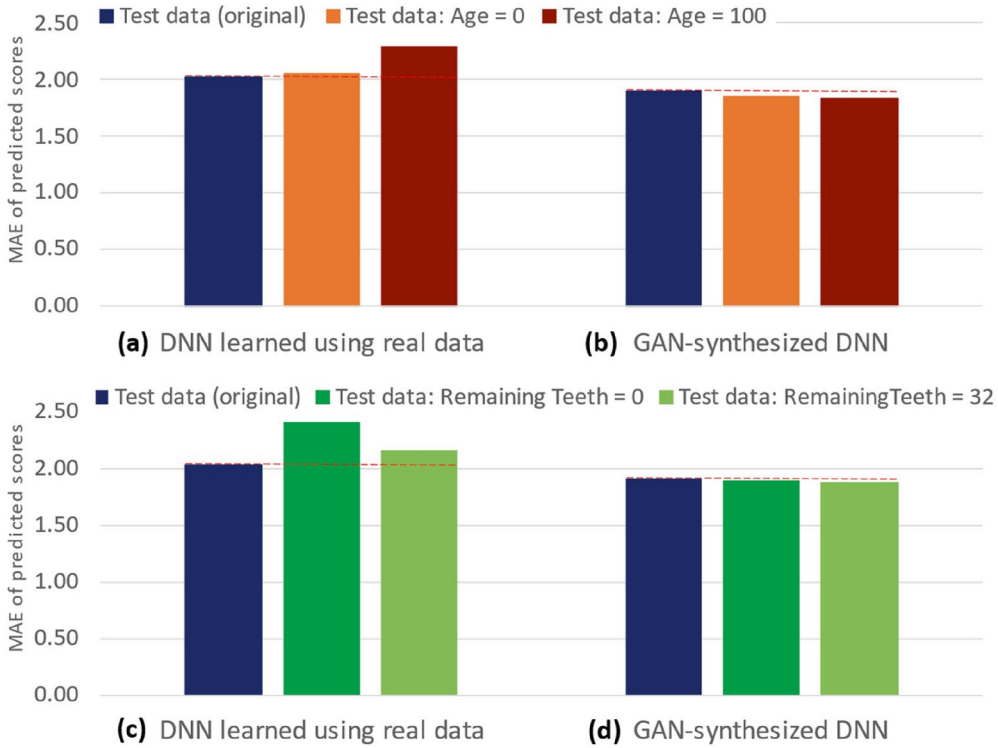


Fig. 1 Comparison of estimation errors in MAE by five-fold cross validation

trained with synthesised data created by a GAN for the same task of MMSE score prediction attained an MAE of 1.91 ± 0.30 (Fig. 1). This suggests that the GAN-synthesised data provided comparable or slightly improved accuracy relative to the real data, despite the limited sample size.

To evaluate model robustness against insertion of anomalous data, validation was conducted for cases where the input age was either 0 or 100 and the number of remaining teeth was either 0 or 32. We compared variable importances calculated using SHAP values and then focused on age and the number of remaining teeth for this evaluation. It was observed that the DNN trained with real data exhibited a significant increase in prediction error in these cases, as shown in (a) and (c) of Fig. 1. In contrast, the DNN trained with synthesised data maintained a stable MAE, as shown in (b) and (d) of Fig. 1. This indicates that the model trained with synthesised data is more robust, even when key variables, such as age and number of remaining teeth, are subject to substantial noise.

Upon comparing the age distribution between real and synthesised data, we found that the real data was concentrated in the age range of 50–70, while the synthesised data exhibited a more uniform distribution across all age groups, as shown in Fig. 2. This illustrates the capability of GANs to train models that can address challenges such as imbalanced data and missing values, which are common issues in medical datasets.

4 Discussion and Conclusion

This study confirms that GANs effectively generate synthesised data, mitigating sample data limitations in predicting cognitive function and MMSE scores. Findings reveal that DNN models trained on synthesised data maintain robustness against anomalies and can outperform models trained on limited data. This underscores a promising new application for GANs in cognitive screening, addressing challenges such as data imbalance and missing values.

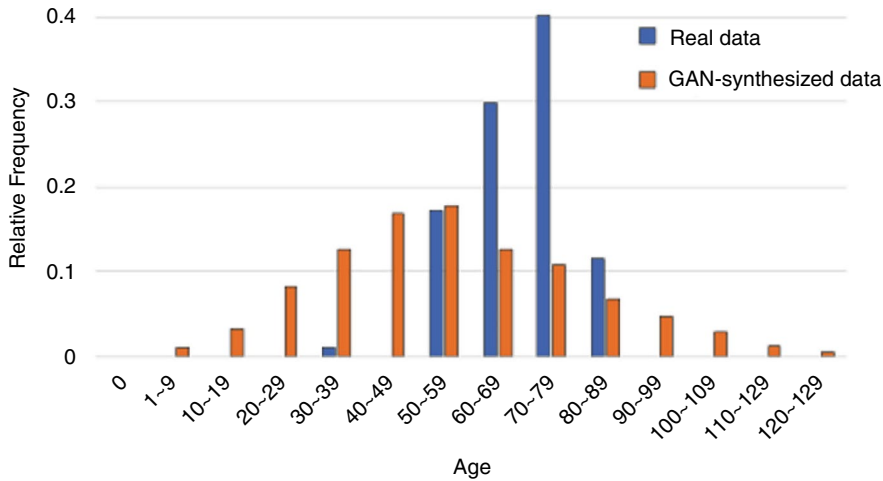


Fig. 2 Age distribution in relative frequency between real and synthesised data

Nonetheless, we acknowledge certain limitations of this study. Although five-fold cross-validation was used, future research should extend to external validation for a thorough performance assessment. Additionally, training GANs is often a time-intensive process and is highly sensitive to learning parameters and the intricate balance between the two competing networks, which can affect the consistency of the augmentation outcomes. Recent advancements in the field of GAN training may provide methodologies to mitigate these challenges. The success of GANs in this study advocates for their exploration in other domains of healthcare where data limitations are a significant barrier to innovation.

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References

1. Lynch C (2020) World Alzheimer report 2019: attitudes to dementia, a global survey: public health: engaging people in ADRD research. *Alzheimers Dement* 16:e038255
2. Sakatani K, Oyama K, Hu L (2020) Deep learning-based screening test for cognitive impairment using basic blood test data for health examination. *Front Neurol* 11:588140
3. Oyama K, Sakatani K (2022) Machine learning-based assessment of cognitive impairment using time-resolved near-infrared spectroscopy and basic blood test. *Front Neurol* 12:624063
4. Karako K, Chen Y, Oyama K, Hu L, Sakatani K (2022) Relationship between cognitive function, Oral conditions and systemic metabolic function in the elderly. *Adv Exp Med Biol* 1438:27–31
5. Ali H, Biswas MR, Mohsen F, Shah U, Alamgir A, Mousa O, Shah Z (2022) The role of generative adversarial networks in brain MRI: a scoping review. *Insights Imaging* 13(1):98

Cerebral Oxygen Monitoring in Extremely Low-Birth-Weight Infants Using Time-Domain Near-Infrared Spectroscopy in Transmittance Mode

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Abstract

Recent advances in neonatal intensive care have improved the survival rates of extremely low-birth-weight infants (ELBWIs). However, there has been no obvious improvement in the proportion of survivors without sequelae. Therefore, the development of appropriate management methods for ELBWIs in the neonatal intensive care unit is important to improve outcomes. In this study, we utilised time-domain near-infrared spectroscopy (TD-NIRS) for deep brain monitoring in premature infants in the clinical setting and mea-

sured the heads of three ELBWIs once weekly using a TD-NIRS system in transmittance mode. We found that optical signals transmitted through the head were detectable in all ELBWIs. We also confirmed that the total haemoglobin concentration and tissue oxygen saturation decreased in the first month after birth, while the reduced scattering coefficient was not correlated with postmenstrual age. We anticipate that this TD-NIRS technique will be useful for clinical assessment of deep brain tissues for appropriate management of cerebral circulation of ELBWIs in the neonatal intensive care unit.

Keywords

TD-NIRS · ELBWI · Transmittance mode · Deep brain · NICU

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1 Introduction

While the survival rate of extremely low-birth-weight infants (ELBWIs; birth weight < 1000 g) has improved with modern advances in neonatal care, the percentage of ELBWIs without

sequelae has not. Therefore, it is important to develop appropriate management methods for ELBWIs in the neonatal intensive care unit to improve neonatal outcomes [1, 2].

Time-domain near-infrared spectroscopy (TD-NIRS) systems have numerous advantages over continuous wave and frequency-domain NIRS systems because time-resolved data measured by TD-NIRS contain the most information about the measurement target. These data include absolute values of mean optical path length, absorption coefficient, reduced scattering coefficient, oxygenated haemoglobin (O_2Hb), deoxygenated haemoglobin (HHb), total haemoglobin (tHb) concentration, and tissue oxygen saturation (StO_2) [3–5]. We previously developed a highly sensitive TD-NIRS system and used transmittance mode to examine the heads of seven healthy adult volunteers during hyperventilation [6]. TD-NIRS data measured in transmittance mode capture more information in the brain compared with data measured in reflectance mode, and may provide new biomarkers for cerebral circulation management.

In the present study, to monitor deep brain haemodynamics in ELBWIs in the clinical setting, we measured three ELBWIs without intraventricular haemorrhage (IVH) once weekly using the TD-NIRS system in transmittance mode until 35 weeks of postmenstrual age (PMA).

2 Methods

2.1 TD-NIRS Instrumentation

A photograph and schematic diagram of the TD-NIRS system (TRS-21; Hamamatsu photonics K.K., Shizuoka, Japan) are shown in Fig. 1. The TD-NIRS system consisted of three picosecond light pulsers of different wavelengths (760 ± 5 , 800 ± 5 , and 830 ± 5 nm); a photomultiplier tube (PMT; H7422P-50MOD; Hamamatsu photonics K.K., Japan) with a gallium arsenide photocathode for single-photon counting; a time-correlated single-photon counting (TCSPC) unit with a constant fraction discriminator (CFD); a time-to-amplitude converter (TAC) and analogue-to-digital (A/D) converter; a histogram memory for measuring the temporal profile function of the sample; and a detection and irradiation optical fibre bundle with a numerical aperture of 0.29. Each picosecond light pulser generated a light pulse with a full-width at half maximum of <100 ps, a pulse rate of 5 MHz, and an average output power at the optical irradiation fibre end of approximately $100 \mu W$. To measure the optical properties more accurately by reducing the effect of the numerical aperture of the detection fibre bundle, we placed a thin diffuser slab (opal diffusing glass; Edmund Optics, New Jersey, USA) in front of the detection fibre bundle during measurements of the instrument response function [7].

The TD-NIRS system can assess the temporal profiles in transmittance mode. For data analysis,

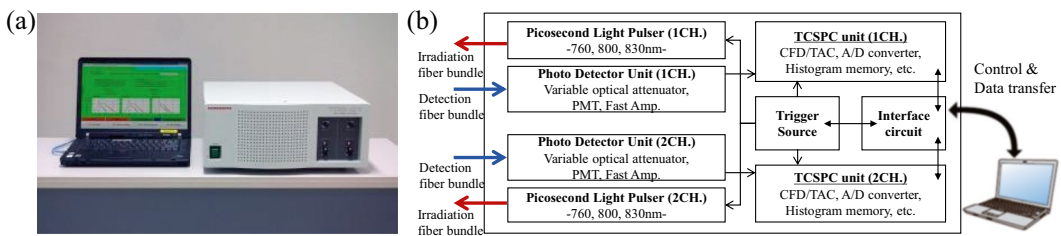


Fig. 1 (a) Photograph of the TD-NIRS system. (b) Block diagram of the TD-NIRS system

we used the solution $T(d,t)$ [8] of an infinite homogeneous slab. The solution $T(d,t)$ is expressed by a function of the fibre distance d and time t , as shown in Eq. (1):

$$T(d,t) = \frac{1}{2} \left((4\pi Dc)^{-\frac{3}{2t}-\frac{5}{2}} \exp(-\mu_a ct) \right) \times \sum_{m=-1}^2 \left[z_{1,m} \exp\left(-\frac{z_{1,m}^2}{4Dct}\right) - z_{2,m} \exp\left(-\frac{z_{2,m}^2}{4Dct}\right) \right],$$
$$D = \left\{ 3(\mu_s') \right\}^{-1}, z_0 = (\mu_s')^{-1} \tag{2}$$
$$\begin{cases} z_{1,m} = d(1-2m) - 4mz_e - z_0 \\ z_{2,m} = d(1-2m) - (4m-2)z_e + z_0 \end{cases} \tag{3}$$

where c is the speed of the light in the medium and z_e is the linear extrapolation distance. Using the non-linear least squares method based on the Levenberg-Marquardt method [9], we fitted Eq. (1) that convolved the instrument response function into the observed temporal profiles obtained from the TD-NIRS system, and then determined the absorption coefficient μ_a and the reduced scattering coefficient μ_s' at each wavelength. Then, O₂Hb and HHb were determined by referring to the haemoglobin spectroscopic data [10] with an assumed water concentration of 85%. Additionally, we calculated the tHb(μM) = O₂Hb + HHb and StO₂(%) = O₂Hb / tHb × 100.

In our previous study, we measured the solid phantom model (Biomimic optical phantoms;

INO, Quebec, Canada) in reflectance mode and transmittance mode, and confirmed the validity of the performance of the system [11].

2.2 Subjects and Protocol

Three ELBWIs without IVH were each measured once weekly using the TD-NIRS system in transmittance mode until 35 weeks of PMA. This study was approved by the Institutional Research Ethics Board of Saitama Children’s Medical Center. Informed consent was obtained from the families of all ELBWIs included in the study. Demographics and perinatal characteristics of the three ELBWIs are shown in Table 1. The characteristics of the three ELBWIs at each measurement are shown in Table 2. As shown in Fig. 2, the irradiation and detection optical fibre bundles were placed near the left and right temples. We then performed the transmittance measurements for 10 minutes (sampling rate of 0.2 Hz) using the TD-NIRS system. The data was then analysed using the averaged value. The fibre distance d was measured with calipers, and the value was used for data analysis.

3 Results

We confirmed that the optical signals transmitted through the head were detectable in all three ELBWIs. The measurement results in transmittance mode for the three ELBWIs are shown in

Table 1 Demographics and perinatal characteristics of the three ELBWIs

ELBWIs	Gestational age (weeks)	Birth weight (g)	AGA ^a /SGA ^b	Sex	Multiplet	Apgar score	
						1 min	5 min
No.1	24.4	683	AGA	Male	Singleton	4	7
No.2	27.7	951	AGA	Male	Singleton	8	9
No.3	23.1	595	AGA	Female	Singleton	5	6
Mean ± S.D. or %	25.1 ± 2.4	743 ± 185	AGA 100%	Male 67%	Singleton 100%	5.7 ± 2.1	7.3 ± 1.5
			SGA 0%	Female 33%			

^aAppropriate for gestational age (AGA)
^bSmall for gestational age (SGA)

Table 2 Characteristics of three ELBWIs (infant no. 1, 2, and 3) at each measurement

	Infant No.1				Infant No.2				Infant No.3			
	PMA [weeks]	CA ^a [days]	Weight [g]	Distance [cm]	PMA [weeks]	CA [days]	Weight [g]	Distance [cm]	PMA [weeks]	CA [days]	Weight [g]	Distance [cm]
Measurement 1	25.4	6	642	4.5	28.3	4	854	4.5	24.0	6	536	4.2
Measurement 2	26.1	12	668	4.9	29.7	14	973	4.6	25.0	13	585	4.3
Measurement 3	27.1	19	665	4.6	30.7	21	1048	4.8	26.0	20	630	4.5
Measurement 4	28.3	27	770	4.8	31.6	27	1231	5.0	27.0	27	674	4.6
Measurement 5	29.0	32	736	4.8	32.7	35	1430	5.2	28.1	35	782	5.0
Measurement 6	30.0	39	839	4.6	33.7	42	1596	5.3	29.1	42	815	5.0
Measurement 7	31.0	46	977	5.5	35.0	51	1886	5.8	30.6	52	1026	5.4
Measurement 8	32.4	56	1092	5.2	35.7	56	2122	5.8	31.4	58	1111	5.8
Measurement 9	33.7	65	1245	5.4	36.7	63	2230	6.1	32.1	63	1196	5.8
Measurement 10	34.6	71	1364	5.5	—	—	—	—	33.1	70	1422	6.1
Measurement 11	—	—	—	—	—	—	—	—	35.1	84	1775	6.4

^achronological age (CA)

Fig. 3. The mean path length increased in proportion to PMA, while tHb and StO₂ show a trend towards a decrease in the weeks following birth. The differential path length factor (DPF) and reduced scattering coefficient were not correlated with PMA.

4 Discussion and Conclusion

Using the TD-NIRS system in transmittance mode, we obtained absolute values of the absorption coefficient, reduced scattering coefficient, haemoglobin concentrations (O₂Hb, HHb, and tHb), and StO₂ of the premature infants' heads. The TD-NIRS system could detect the increase in mean path length caused by growth of the ELBWIs, which indicates the utility of this transmittance measurement. However, we have not been able to validate the analytical solution of Eq. (1) for the ELBWIs' inhomogeneous head measurements in transmittance mode. Therefore,

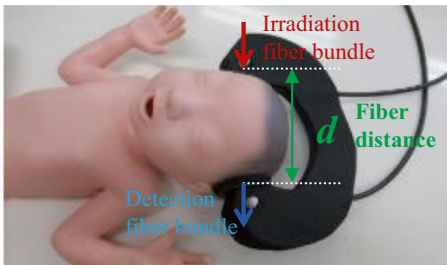


Fig. 2 Image of the irradiation and detection optical fibre bundles for the transmittance measurement of the premature infant (doll) using the TD-NIRS system

our results may contain errors, and further study with simulation or measurements of inhomogeneous phantom models is required.

The trend of variation in tHb and StO₂ in the first month after birth is similar to a prior reflectance mode study [12]. The authors of that study suggested that it is dependent on systemic changes and does not reflect changes associated with brain development. This is consistent with the findings that StO₂ correlates with the heart and respiratory rate in newborns [13]. Our results, however, were not consistent with those of Ref. [1]. We think that differences in the experimental protocols have influenced on the results. In Ref. [12], measurements are performed once weekly on the same infants with an experimental protocol that is similar to our present study. In Ref. [1], measurements were performed only at 32.9 ± 2.1 hours after birth. Furthermore, differences in the measurement positions, as well as the limitations of the analytical model as described above, have an impact on the results. The reduced scattering coefficient was not correlated with PMA ($R^2 = 0.21$ at 796 nm; data not shown). However, because our study only included three infants, further studies with more infants are required. It is also important to collect and analyse data on infants with various pathological conditions such as IVH, in which symptoms appear in the deep brain region, to further validate the utility of our TD-NIRS system in transmittance mode for deep brain monitoring. Our ultimate goal is to develop new biomarkers for the appropriate management of cerebral circulation in ELBWIs.

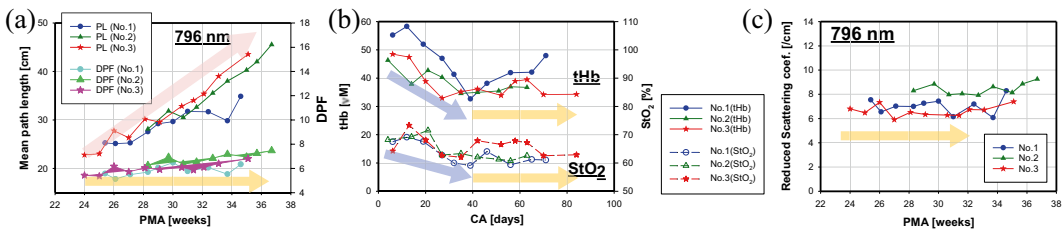


Fig. 3 Measurement results of (a) mean path length and DPF (796 nm), (b) total haemoglobin concentration (tHb) and tissue oxygen saturation (StO₂), and (c) reduced scat-

tering coefficient (796 nm) using the TD-NIRS system in transmittance mode for the three ELBWIs

References

1. Ijichi S, Kusaka T, Isobe K et al (2005) Developmental changes of optical properties in neonates determined by near-infrared time-resolved spectroscopy. *Pediatr Res* 58(3):568–573
2. Imanishi T, Sumiya W, Kanno C et al (2023) Relationship of cerebral blood volume with arterial and venous flow velocities in extremely low-birth-weight infants. *Eur J Pediatr* 182(6):2821–2832
3. Chance B, Nioka S, Kent J et al (1988) Time-resolved spectroscopy of hemoglobin and myoglobin in resting and ischemic muscle. *Anal Biochem* 174:698–707
4. Delpy DT, Cope M, van der Zee P et al (1988) Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys Med Biol* 33:1433–1442
5. Patterson MS, Chance B, Wilson BC (1989) Time resolved reflectance and transmittance for the non-invasive measurement of tissue optical properties. *Appl Opt* 28:2331–2336
6. Suzuki H, Oda M, Ohmae E et al (2016) Hemodynamic measurements of the human adult head in transmittance mode by near-infrared time-resolved spectroscopy. *Adv Exp Med Biol* 876:399–406
7. Liebert A, Wabnitz H, Grosenick D et al (2003) Fiber dispersion in time domain measurements compromising the accuracy of determination of optical properties of strongly scattering media. *J Biomed Opt* 8(3):512–516
8. Contini D, Martelli F, Zaccanti G (1997) Photon migration through a turbid slab described by a model based on diffusion approximation. I Theory. *Appl Opt* 36(19):4587–4599
9. Suzuki K, Yamashita Y, Ohta K et al (1994) Quantitative measurement of optical parameters in the breast using time-resolved spectroscopy. Phantom and preliminary in vivo results. *Investig Radiol* 29:410–414
10. Matcher SJ, Elwell CE, Cooper CE et al (1995) Performance comparison of several published tissue near-infrared spectroscopy algorithms. *Anal Biochem* 227(1):54–68
11. Suzuki H, Oda M, Yamaki E et al (2014) Hemodynamic measurements in deep brain tissues of humans by near-infrared time-resolved spectroscopy. *Proc SPIE*:8928, 89280O
12. Roche-Labarbe N, Fenoglio A, Aggarwal A et al (2012) Near-infrared spectroscopy assessment of cerebral oxygen metabolism in the developing premature brain. *J Cereb Blood Flow Metab* 32(3):481–488
13. Tina LG, Frigiola A, Abella R et al (2009) Near infrared spectroscopy in healthy preterm and term newborns: correlation with gestational age and standard monitoring parameters. *Curr Neurovasc Res* 6(3):148–154

Development of a Remote Health-Monitoring System for Frail People: A Preliminary Study

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Abstract

Respiratory and cardiovascular functions decline with age in elderly individuals. Consequently, the incidence of chronic respiratory and cardiovascular diseases increases with age. Heart disease and pneumonia are the leading causes of death in Japan. Given the pathophysiological nature of these diseases, patients inevitably require monitoring of their cardiac and pulmonary functions, such as heart rate and arterial blood oxygenation, as

systemic parameters. In addition, monitoring skin temperature and humidity as local parameters is preferable for elderly individuals to maintain healthy daily conditions. In the present study, we developed a wearable vital sign monitoring system and validated the accuracy of the device under development as compared to authorised medical devices that measure these systemic and local parameters in the peripheral tissue of the palm. For the systemic parameters, mean values showed no significant differences between the two devices, but the data bias was greater for the device under development. For the local parameters, mean values showed significant differences between the two devices; however, the data bias was the same for both devices. The acceptable data acquisition of the device under development was approximately 89%, with error acquisition mainly caused by the measurement of systemic parameters. We conclude that further improvements in measurement of systemic parameters are required to increase the data acquisition beyond 90%.

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Keywords

Remote health monitoring system · Arterial
blood oxygenation · Pulse rate · Skin tem-
perature · Skin humidity

1 Introduction

Japan's health boom began in the 1970s, coinciding with a decrease in the poverty rate and an increase in the population aged 65 and over [1]. However, studies conducted since 2000, when healthy life expectancy was introduced, show that the gap between average life expectancy and healthy life expectancy (approximately 10 years) has only changed slightly over the past 20 years [1]. This suggests that our bodies and minds are vulnerable and unable to adapt to rapid environmental and economic changes. This problem is likely to become more serious in the future in areas facing depopulation, a shortage of medical professionals, and a lack of physical fitness facilities such as exercise gyms.

However, recent advances in information and communication technology (ICT) and the Internet of Things (IoT) have enabled the development of health-monitoring [2–5] and lifelogging technologies [6, 7]. The ultimate goal of this study is to develop a remote health monitoring system consisting of the following three functions: (1) a health monitoring device that allows people to track their health status and incorporate preventive health measures into their daily lives (especially for the elderly), (2) data analysis tools to enable early detection of frailty and disease prevention (for medical researchers), and (3) a database system to enable postoperative patient management (for healthcare professionals).

In the present study, we reported the function of a health monitoring device under development in comparison with approved medical devices.

2 Material and Methods

2.1 Basic Structure of System

Figure 1 presents a block diagram of the system under development. The system comprises the following two units:

1. Sensing system: An ESP-32-based microcomputer was purchased from Switch Science Inc., and Arduino IDE-based firmware was

used to connect a pulse oximeter (SpO₂) and heart rate (Pulse) sensor (MAX30102, Analog Devices, Inc.) and temperature (Temp) and humidity (Hum) sensor (HTU21D-F, Adafruit Industries). The basic firmware was downloaded from GitHub sites [8, 9] and modified for digital data acquisition every 1.0 s and UDP data transfer at 0.1 s.

2. Operating system: A personal computer with Windows 10 was used. The ESP-32 system has a static random-access memory (SRAM) of 520 KB and enabled the signal processing with a 3-point moving average. The processing-based application enables data saving and waveform displays. The sensing limits of the individual sensors were set to $80 < \text{SpO}_2 (\%) < 100$, $50 < \text{Pulse (bpm)} < 150$, $25 < \text{Temp (}^\circ\text{C)} < 45$, and $10 < \text{Hum} (\%) < 100$. The limitations were set referring to the referenced medical grade devices (see Sect. 2.2). Data acquisition (%) of the device under development was defined considering that all these physiological values measured with the testing devices were within the setting ranges.

2.2 Simultaneous Measurement of Physiological Parameters Using Standard Medical Devices and the Device Under Development

Figure 2 illustrates the experimental setup for measuring the above four physiological parameters compared with the device under development and the standard medical-grade devices. We used a medical-grade pulse oximeter, OX-200 (Dretec, Co., Ltd.), for SpO₂ and Pulse (maximum sampling rate 8 Hz) measurements, attached to the distal phalanx tissue of the ring finger. The MAX30102 was gently placed on the palm of the distal phalanx on the middle phalanx tissue. Skin conductance was measured between the palms of the distal and proximal phalanx tissues and on the index finger tissue using Procomp2 (SA9309M, Thought Tech., LTD). The skin temperature was measured on the palm of the proximal phalanx and index finger tissues using ProComp2 (SA9310M, Thought Tech., LTD). The

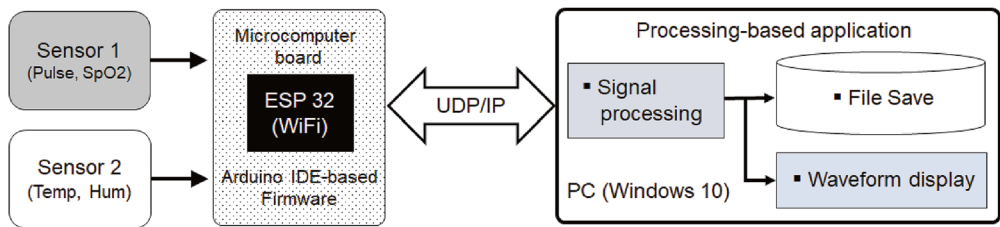


Fig. 1 Block diagram of system under development

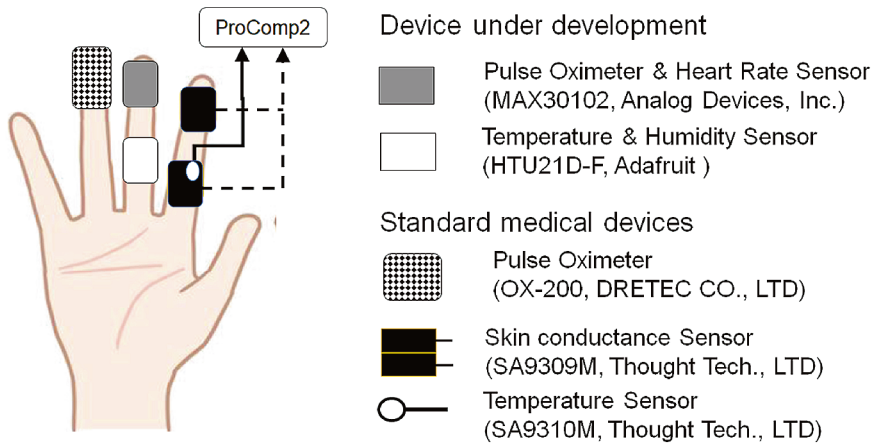


Fig. 2 Sensor positions

maximum sampling rate of the ProComp2 was 32 Hz. The sensors were tightly bundled and taped with Velcro tape, and the HTU21D-F was gently placed on the palm of the proximal phalanx of the middle phalanx tissue. We conducted 11 measurement sessions on two participants (man aged 67 and woman aged 47 years) for 3 min. The sampling rates of data for the reference devices were down-sampled to 1 Hz to compare with the device under development whose sampling rate was set to 1 Hz in the present experiment. All data were stored as CSV files in their operating PC for post statistical analyses. This study was approved by the Kyoto University Medical Ethics Committee. Furthermore, the participants were fully informed before the start of the experiment, with informed consent obtained verbally and in writing.

3

Results

3.1

Comparison of Systemic Parameters (Pulse Rate and SpO₂)

Table 1 compares the pulse rate and arterial blood oxygenation (SpO₂) measured using a reference medical device (OX-200) and testing device. Although no significant differences were observed between the mean values (column comparison), the data deviation was greater for the device under development (raw comparison). As pulse rate and arterial blood oxygenation are systemic parameters, they should yield the same values regardless of the fingers chosen. The present results support this assumption.

Table 1 Comparison of systemic parameters between reference medical device and testing device under development

Exp. No.	Reference device: OX-200				Testing device: MAX30102				Data acquisition (%)
	Pulse(bpm)	SD	SpO2 (%)	SD	Pulse(bpm)	SD	SpO2(%)	SD	
Exp_1	63.7	3.6	97.2	0.4	70.8	11.2	95.9	2.6	77
Exp_2	59.6	2.4	97.1	0.3	62.3	8.2	98.1	1.3	95
Exp_3	61.1	2.9	97.1	0.7	66.2	15.5	97.7	1.0	84
Exp_4	59.1	2.9	96.4	0.9	61.7	17.1	96.4	2.3	84
Exp_5	59.0	3.1	96.7	0.6	59.1	7.0	98.1	1.0	94
Exp_6	73.6	2.2	96.9	0.7	59.1	8.4	97.0	2.6	80
Exp_7	70.1	1.6	97.3	0.5	71.1	14.4	96.9	1.4	81
Exp_8	69.2	1.8	97.5	0.6	70.0	7.4	98.3	0.9	98
Exp_9	59.5	2.5	97.9	0.6	61.4	3.8	98.8	0.5	99
Exp_10	61.9	1.5	97.6	0.5	63.5	11.7	98.1	1.3	93
Exp_11	63.7	2.5	97.2	0.8	63.5	10.0	97.9	1.9	90
Mean	63.7	2.5	97.2	0.6	64.4	10.4	97.6	1.5	89
SE	1.5	0.2	0.1	0.1	1.3	1.2	0.3	0.2	2.3

Table 2 Comparison of local parameters between reference medical device and testing device under development

Exp. No.	Reference device: Procomp2				Testing device: HTU21D-F				Data acquisition (%)
	Temp (°C)	SD	Hum (%)	SD	Temp (°C)	SD	Hum (%)	SD	
Exp_1	33.2	0.1	67.6	1.2	34.1	0.2	75.7	3.0	77
Exp_2	33.2	0.1	71.8	0.9	34.3	0.1	77.6	0.6	95
Exp_3	31.8	0.1	53.7	0.7	34.0	0.3	77.5	1.1	84
Exp_4	31.2	0.1	53.6	0.5	34.3	0.1	76.1	1.4	84
Exp_5	31.6	0.1	53.7	0.7	33.4	0.3	71.4	1.9	94
Exp_6	34.1	0.1	76.6	3.6	34.3	0.2	75.8	0.7	80
Exp_7	31.6	0.3	65.4	1.2	33.8	0.3	79.5	1.1	81
Exp_8	31.7	0.5	77.3	2.3	33.9	0.2	71.8	1.5	98
Exp_9	31.5	0.3	72.6	2.2	30.7	0.3	71.6	3.8	99
Exp_10	31.2	0.6	57.8	1.9	32.1	0.3	77.4	0.5	93
Exp_11	31.5	0.1	64.0	1.2	32.6	0.4	69.9	3.2	90
Mean	32.1	0.2	64.9	1.5	33.4	0.2	74.9	1.7	89
SE	0.3	0.1	2.8	0.3	0.3	0.03	1.0	0.3	2.3

The following equation was used to convert skin conductance to humidity [10], assuming that the interface between the

palm and the sensor was filled with saturated water vapor: $\text{Hum}(\%) = \left\{ \left(\frac{C}{0.002} \right)^{\frac{1}{3.113}} \right\} \times 10$, where C is the skin conductance (mS)

3.2 Comparison of Local Parameters (Skin Temperature and Humidity)

Table 2 compares the skin temperature and humidity measured using the reference medical device (ProComp2) and testing device. Although significant differences were observed between

the mean values (column comparison), the data deviations were the same for both devices (raw comparison). As skin temperature and humidity are local parameters, these values may differ for the individual fingers chosen. Therefore, the present results suggest that the test device operates with the same level of precision as the reference medical device.

4 Discussion and Conclusion

The data acquisition (%) of the device under development was $89 \pm 7.7\%$ (Tables 1 and 2), which indicates that all of the physiological values measured with the testing devices were within the setting range. While the sensors of the standard medical devices used in the present study were fixed to the skin surface, the sensors of our device were gently placed on the palm of the finger. This configuration is a possible cause of unstable acquisition of data, especially the pulse rate and arterial blood oxygenation sensed on the palm of the distal phalanx in the middle phalanx tissue. There are several limitations in the present preliminary study. The measurement time adopted here was 2 min with a sampling rate of 1 Hz. To analyse the heart rate variability, it was reported that the Poincaré plot could be adequately computed by small data size (60–100 R peaks) [11]. However, further examinations using sufficiently lengthy measurement time and increased number of participants will indicate whether the system under development is useful as a health-monitoring system.

Therefore, we conclude that further improvements in measurements of systemic parameters are required to increase data acquisition beyond 90%.

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Declaration of Competing Interest There is no conflict of interest.

References

1. <https://www8.cao.go.jp/kourei/english/annualreport/index-wh.html> (2023/09/27, view). Annual Report on the Ageing Society [Summary] FY2021
2. Nitzan M et al (2014) Pulse oximetry: fundamentals and technology update. *Med Devices (Auckl)* 7:231–239. <https://doi.org/10.2147/MDER.S47319>. eCollection 2014
3. Yossef HO et al (2018) Pulse oximetry with two infrared wavelengths without calibration in extracted arterial blood. *Sensors (Basel)* 18(10):3457. <https://doi.org/10.3390/s18103457>
4. Gao F et al (2023) Wearable and flexible electrochemical sensors for sweat analysis: a review. *Microsyst Nanoeng* 9:1. <https://doi.org/10.1038/s41378-022-00443-6>
5. Lyzwinski L et al (2023) Opportunities and challenges for sweat-based monitoring of metabolic syndrome via wearable technologies. *Commun Eng* 2:48. <https://doi.org/10.1038/s44172-023-00097-w>
6. Offermann J et al (2021) Acceptance and preferences of using ambient sensor-based life logging technologies in home environments. *Sensors* 21:8297. <https://doi.org/10.3390/s21248297>
7. Facchinetti G et al (2023) Can smart home technologies help older adults manage their chronic condition? A systematic literature review. *Int J Environ Res Public Health* 20:1205. <https://doi.org/10.3390/ijerph20021205>
8. https://github.com/sparkfun/SparkFun_MAX3010x_Sensor_Library/tree/master/examples/Example8_SPO2 (2020/07/21, view)
9. https://github.com/adafruit/Adafruit_HTU21DF_Library (2020/07/21, view)
10. <https://patents.google.com/patent/JPH1119060A/ja> (2023/07/28, view)
11. Chou et al (2021) Effects of ECG data length on heart rate variability among young healthy adults. *Sensors (Basel)* 21(18):6286. <https://doi.org/10.3390/s21186286>

Eumelanin and Pheomelanin Modelling in Optical Oximetry Using Pulse Oximetry (for 540 nm and 660 nm): DC Component

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Abstract

Oximetry is used to quantify the presence of oxygen in soft tissues. It can be expressed as, for example, tissue oxygen saturation (StO_2), arterial oxygen saturation (SaO_2) and pulsatile oxygen saturation (SpO_2), among others. Non-invasive medical devices are used to estimate (SaO_2). Their accuracy is compromised in individuals with highly pigmented skin. The aim of this initial work is to go back few steps into the understanding of the light absorption for the DC component in pulse oximeters, by using a mixtures model for different hypothet-

ical scenarios of normoxia and hyperoxia. Under hypoxic states, an initial and simple multi-wavelength approach could be established to identify the impact of eumelanin (EuM) and pheomelanin (PhM), which are directly related to skin pigmentation in dark skin colour individuals. We used public spectra for water (H_2O), haemoglobin (HHb), oxy-haemoglobin (HbO_2), eumelanin and pheomelanin, to create 1000 possible absorption combinations. These spectra simulations were used to understand the hypothetical limits, across a 450–800 nm wavelength range. These results have outlined the maximum oxy-haemoglobin concentrations that can be detected without interfering with eumelanin and pheomelanin. This initial and simple approach helped us to understand how eumelanin and pheomelanin absorption interferes and overlaps with low oxy-haemoglobin, which is a key biomarker for oxygen quantification in pulse oximeters and other non-invasive biomedical devices.

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Keywords

Arterial oxygen saturation (SaO_2) · Pulsatile oxygen saturation (SpO_2) · Haemoglobin (HHb) · Oxy-haemoglobin (HbO_2) · Skin pigmentation · Photoplethysmogram (PPG)

1 Introduction

Pulse oximeters are commonly used in clinical settings. However, during the calibration process, only a limited number of highly pigmented skin participants were taken into consideration. The devices are also calibrated within a limited range of blood-oxygen saturation (~70% to 100%). Furthermore, Sjoding [1] and Dyer [2] performed a wide cohort study (with more than 1000 volunteers with high skin pigmentation) and identified that pulse oximeters can overestimate the blood oxygen level for people with highly pigmented skin. This issue is now officially recognised by the US Food and Drug Administration (FDA) [3]. Therefore, the scope of this work was to look at the DC component of the signal used for oximetry calculations as a starting point. Considerations such as noise were not accounted for in the simulated spectra profiles since it may interfere with this simple and initial proposed model. Therefore, the aim was to develop this simple mixing model in order to identify and provide opportunities in the wavelength range currently available, 450–800 nm. However, the AC component of pulse oximeter signals was not considered, and wavelength range from 800 up to 940 nm was not available. Moreover, the aim of our model is to have a better understanding of the key role that eumelanin and pheomelanin have on the optical measurements. We also introduce the benefits of a multi-wavelength approach on hypothetical scenarios, where it could avoid experimental calibration procedures, since current oximetry principles do not include pigmentation (eumelanin and pheomelanin) detection in pulse oximeter technology. This had an impact in many countries during the COVID-19 pandemic [4–6] especially in highly pigmented populations.

2 Material and Methods

We used public data sets and methods from Prahla and Jacques [7] to create an initial linear mixtures model for the DC component for oxygen mea-

surements (no noise was considered), using water, HbO₂, HHb, eumelanin and pheomelanin molar extinction coefficients (Fig. 1). Eumelanin and pheomelanin absorbances were calculated (Eqs. 1 and 2) by using concentrations of 1% constant for pheomelanin and 1–3.5% for eumelanin (Fig. 3). These values are based on Wakamatsu and Ito [8] where the typical ranges are eumelanin (25–33%) and pheomelanin (10–14%).

These initial assumptions were made to understand how their presence in the epidermis can interfere in oximetry DC signals (Figs. 2 and 3), calculation of absorbances across the spectrum, where A is the absorbance, $\epsilon(\lambda)$ epsilon lambda represents the molar extinction coefficient, C the concentration and L is the path length.

$$A = \epsilon(\lambda)CL \quad (1)$$

All percentages shown in this work were based on the value ranges mentioned by Steven Jacques [7]. Data were processed and analysed with MATLAB 2019a (statistics toolbox). Multi-wavelength data usage was divided into multiple steps as follows. (A) Data were used from plain text files containing molar extinction coefficients versus wavelength. (B) Data curation; HbO₂, HHb, water, eumelanin and pheomelanin, molar extinction coefficients data was matched similarly at every single wavelength position, allowing molar extinction coefficients data at the same wavelength range. (C) Data preparation; multiple absorbance combinations (1000 hypothetical combinations for each biomarker) of HbO₂ and eumelanin and pheomelanin were produced using eqs. 1 and 2 (linear mixtures) [6], where tA is the total light absorbance, $\epsilon(\lambda)_{\text{HbO}_2}$ is the molar extinction coefficient for oxy-haemoglobin, $\epsilon(\lambda)_{\text{HHb}}$ is the molar extinction coefficient for haemoglobin, $\epsilon(\lambda)_{\text{EuM}}$ is the molar extinction coefficient for eumelanin, $\epsilon(\lambda)_{\text{PhM}}$ is the molar extinction coefficient for pheomelanin, $\epsilon(\lambda)_{\text{H}_2\text{O}}$ is the molar extinction coefficient for water, C is the corresponding concentration for each compound and L is the pathlength (length was implemented making the assumption of 1 cm length standard cuvette).

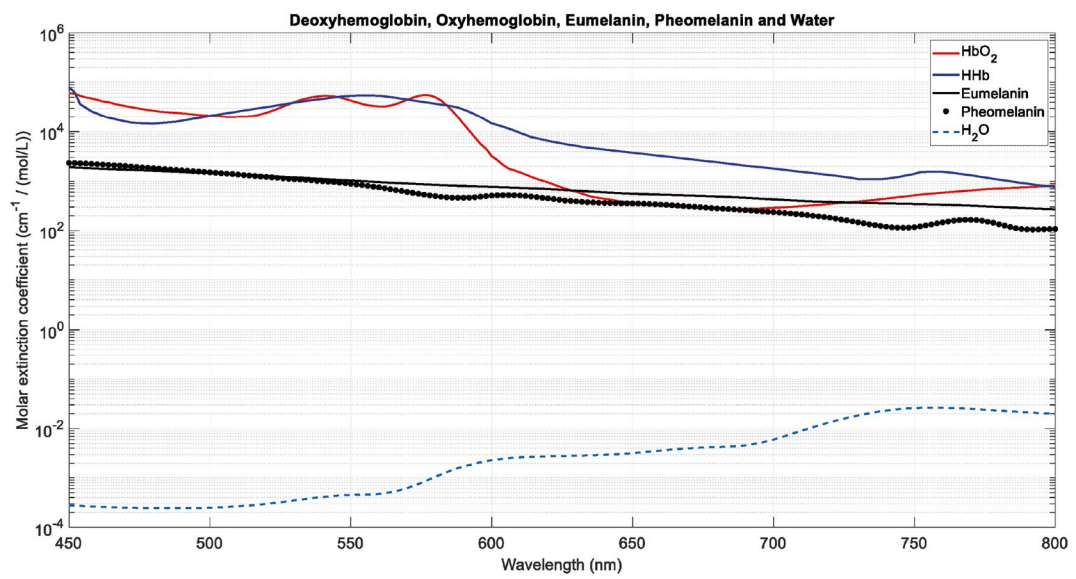


Fig. 1 Log plot of molar extinction coefficients of deoxy-haemoglobin, oxy-haemoglobin, eumelanin, pheomelanin and water from 450 nm to 800 nm. (Modified from Ref. [7])

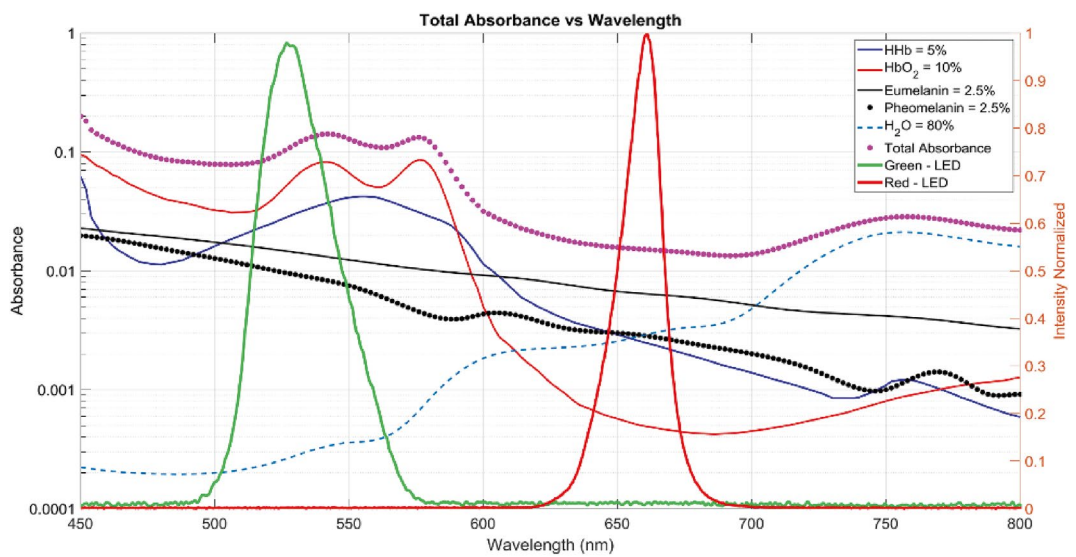


Fig.2 Log scale plot of absorbances of oxy-haemoglobin, deoxy-haemoglobin, eumelanin, pheomelanin and water obtained through simulations. Initial assumption was HHb=5%, HbO₂ = 10%, EuM = 2.5%, PhM = 2.5%, H₂O = 80%. Spectra of green (typically centered at 540 nm) and red (typically centred at 660 nm) LEDs are shown. These wavelengths are used as candidate of the reference wavelength in the visible range for many dual wavelength oximeters. The pink dots indicate the total absorbance from 450 nm to 800 nm

$$tIA = [\epsilon(\lambda)_{HbO_2} C_{HbO_2} + \epsilon(\lambda)_{HHb} C_{HHb} + \epsilon(\lambda)_{EuM} C_{EuM} + \epsilon(\lambda)_{PhM} C_{PhM} + \epsilon(\lambda)_{H_2O} C_{H_2O}] L \quad (2)$$

(D) Data analysis: our modelled absorbance data was used to create new hypothetical scenarios without performing experiments that will result in long hours, repetition validation and costs associated to laboratory equipment, consumables, and time efforts. However, specific experiments will be required in the future. An initial understanding of eumelanin and pheomelanin linear mixing was split into rational simulations. The simulation in Fig. 2 identifies the main optical window where HbO₂ and HHb can be detected without eumelanin and pheomelanin mixing. However, there is an optical interference or overlapping; as we increase the concentration of eumelanin and pheomelanin, they become the dominant biomarkers over the optical range where common pulse oximeters and other non-invasive biomedical devices are operational. Figures 2 and 3 show situations where the pres-

ence of both in similar quantity can be seen as interaction of eumelanin and pheomelanin versus HbO₂ [7].

3 Results

Simulations (Figs. 2 and 3) were performed with a combination of multiple absorbances versus multiple concentrations of EuM and PhM accordingly, where arrows A-B in Fig. 3 indicate the optical gap range that affects especially the green channel for many dual wavelength oximeter devices, especially between 500 and 570 nm, C-D, in Fig. 3, indicating the optical gap range that affects especially the red channel for many dual wavelength oximeters devices, especially between 620 and 660 and > 680 nm. These simulations had hypothetical concentrations, suggest-

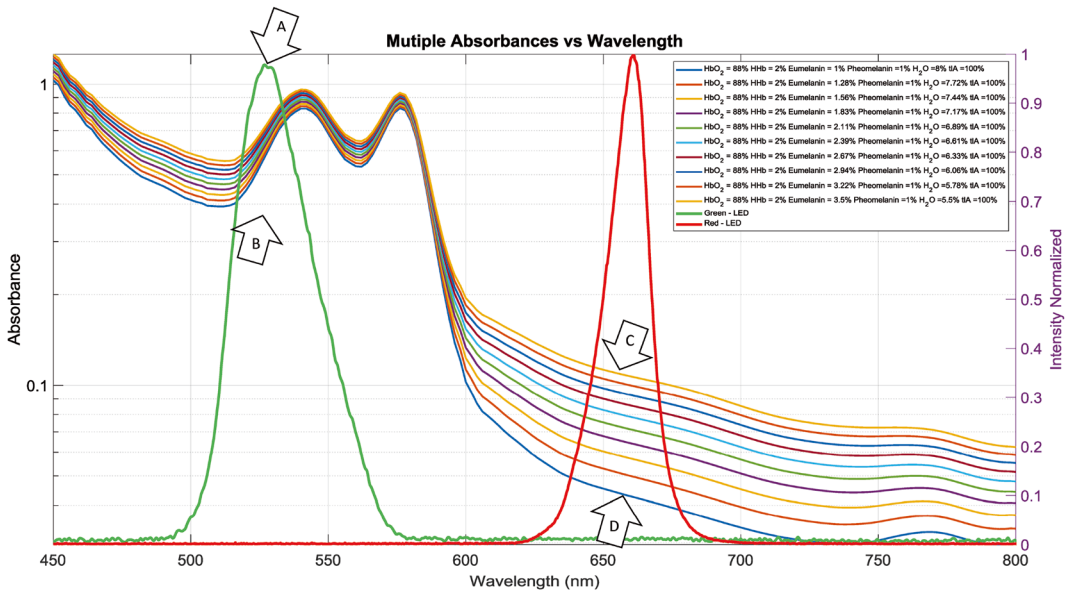


Fig. 3 Log plot of multiple absorbances possible combinations with HbO₂ = 88%, HHb=2%, EuM = 1%–3.5%, PhM = 1% and H₂O = 5.5%–8%, tIA of 100% obtained through simulations. Spectra of green (typically centred at

540 nm) and red (typically centred at 660 nm) LEDs are shown. These wavelengths are used as candidate of the reference wavelength in the visible range for many dual wavelength oximeters. (A–D) Arrows indicates eumelanin increments

ing that another wavelength range must be used, or that compensation in the DC component should be taken into consideration.

A simulation (Fig. 3) where mixed biomarkers could be considered as a realistic scenario by having changing concentrations of EuM and PhM at the same time demonstrates how their presence interferes with HbO₂ detection. The correlation analysis took mixed spectral profiles (Fig. 3C, orange line) HbO₂ = 88%, EuM = 3.5%, PhM = 1% and compared them against mixed spectral profile (Fig. 3D, blue line) HbO₂ = 88%, EuM = 1%, PhM = 1%, without taking into consideration the scattering effect or other dis-haemoglobins.

4 Discussion and Conclusions

These calculations serve as an approach to enable future oximetry calculations for DC signals in pulse oximetry technology. However, any oximetry calculation should include water, eumelanin, and pheomelanin, where pigmentation is a confounder and two wavelengths cannot compensate for its detection, but a multi-wavelength approach provides an opportunity [9, 10] to also improve motion artifact, quick response, etc. Future work will be to perform experiments using phantoms and extend this model into photoplethysmogram (PPG) signals (AC component). We also conclude that skin types (Fitzpatrick scale) should be avoided for any oximetry purposes [6], since it leads to misinterpretations and lack of accuracy in referring to skin pigmentation quantification. More accurate methods for skin pigmentation should be developed. All previous calculations can be improved further by adding the PPG component.

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References

1. Sjoding MW, Dickson RP, Iwashyna TJ, Gay SE, Valley TS (2020) Racial bias in pulse oximetry measurement. *N Engl J Med* 383:2477–2478
2. Dyer O (2020) Pulse oximetry may underestimate hypoxaemia in black patients, study finds. *BMJ* 371:m4926
3. Food, U. S. & Administration, D (2021) FDA – Pulse oximeter accuracy and limitations: FDA safety communication
4. Ferrari M, Quaresima V, Scholkmann F (2022) Pulse oximetry, racial bias and statistical bias: further improvements of pulse oximetry are necessary. *Ann Intensive Care* 12:1–2
5. Kyriacou PA, Charlton PH, Al-Halawani R, Shelley KH (2023) Inaccuracy of pulse oximetry with dark skin pigmentation: clinical implications and need for improvement. *Br J Anaesth* 130:e33–e36
6. Cabanas AM, Fuentes-Guajardo M, Latorre K, León D, Martín-Escudero P (2022) Skin pigmentation influence on pulse oximetry accuracy: a systematic review and bibliometric analysis. *Sensors* 22:3402
7. Prah S, Jacques S. OMLC (<https://omlc.org/index.html>, 16 March 2023)
8. Wakamatsu K, Ito S (2002) Advanced chemical methods in melanin determination. *Pigment Cell Res* 15:174–183
9. Norton HL (2022) Variation in pulse oximetry readings: melanin, not ethnicity, is the appropriate variable to use when investigating bias. *Anaesthesia* 77:354–355
10. Aoyagi T, Fuse M, Kobayashi N, MacHida K, Miyasaka K (2007) Multiwavelength pulse oximetry: theory for the future. *Anesth Analg* 105:S53–S58

Fabrication of Tuneable Tissue-Mimicking Phantom for Optical Methods

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AbstractBackground

Tissue mimicking optical phantoms are commonly used to calibrate or validate the performance of near-infrared spectroscopy or tomography. Human tissue is not only irregular in shape, but also exhibits dynamic behaviour, which can cause changes in optical properties. However, existing phantoms lack complex structures and/or continuously varying optical properties. Aim

The project aimed to design, fabricate and characterise a novel phantom system for testing near-infrared imaging devices. Material and Methods

We designed a dynamic tissue-mimicking phantom platform which features arbitrary internal shapes and variable optical properties. The solid part of phantom was made of silicone material with absorbing and scattering properties similar to the brain. We printed a semi-ellipsoidal sphere (a major axis = 20 mm and a minor axis = the third axis = 12 mm) using a water-soluble material polyvinyl alcohol (PVA). The shape was placed at the

depth of 5 mm in the silicone bulk. The desired internal hollow structure was formed after curing and submerging the phantom in water. The liquid part contained dyes and Intralipid. The optical properties within the internal shape were adjusted by injecting the liquid solutions of varying dye concentrations with a syringe pump at a constant rate. The phantom was measured by a frequency domain near-infrared spectroscopy (FD NIRS) and imaged by a time domain near-infrared optical tomography (TD NIROT). Results and Discussion

A dynamic phantom system with a complex internal structure and varying optical properties was created. Changes in light intensity were detected by the FD NIRS. The internal structure of this phantom was accurately recovered by NIROT image reconstruction. Conclusion

We successfully developed a novel phantom system with an internal complex shape and continuously adjustable optical properties. This phantom was accurately imaged using NIROT, and the changing light intensity was detected by NIRS. It is a valuable tool for validating optical technologies.

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Keywords

Optical phantoms · Dynamic phantoms ·
Near-infrared spectroscopy · Near-infrared
tomography · Optical devices

1 Introduction

Near-infrared tomography and spectroscopy are widely used to measure the optical properties of tissue [1]. Biological tissue not only has an irregular shape, but also changes dynamically, for example, through cellular damage, genetic mutations [2], or changes in activity or oxygenation, which cause changes in optical properties of this tissue [3]. For the evaluation of the performance of the near-infrared devices, realistic tissue-mimicking phantoms need to be used. Tissue phantoms mimic the optical properties of biological tissues, along with other essential elements of the imaging process [4]. The control in absorption and scattering properties is the main design objective for these phantoms; meanwhile, fabrication ease, cost, and long-term stability are also important [5]. We have previously designed inhomogeneous solid phantoms to simulate brain hypoxia and haemorrhage [6], and a liquid phantom incorporating motor-controlled movable solid inclusions [7]. Nevertheless, there is a need to image local haemodynamic changes and a respective phantom is missing. To increase the phantom complexity in this aspect, we previously created a phantom that consisted of an internal structure based on microfluidic chips with a controllable dynamic flow [8]. However, the internal structure of the phantom is not ideal for NIROT due to micrometre scale of the internal structure. In this paper, the aim is to introduce a new dynamic phantom featuring both complex internal structures and changeable local optical properties.

2 Materials and Methods

2.1 Phantom Fabrication

To fabricate the solid bulk of phantom, we used SILPURAN® 2420 two-component silicone (Wacker, Germany) to produce the solid bulk of the phantom. Silicone-compatible white ink ELASTOSIL® RAL 9010 (Wacker, Germany) was added to obtain the target reduced scattering coefficient. The absorption coefficient was

adjusted by adding carbon black powder (Wacker, Germany). The India ink was first mixed with component A of the silicone and then component B was added to the mixture. The mixture was degassed with a vacuum pump to avoid air bubbles and then cured in a mould. The absorption coefficient μ_a of the homogeneous silicone bulk was 0.0618 cm^{-1} , 0.0611 cm^{-1} , 0.0589 cm^{-1} and 0.0566 cm^{-1} at 689 nm, 725 nm, 800 nm and 838 nm, respectively while the reduced scattering coefficient $\mu'_s = 4.7971 \text{ cm}^{-1}$, 4.4593 cm^{-1} , 4.0048 cm^{-1} and 3.9760 cm^{-1} . Then the silicone mixture with same optical properties was poured in the mould at the depth of 5 mm from the measurement surface. There was a 3D PVA-printed (Ultimaker, Netherlands) semi-ellipsoidal (major axis = 20 mm, minor axis and the third axis = 12 mm). The rest of the silicone mixture was poured on the top of the printing tube then cured. This bulk was immersed in water to dissolve the PVA material, thus creating a cavity in the middle of the bulk in the shape of the printed part. The whole fabrication procedure is depicted in Fig. 1.

2.2 Measurement with FD NIRS and NIROT

As shown in Fig. 2a, the dynamic phantom was measured using a NIROT system while the concentration of the injected ink solution increased over time. The imaging system and image reconstruction method were described in [9, 10]. The measurement was performed using laser emissions with an exposure time of 500 ms at a wavelength of 689 nm. The initial liquid in the container was a mixture of 1.1 g of 50 g/mL dye (Trodax AG, Austria), 60 mL Intralipid (Fresenius Kabi AG, Bad Homburg, Germany) and 1940 mL distilled water, was $\mu_a = 0.095 \text{ cm}^{-1}$ and $\mu'_s = 6.2 \text{ cm}^{-1}$. Additionally, to characterise the μ_a of the ink solution at different concentrations, unscattered transmittance measurements were made for diluted ink at 0.001 g/mL in the range 350 to 1100 nm using a spectrometer system (PerkinElmer, US) to calculate the absorbance of the attenuat-

Fig. 1 (a) The fabrication process of a solid phantom with a hollow structure inside. (b) The 3D illustration of the phantom used in this study

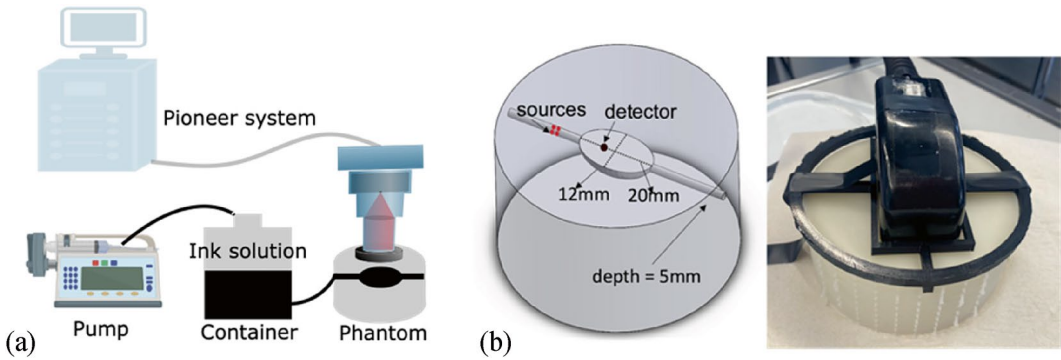
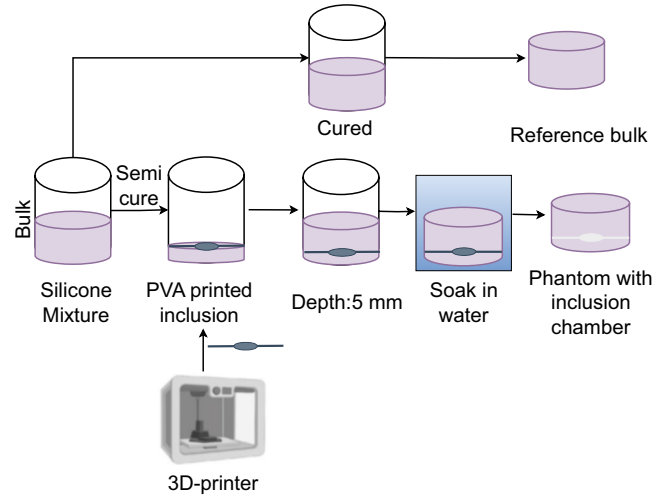


Fig. 2 (a) Schematic of dynamic phantom system. It consisted of a pump to inject high absorbing liquid into a container at a controlled speed. Then the ink solution from the container flowed into the silicone phantom. The concentration of the ink in the solution steadily increased and therefore the absorption μ_a of the internal

chamber also rose. (b) 4 sources (red dots) and a detector (black dot) of the probe of the NIRS instrument were used in the measurement. The source-detector separation was 1.5 cm for the sources at wavelength 800 nm and 725 nm, 1.7 cm for those at 689 nm and 838 nm

ing species. To achieve the concentration gradient, the pump introduced 50 g/mL of ink into the container at a rate of 99 mL per hour. As the ink flowed through the container, it gradually entered the inclusion chamber of the solid phantom. Increasing ink concentrations from 0.55 to 5.5×10^{-4} g/mL at a step of 0.55×10^{-4} g/mL were injected into the phantom once manually and once controlled by the pump. The ISS Imagent™ FD NIRS (ISS, USA) measured the corresponding optical changes at wavelengths of 689 nm, 725 nm, 800 nm and 838 nm, as shown in Fig. 2b.

3 Results and Discussion

We designed and developed a NIRS and NIROT phantom system that combined the advantages of liquid and solid phantoms. This phantom system had a stable internal structure, while the liquid in the internal structure varied with a stable concentration gradient. As shown in Fig. 3, the semi-ellipsoidal structure inside the phantom was successfully reconstructed under the NIROT test, and the position and shape of the semi-ellipsoidal sphere were accurate. The concentration change

Fig. 3 The internal structure of this phantom was reconstructed from a NIROT measurement. The black dots represent the image reconstruction and the grey shape the ground truth of internal structure

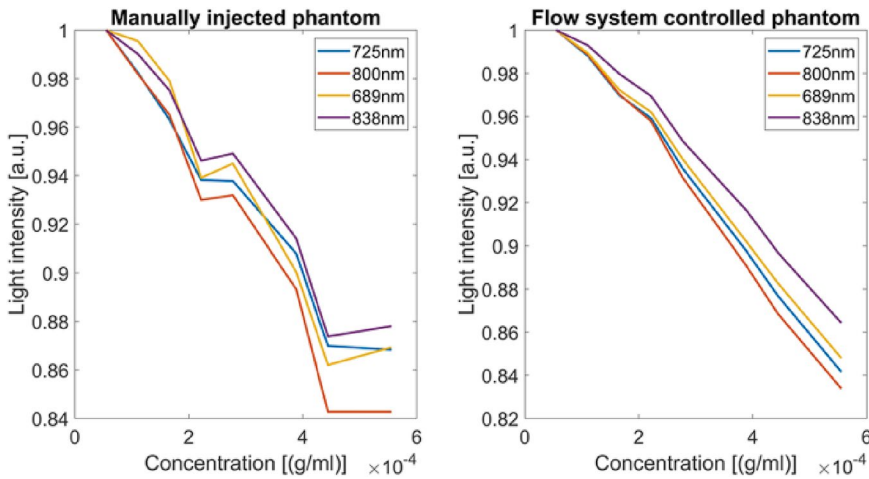
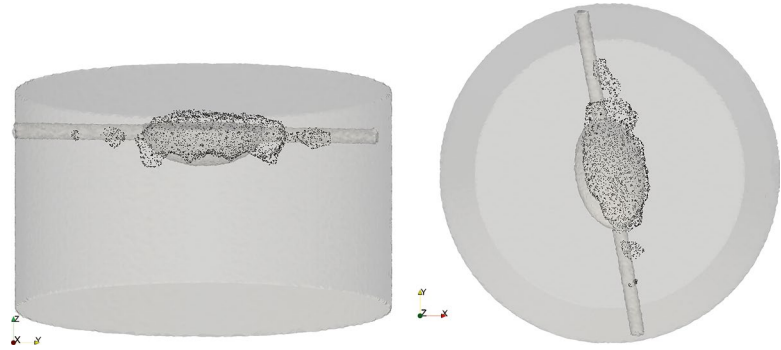


Fig. 4 Light intensity was measured with an FD NIRS instrument at various ink concentrations, manual (left) and pump controlled (right) injection

and trend of the liquid inside the phantom were also accurate under FD NIRS test.

The dye of the ink solution was characterised, and the extinction coefficient of the ink was $152.0, 132.0, 172.7$ and $125.6 \text{ cm}^{-1} / (\text{g/mL})$ at $725 \text{ nm}, 800 \text{ nm}, 689 \text{ nm}$ and 838 nm , respectively, when the ink concentration was 0.001 g/mL . The ink solution with different absorption coefficients could be easily prepared to simulate the different tissue types.

As illustrated in Fig. 4a, the phantom with the manually injected liquid and the phantom with the pump-controlled injection system were also compared. The changes in light intensity were not smooth during the manual injection because the ink solution could not be prepared accurately at some points. In contrast, the pumped ink solution gave a smooth decreasing trend in intensity

because the 50 g/mL dye was pumped into the container at a constant speed by a pump. This resulted in a stable increase in the concentration in the ink solution. Figure 4 shows the changes in the intensity at different wavelengths. In summary, a phantom with an arbitrary shape and a stable dynamic optical property has been successfully designed and produced. It can be used to simulate the complex structure and the dynamic behaviour of biological tissues.

To quantify the concentration of the ink solution, the measured light intensity decreased proportionally with the concentration of the ink solution while the phase from FD NIRS increased. To account for the delay of the ink reaching the field of view of the NIRS instrument, future studies could measure the ink solution at the inlet and outlet of the phantom. In

addition, the blood-containing fluid can be injected into the phantom to validate oximeters or other optical devices [11].

4 Conclusion

We successfully created a dynamic phantom with a complex internal structure. An image of the internal structure was accurately reconstructed by NIROT. Dynamic changes were visible in the NIRS measurement. The flow system created changes in ink concentration that were more precise, and smoother compared to the manual method. This phantom can test and validate multiple optical modalities.

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Conflicts of Interest Martin Wolf declares that he is the president of the board and co-founder of OxyPrem AG and Alexander Kalyanov works partially for this company.

References

1. Hacker L, Wabnitz H, Pifferi A et al (2022) Criteria for the design of tissue-mimicking phantoms for the standardization of biophotonic instrumentation. *Nat Biomed Eng* 6:541–558
2. Mateus R, Fuhrmann JF, Dye NA (2021) Growth across scales: dynamic signaling impacts tissue size and shape. *Curr Opin Cell Biol* 73:50–57
3. Nordstrom RJ (2011) Phantoms as standards in optical measurements. In: Nordstrom RJ, Coté GL (eds). San Francisco, California, USA, p 79060H
4. Pekar J, Patterson MS (2010) Fabrication and characterization of phantoms with tissue-like optical properties from 500 to 700nm. *Med Laser Appl* 25:147–153
5. Jiang J, Lindner S, Di Costanzo-Mata A et al (2021) In phantom validation of time-domain near-infrared optical tomography Pioneer for imaging brain hypoxia and hemorrhage. In: Nemoto EM, Harrison EM, Pias SC et al (eds) *Oxygen transport to tissue XLII*. Springer International Publishing, Cham, pp 341–346
6. Jiang J et al (2022) Resolution and penetration depth of reflection-mode time-domain near infrared optical tomography using a ToF SPAD camera. *Biomed Opt Express* 13:6711
7. Li T, Kalyanov A, Wolf M et al (2023) BIAN: a multilayer microfluidic-based tissue-mimicking phantom for near-infrared imaging. In: Scholkmann F, LaManna J, Wolf U (eds) *Oxygen transport to tissue XLIV*. Springer International Publishing, Cham, pp 179–183
8. Ayers F, Grant A, Kuo D et al (2008) Fabrication and characterization of silicone-based tissue phantoms with tunable optical properties in the visible and near-infrared domain. In: Nordstrom RJ (ed). San Jose, CA, p 687007
9. Jiang J, Di Costanzo Mata A, Lindner S et al (2020) Image reconstruction for novel time domain near infrared optical tomography: towards clinical applications. *Biomed Opt Express* 11(8):4723–4734
10. Jiang J, Di Costanzo Mata A, Lindner S et al (2022) 2.5 Hz sample rate time-domain near-infrared optical tomography based on SPAD-camera image tissue hemodynamics. *Biomed Opt Express* 13(1):133–146
11. Kleiser S, Hyttel-Sorensen S, Greisen G, Wolf M (2016) Comparison of near-infrared oximeters in a liquid optical phantom with varying intralipid and blood content. In: Elwell CE, Leung TS, Harrison DK (eds) *Oxygen transport to tissue XXXVII*, vol 876, pp 413–418

Imaging Deep Haemorrhage and Ischaemia in Preterm Infants' Heads with Time-Domain Near-Infrared Optical Tomography: Phantom Study

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Abstract Background and Aim

The occurrence of brain lesions in preterm infants is common and can result in lasting disabilities. To prevent these and to safeguard the brain through therapeutic measures or neuroprotective treatments, it is important to identify cerebral ischaemia/hypoxia and haemorrhage at an early stage. For this purpose, we have successfully developed a cutting-edge time-domain near-infrared optical tomography (TD-NIROT) system, which offers diagnostic imaging for neonatal brain oxygenation. Our objective is to validate the effectiveness of the TD-NIROT in detecting deep ischaemia/hypoxia and haemorrhages through phantom experiments. Methods

Spherical silicone phantoms were fabricated to replicate the head of preterm infant. To simulate the lesions, we made two head phantoms and embedded small inclusions mimicking ischaemia and haemorrhage at the

depth of 30 mm. Additionally, a spherical interface was constructed to connect the spherical phantom to the imaging system, allowing us to collect time-domain data. Following the data acquisition, we proceeded with image reconstruction. Dice similarity was used as an indicator of the accuracy and similarity between the reconstructed images and the ground truth. Results and Discussion

The resulting images exhibited an accurate location of haemorrhage and detected the ischaemia with a slightly shifted position with Dice similarity of 0.47 and 0.27. Conclusion

Our experiment validates the capability of our TD-NIROT system in successfully detecting deep haemorrhages and ischaemia within the phantom model. The achieved results suggest a promising level of accuracy in the imaging process. These findings are encouraging to continue this work to ultimately achieve clinical application of the TD-NIROT system in diagnosing and monitoring neonatal brain injuries.

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Keywords

Brain oxygen saturation · Diffuse optical tomography · Time of flight imaging · Brain hypoxia

1 Introduction

Premature birth is a critical medical condition that affects millions of infants worldwide. Unfortunately, preterm infants are particularly vulnerable to various health complications, with intracranial haemorrhage and white matter disease being two of the most critical and potentially life-threatening conditions. Prompt and accurate diagnosis of head haemorrhage is crucial for early intervention [1, 2]. While haemorrhage is visible immediately in ultrasound scans, white matter disease is only visible in ultrasound ~3 weeks after the incidence when irreversible damage occurred. Hypoxia is one of the key factors leading to haemorrhage and white matter disease. Our rationale is that if hypoxia was detected in time, it could be treated and brain lesions could be prevented. Since brain lesions are localised, we expect that hypoxia is a localised event as well, and therefore, an imaging tool is required to detect such and adverse event. In recent years, near-infrared imaging (NIRI) has emerged as a promising non-invasive technique for studying cerebral haemodynamics in infants [2]. By utilising near-infrared (NIR) light, NIRI enables the assessment of tissue oxygenation and haemoglobin concentration changes [3], providing valuable insights into cerebral oxygenation and detecting possible intracranial haemorrhage. Among the various NIRI modalities, time-domain near-infrared optical tomography (TD-NIROT) has gained increasing attention due to its ability to reconstruct images with high accuracy and depth sensitivity [3–6]. TD-NIROT utilises pulsed near-infrared light and measures the time-of-flight of photons to create detailed 3D volumes of tissue absorption and scattering properties. We have recently developed a single-photon avalanche diode (SPAD) array-based TD-NIROT [7, 8]. This technology offers the potentials for improved accuracy in detecting and characterising intracranial haemorrhage and hypoxia in preterm infants [9], enhancing clinicians' ability to deliver targeted interventions and monitor therapeutic responses. We have previously performed a simulation study on the detection of ischaemia and haemorrhage at various

depths with a TD-NIROT and showed its feasibility [9]. The aim of this study was to evaluate the performance of the TD-NIROT system through an experimental study simulating deep ischaemia and haemorrhage in neonatal brain phantom.

2 Methods and Materials

2.1 Experimental Setup

The experimental setup is briefly described in Fig. 1. The imaging instrument comprises a light source, which is a super-continuum laser SuperK Extreme EXR-15 (NKT, Denmark). This laser emits picosecond light pulses with a wide spectrum in the near-infrared (NIR) region. To perform spectral measurements, an acousto-optic tuneable filter (AOTF) is employed, which allows for the selection of specific wavelengths. Furthermore, a fibre switch is utilised to distribute these pulses to the optical fibres, which then direct the light towards the tissue. The detailed description of the TD-NIROT system is in [8]. Curved interfaces are fabricated to securely attach the SPAD camera and optical fibres onto the surface of the head phantoms. Nine optical fibres are utilised to direct laser light to the surface of the phantom and the detection probe is placed on the opposite side, as shown in Fig. 1, creating a transmission-mode modality. One of the nine sources is placed at the centre, while the other eight sources are evenly distributed along a circle with a radius of 12 mm. The detection probe comprises a NIR lens SY110M 1.68 mm F1.8 (Theia Technologies, US) to project the light emitted from the tissue within the field of view onto a custom-made 32 × 32-pixel single photon avalanche diodes (SPAD) sensor [7]. Each pixel of the SPAD array was projected to a size of approx. 1.07 mm in this experiment. The SPAD sensor detects the arrival times of photons. These photon arrival times are processed by a Xilinx Kintex 7 FPGA board (XEM7360, Opal Kelly, USA) to construct the probability functions of photon arrival times for each individual pixel. This time-domain data set is sent to a workstation for further analysis.

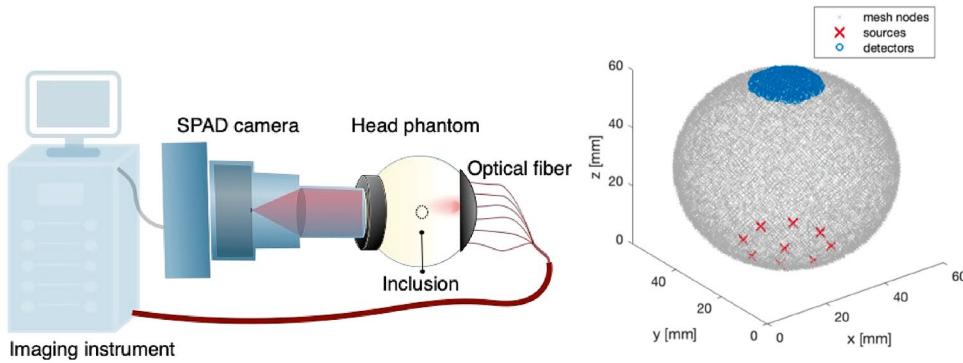


Fig. 1 Left: Schematic of the measurement on the head phantom with a haemorrhage mimicking inclusion in the centre. Right: Mesh model with nine sources and selected

132 detectors placed in transmission mode as in the measurement

To mimic the cases of hypoxia and deep haemorrhage in a preterm head, we produced a spherical silicone phantom of 60 mm diameter and placed a strong spherical absorber of 10 mm in diameter in the centre to represent haemorrhage. The bulk material optical properties were absorption coefficient $\mu_a^B = 0.078 \text{ cm}^{-1}$ and reduced scattering coefficient $\mu_s'^B = 4.1 \text{ cm}^{-1}$ at 689 nm. The optical properties were obtained from [2] for the head of a preterm neonate at the gestational age of 32 weeks. We made an estimate for the two μ_a values of haemorrhage and ischaemia. The hypoxia–ischaemia means that oxy-Hb was converted to deoxy-Hb, resulting in high deoxy-Hb and low oxy-Hb concentration, which leads to an elevated μ_a by a factor of 2.6 at 689 nm. Haemorrhage means pockets of pure blood in the brain. Since the concentration of blood is $\sim 2 \text{ ml/100 g}$ of brain, pure blood will have ~ 50 times more haemoglobin than brain tissue, which corresponds to 50 times higher μ_a , since blood is the main absorber in the NIR. Therefore, μ_a of the ischaemia and haemorrhage are ~ 2.6 and ~ 50 times higher than that of the surrounding material and μ_s' remains the same as the bulk. The recipe and procedures of the phantom creation are described in [5].

2.2 Image Reconstruction and Data Processing

We employed a model-based image reconstruction method. The light propagation in tissue was approximated by diffusion equations (DE) and we employed a finite-element method based on the open-source software NIRFAST to solve the DE [10].

A spherical mesh was created in NIRFAST consisting of 83,721 nodes (Fig. 1, right). A total of nine sources, with one centrally located and the remaining eight evenly arranged in a circle, were utilised. Additionally, 132 detectors were selected and positioned on the opposite side, as depicted in Fig. 1, right. A total of 1188 temporal point spread functions (TPSFs) were selected. The TPSFs were first transformed into Fourier domain and the amplitude and phase data at 100 MHz were used in the image reconstruction. The optical properties were retrieved using modified Tikhonov minimisation approach, starting with an initial guess of a homogeneous phantom characterised by the optical properties of the bulk material. The initial regularisation parameter lambda was set to 10, and a coarser mesh of 1473 nodes was generated for the reconstruction pro-

cess to reduce the memory burden and speed up the process. The reconstructed results were segmented using a threshold of half of the maximum value, and the recovered inclusion was compared with the actual inclusion location. The detailed image reconstruction and pre-processing procedures are described in [5]. We used the Dice similarity to evaluate the performance of the image reconstruction [9]. A Dice index close to 1 indicates high similarity between the reconstructed and ground-truth images.

3 Results and Discussion

In this study, we employed transmission-mode configuration of sources and detectors. The SPAD camera received 3.8×10^5 photons/second on average for the source placed at the

centre of the opposite side. The exposure time in this experiment was 2 min. Image reconstruction was performed, and the optimisation process finished at the 14th iteration for the haemorrhage case (Fig. 2) and fifth iteration for the ischaemia case (Fig. 3), as it achieved the minimum deviation between the estimated forward results and the measured values. The reconstructed μ_a in the region of the inclusion in Fig. 2 displayed significantly lower values compared to the actual values. This phenomenon arises from the optimisation algorithm with Tikhonov regularisation in image reconstruction, which tends to converge more readily towards a solution with smaller variations in μ_a s. The resulting 3D volumes of absorptions were then segmented through thresholding with a threshold of half of its maximum value to obtain the final estimation of the

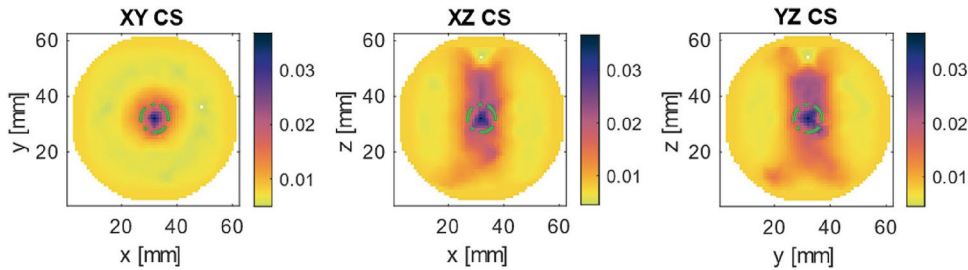


Fig. 2 Reconstructed images for the haemorrhage case displaying absorption coefficients in three central cross-sectional views, with the ground truth location highlighted

by a green dashed circle. The colour bars on the right indicate the values of μ_a in mm^{-1}

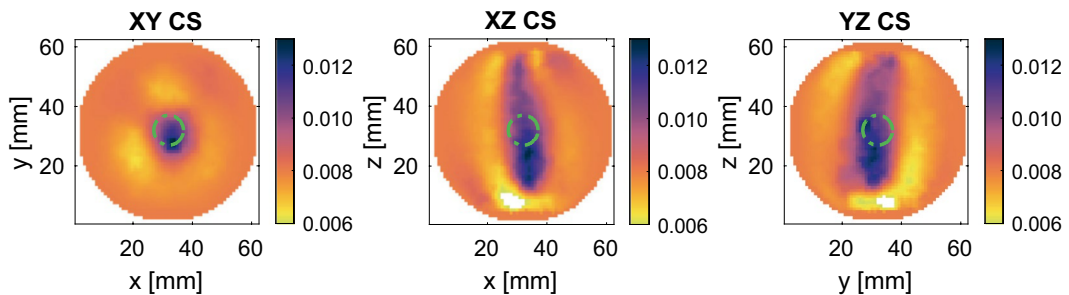


Fig. 3 Reconstructed images for the ischaemia case displaying absorption coefficients in three central cross-sectional views, with the ground truth location highlighted

by a green dashed circle. The colour bars on the right indicate the values of μ_a in mm^{-1}

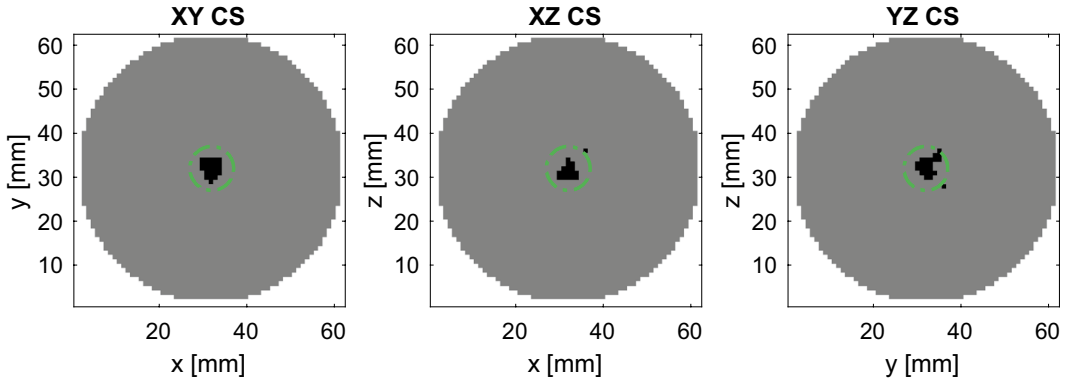


Fig. 4 Reconstruction results after segmentation for the haemorrhage-phantom experiment in three cross-sectional views. The ground truth inclusions are indicated

by green dashed circles, while the reconstructed inclusion is depicted as a black region

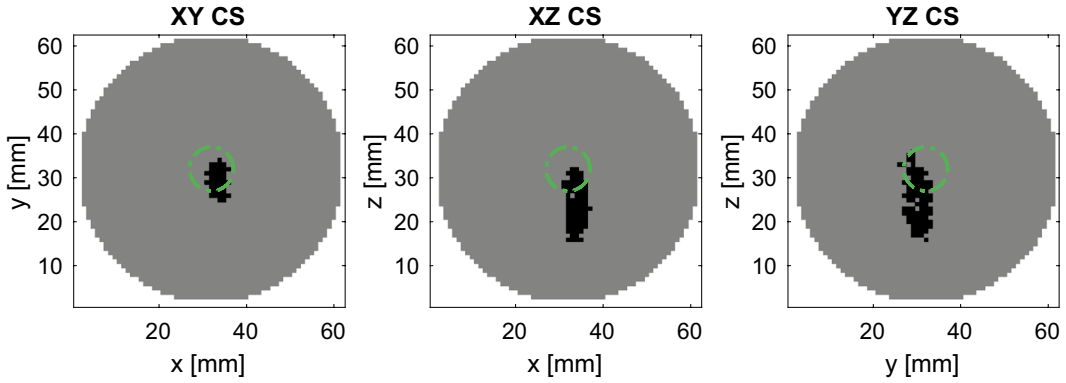


Fig. 5 Reconstruction results after segmentation for the ischaemia-phantom experiment in three cross-sectional views. The ground truth inclusions are indicated by green

dashed circles, while the reconstructed inclusion is depicted as a black region

haemorrhage (Fig. 4) and ischaemia regions (Fig. 5). In the case of haemorrhage (Fig. 4), even though the segmented inclusion region is smaller than the actual one, there is a high agreement between the recovered inclusion and the ground-truth inclusion, with Dice index of 0.47. In the case of ischaemia, the Dice index was 0.27. The lower Dice index in this case resulted from the lower contrast ($2.6\times$) in μ_a and the mismatch in the model positions of the sources and detectors during data processing, compared to those in the measurements. In the future, the accurate determination of the placement of sources and detectors on a curved surface can be further

enhanced to achieve greater accuracy in the reconstructed images.

4 Conclusion

The TD-NIROT has demonstrated remarkable capabilities in detecting deep haemorrhage and ischaemia mimicking inclusions with a high level of accuracy in phantom experiments. With its transmission-mode measurement, we have successfully detected inclusions at a depth of 30 mm. These results highlight the effectiveness and potential of the system to be employed in clinical applications.

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Conflicts of Interest Martin Wolf declares that he is president of the board and co-founder of OxyPrem AG, and Alexander Kalyanov works partially for this company.

References

1. Wolf M, Bucher HU, Dietz V et al (1997) How to evaluate slow oxygenation changes to estimate absolute cerebral Haemoglobin concentration by near infrared spectrophotometry in neonates. In: Nemoto EM et al (eds) Oxygen transport to tissue XVIII. Advances in experimental medicine and biology, vol 411. Springer, Boston
2. Arri S, Muehlemann T, Biallas M et al (2011) Precision of cerebral oxygenation and hemoglobin concentration measurements in neonates measured by near-infrared spectroscopy. *J Biomed Opt* 16(4):047005
3. Jiang J, Di Costanzo Mata A, Lindner S et al (2022) 2.5 Hz sample rate time-domain near-infrared optical tomography based on SPAD-camera image tissue hemodynamics. *Biomed Opt Express* 13(1):133–146
4. Jiang, J., Ackermann, M., Russomanno, E., et al. (2022). Resolution and penetration depth of reflection-mode time-domain near infrared optical tomography using a ToF SPAD camera. *Biomed Opt Express*, 13(12), 6711–6723
5. Jiang J, Di Costanzo Mata A, Lindner S et al (2020) Image reconstruction for novel time domain near infrared optical tomography: towards clinical applications. *Biomed Opt Express* 11(8):4723–4734
6. Jiang J, Wolf M, Sánchez Majos S (2017) Fast reconstruction of optical properties for complex segmentations in near infrared imaging. *J Mod Opt* 64(7):732
7. Lindner S, Zhang C, Antolovic IM et al (2018) A novel 32× 32, 224 Mevents/s time resolved SPAD image sensor for near-infrared optical tomography, optics and the brain, *JTh5A*. 6, Optical Society of America
8. Jiang J, Di Costanzo Mata A, Lindner S et al (2020) Dynamic time domain near-infrared optical tomography based on a SPAD camera. *Biomed Opt Express* 11(10):5470–5477
9. Jiang J, Di Costanzo Mata A, Lindner S et al (2021) Localization of deep ischemia and hemorrhage in pre-term Infants' head with near-infrared optical tomography: a numerical case study. *Adv Exp Med Biol* 1269(131–136):11
10. Dehghani H, Eames ME, Yalavarthy PK et al (2009) Near infrared optical tomography using NIRFAST: algorithm for numerical model and image reconstruction. *Commun Numer Methods Eng* 25:711–732

Importance of Serum Albumin in Deep Learning–Based Prediction of Cognitive Function Data in the Aged Using a Basic Blood Test

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AbstractBackground

Recently, a method using deep learning has been developed to estimate the risk of developing dementia. This method uses general blood test data from routine health examinations that reveal lifestyle-related diseases, which can lead to vascular cognitive impairment via arteriosclerosis, as well as systemic metabolic disorders that are unrelated to lifestyle, such as nutritional disorders. In this study, we investigated the differences in the accuracy of estimating the risk of dementia based on the presence or the absence of blood

test parameters reflecting nutritional disorders while focusing on the association between malnutrition and the risk of dementia in frail, elderly individuals.Objectives

The objective of this study was to evaluate the impact of including or excluding serum albumin, which reflects nutritional status, on the accuracy of predicting cognitive function in older adults using blood test data.Methods

We estimated cognitive function, as measured by the Mini-Mental State Examination (MMSE), using the deep learning model (DLM). The estimation was performed based on general blood test data, including complete blood tests and basic metabolic panels, obtained from a selection of 1287 patients admitted to Osaka Medical and Pharmaceutical University Hospital. The data were divided into two groups: individuals aged 65 and

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above and those aged below 65. The impact of including or excluding serum albumin on the predictive performance of MMSE was examined within each group. Results

In those aged below 65, the mean squared error (MSE) of the DLM was 5.33 without albumin and 4.62 with albumin, showing a -0.71 improvement with albumin. In those aged 65 and above, the MSE of the DLM was 6.38 without albumin and 6.28 with albumin, showing a -0.1 improvement with albumin. Discussion

The present study demonstrated that including serum albumin in the input data resulted in lower estimation errors for MMSE across all applied algorithms in the group aged 65 and above. This is consistent with previously reported studies that have shown the adverse effects of malnutrition on cognitive function in older adults. Conclusions

This study highlighted the significance of serum albumin, which reflects nutritional status, as an important assessment variable for estimating MMSE from blood test data, particularly in individuals aged 65 and above.

Keywords

Dementia · MMSE · Deep learning · Albumin · Prediction of cognitive function

1 Introduction

Dementia currently lacks fundamental treatment, making it crucial to manage the disease appropriately from an early stage to try to halt its progression. Various methods exist for the examination of dementia, such as the use of biomarkers [1] and the Mini-Mental State Examination (MMSE) [2], which measure cognitive function through interviews. However, these require patients to attend medical facilities. This reality presents a hurdle for early detection, since individuals in the early stages who may lack noticeable symptoms find it burdensome to undergo these examinations voluntarily. In light of these challenges, efforts are underway to develop methods to estimate cognitive decline

using more easily obtainable indicators. Recently, the remarkable advancements in machine learning, particularly deep learning, have spurred applied research in the medical field towards new disease diagnostics and early detection. Leveraging deep learning, studies are being conducted to estimate cognitive decline using indicators obtainable relatively easily from speech or blood tests or similar. Our research particularly focuses on estimating cognitive function (MMSE Score) from blood test data. Previous studies [3] have proposed methods using deep learning to estimate a patient's MMSE score at a given time, using age, gender, and 24 blood test parameters as explanatory variables. In this study, we focused on albumin, which reflects nutritional status likely to impact cognitive function [4]. We conducted a comparative investigation to examine the difference in cognitive function estimation with and without albumin.

2 Methods

2.1 Data Set

In this study, we extracted data from patients who underwent MMSE at Osaka Medical and Pharmaceutical University Hospital from 2014 to 2021. The extracted data items include age, sex, white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, total protein, albumin, albumin–globulin ratio, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total cholesterol, triglycerides, blood–urea–nitrogen (BUN), creatinine, uric acid, glucose, sodium, potassium, and chloride. We focused on those without any missing values in all the explanatory variables used in the preceding research, resulting in a total of 1287 subjects. The utilisation of these data was approved after ethical review (Approval ID: 2022–181). Before comparing the cognitive function estimation performance based on the presence or the absence of albumin, we divided the 1287 individuals into two groups: those aged 65 and above and those aged below 65.

Table 1 Explanatory variables for the two age groups

<65 years	≥65 years
Age, year	Age, year
Sex, (male)	Sex, (male)
Platelet, 10 ³ /μL	WBC, 10 ³ /μL
Total protein, g/dL	RBC, 10 ⁶ /μL
Albumin, g/dL	Total protein, g/dL
Triglyceride, mg/dL	Albumin, g/dL
Uric acid, mg/dL	BUN, mg/dL
Sodium, mEq/L	Creatinine, mg/dL
	Potassium, mEq/L

Upon conducting univariate analysis on each group’s data, significant differences were observed in the test items, as shown in Table 1. In this research, we used the items listed in Table 1 as explanatory variables to construct the cognitive function estimation model. For model construction and evaluation, the group aged below 65 was divided into 81 training data and 31 evaluation data, while the group aged 65 and above was allocated as 778 training data and 397 evaluation data.

2.2 Deep Learning Algorithms

To investigate whether the performance varies with the presence or the absence of albumin, cognitive function estimation models were constructed for each group, those aged under 65 and those aged 65 and above. The construction of the estimation models employed the deep learning model (DLM). The DLM was built with MMSE as the target variable, utilising the items listed in Table 1 as explanatory variables. Note that the DLM was constructed using a five-layer feedforward neural network [5] with two output layers to simultaneously estimate MMSE and binary classification problems, categorising MMSE scores of 24 and above as normal and below 24 as positive. The output layer for binary classification was added to assist in the learning of MMSE estimation. During the training of the DLM, learning was conducted with a weight ratio of 1:0.001 between the loss function of the estimated MMSE and the loss function for binary classification, updating the overall parameters of the DLM accordingly. The

output for binary classification was not utilised; instead, only the MMSE was used as the output of the DLM. Furthermore, as the performance of each model could vary with the parameters during training, parameter optimisation was carried out using the Optuna library [6, 7] to optimise the hyperparameters of each model. The impact of the presence or the absence of albumin was evaluated using the best model obtained during this optimisation process. DLM construction was carried out using TensorFlow [8]. To evaluate the models, we calculate the Pearson correlation coefficient, mean absolute error, mean squared error (MSE), and root MSE for the predictions. Furthermore, to analyse the impact of each input variable on the models, we compute SHapley Additive exPlanations (SHAP) values [9]. SHAP values quantify the impact of each input variable on the model’s output when making an estimation with the given input data. By calculating SHAP values for the test data, it is possible to ascertain which features the constructed models deem important for predictions. Since SHAP values take into account the influence among input variables within each model, they represent relative values. To compare the presence of albumin or across age groups, we calculate the mean absolute SHAP value for each input variable within each model, normalise these by dividing by the maximum value within each model, and then compare these standardised values across models.

3 Results

As shown in Table 1, explanatory variables were employed for the two groups, and the performance of DLM was evaluated with and without the inclusion of albumin (refer to Table 2). In those aged below 65, the mean squared error (MSE) of DLM was 5.325 without albumin and 4.618 with albumin, showing a −0.707 improvement with albumin. In those aged 65 and above, the MSE of the DLM was 6.379 without albumin and 6.279 with albumin, showing a −0.1 improvement with albumin. Figure 1 shows scatter plots of the MMSE estimation results for each model on the test data. It can be observed that using albumin improves the

Table 2 Performance of MMSE regression deep learning model

Age group	Used Albumin	corr	MAE	MSE	RMSE
Those aged below 65	No	0.389	2.002	5.325	2.308
Those aged below 65	Yes	0.514	1.706	4.618	2.149
Those aged 65 and above	No	0.382	2.171	6.379	2.526
Those aged 65 and above	Yes	0.413	2.160	6.279	2.506

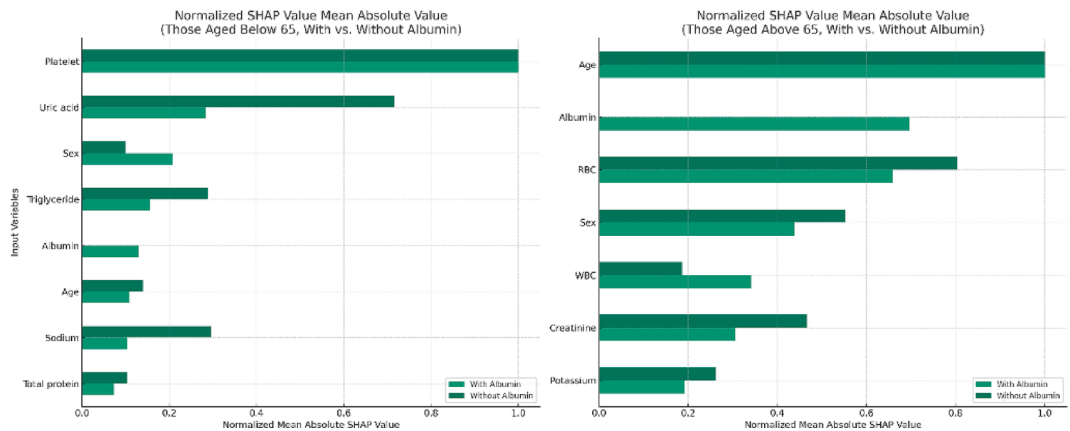


Fig. 1 Scatter plots of the actual and predicted MMSE scores

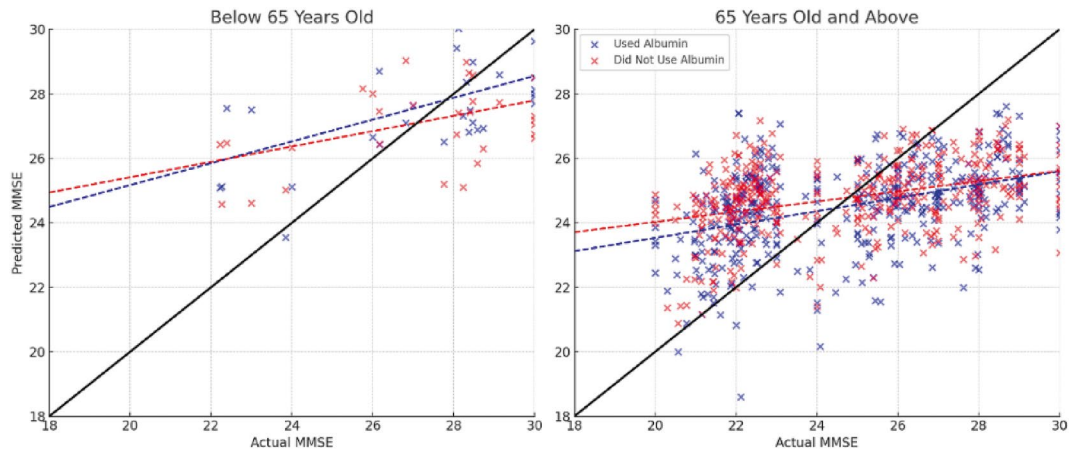


Fig. 2 Impact of SHAP value for each DLM

correlation coefficient in both age groups. Figure 2 shows the impact of SHAP values on the MMSE estimation for each model with the test data. According to the impact of SHAP values, in the group aged 65 and above, adding albumin as an input variable made it the second most influential variable. Compared to the most significant impact

of age, the SHAP value's impact for albumin was about 0.7, making it a major variable. On the other hand, for those under 65, adding albumin as an input variable resulted in the fifth highest SHAP value impact. It was less significant than the most influential variable, platelets, with an impact of less than 0.2.

4 Conclusions and Discussion

The present study demonstrated that including serum albumin in the input data resulted in lower estimation errors for MMSE across all applied algorithms in both the group aged below 65 and aged 65 and above. When comparing the group under 65 years with the group aged 65 and above, the improvement in MSE when albumin was used was more significant in the under-65 group. However, since the amount of training and evaluation data used differs between the two groups, it is not possible to simply compare the change in MSE to determine how albumin affects the groups differently. According to the SHAP value results, which show the importance of variables for each model, albumin is the second most important variable in the group aged 65 and above, but it is ranked fifth in importance and considered a less important variable in the group aged below 65. The reason why adding albumin improved MSE in the under-65 group could be due to the fact that previously significant predictors, such as uric acid, sodium, and triglyceride, had a negative effect on predictions. The addition of albumin may have diluted their impact, leading to an improvement in MSE. The influence of albumin is greater than the 65 and older group is consistent with previously reported studies [10] that have shown the adverse effects of malnutrition on cognitive function in older adults. Albumin levels decrease in conditions of liver dysfunction or malnutrition. A diet rich in vegetables has been reported to prevent cognitive decline associated with aging [4], and the importance of albumin in the group aged 65 and above aligns with this report. Factors other than diet and illness that affect cognitive function, such as physical activity, sleep quality, and sociability, have also been reported [4]. While this study constructed estimation models based on blood test data, further improvement in performance is believed to require information on individuals' lifestyles. Since SHAP values allow for the analysis of which factors influence each prediction, add-

ing lifestyle information could not only lead to dietary improvements but also uncover individual factors contributing to cognitive decline hidden within lifestyles, leading to improvements in exercise and sleep.

In conclusion, this study highlighted the significance of serum albumin, which reflects nutritional status, as an important assessment variable for estimating MMSE from blood test data, particularly in individuals aged 65 and above.

References

1. Dumurgier J, Hanseeuw BJ, Hatling FB, Judge KA, Schultz AP, Chhatwal JP, Blacker D, Sperling RA, Johnson KA, Hyman BT, Gómez-Isla T (2017) Alzheimer's disease biomarkers and future decline in cognitive Normal older adults. *J Alzheimers Dis* 60:1451–1459
2. Folstein MF, Folstein SE, McHugh PR (1975) "Minimal state" A practical method for grading the cognitive state of patients for clinicians. *J Psychiatr Res* 12:189–198
3. Sakatani K, Oyama K, Hu L (2020) Deep learning-based screening test for cognitive impairment using basic blood test data for health examination. *Front Neurol* 11:588140
4. Dominguez LJ, Veronese N, Vernuccio L, Catanese G, Inzerillo F, Salemi G, Barbagallo M (2021) Nutrition, physical activity, and other lifestyle factors in the prevention of cognitive decline and dementia. *Nutrients* 13:4080
5. Goodfellow I, Bengio Y, Courville A (2016) Deep learning. MIT Press, Cambridge
6. Optuna (2018) optuna.samplers.TPESampler. <https://optuna.readthedocs.io/en/stable/reference/samplers/generated/optuna.samplers.TPESampler.html> Accessed 29 Oct 2023
7. Bergstra J, Bardenet R, Bengio Y, Kégl B (2011) Algorithms for hyper-parameter optimization. *Advances in neural information processing systems* 24
8. TensorFlow (n.d.) <https://www.tensorflow.org>. Accessed 26 Feb 2024
9. Lundberg SM, Lee SI (2017) A unified approach to interpreting model predictions. In: *Advances in neural information processing systems* 30. Curran Associates, Inc.
10. González-Gross M, Marcos A, Pietrzik K (2001) Nutrition and cognitive impairment in the elderly. *Br J Nutr* 86:313–321

PSAA–nnUNet: An Efficient Method for CT Carotid Artery Image Segmentation

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Abstract

Carotid artery (CA) stenosis (CAS) constitutes a significant factor to ischaemic cerebrovascular events which exhibiting no overt symptoms in the early stages. Early detection of CAS can prevent ischaemic stroke and improve patient prognosis. In this study, we developed a non-invasive CAS automatic assessment method based on deep learning, intended for the early detection of CAS with CT imaging. The method proposed in this paper consists of three main components. First, we utilised thresholding and the Hessian-based Frangi filter to eliminate irrelevant tissue and enhance vascular structures. Second, we introduced a novel neural network named parameter shared axial attention (PSAA)–nnUNet for the automatic segmentation of CA. Finally, we assessed the degree of CAS with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) formula. The PSAA–nnUNet algorithm proposed in this study achieved a segmentation accuracy of 0.82. The non-invasive CAS automatic assessment method based on PSAA–nnUNet exhibits excellent accuracy and great application potential.

Keywords

Carotid artery stenosis (CAS) · Neural network · Cerebral vascular accident (CVA) · Dice similarity score (DSC) · Ischaemic stroke

1 Introduction

Cerebral vascular accident (CVA) is a cerebrovascular disorder caused by vascular occlusion or rupture in the brain, leading to impaired blood supply to brain tissue. Ischaemic strokes account for approximately 80% of all stroke cases [1]. Ischaemic stroke is a common medical emergency, characterised by an exceptionally high incidence, disability rate, and mortality rate. Additionally, there is an emerging trend of ischaemic strokes occurring in younger individuals [2]. Notably, the narrowing of intracranial carotid arteries (ICA) stand as the primary aetiological factors responsible for ischaemic strokes [3]. Early identification of carotid artery (CA) stenosis (CAS) and targeted intervention can significantly enhance patient survival rates and improve long-term prognosis [1].

Medical imaging is currently a popular diagnostic method in clinical practice. CT is characterised by its rapid imaging, cost-effectiveness, and wide-ranging utility, making it superior in the early identification of diseases. However, CT

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imaging exhibits relatively lower vascular resolution and blurred boundaries, which enables only the observation of vascular calcification [4]. Consequently, its diagnostic accuracy for CAS seems less proficient compared to CTA and MRI. Due to the blurry boundaries of carotid arteries in CT images, segmentation of the carotid artery (CA) region has primarily relied on CTA [5] and MRI images [6]. However, with the advancements in medical imaging and artificial intelligence technology, the segmentation accuracy of carotid arteries from CT images has significantly improved [7, 8].

The initial step in quantitatively assessing imaging of CAS is the segmentation of the carotid arteries. It is a challenge for radiologists to perform this task, and there is significant segmentation variability among individuals. Consequently, there is an urgent demand for an automatic and precise carotid artery segmentation approach to enhance the accuracy of CAS quantification [9, 10].

This research aims to utilise deep learning techniques to achieve precise segmentation of carotid arteries from complex head and neck CT images and to evaluate the severity of CAS, thus providing aid in the early diagnosis of ischaemic strokes.

2 Methods

2.1 Data Set

This study employed a data set provided by the Affiliated Hospital of Nantong University; this data set comprises 42 head and neck non-contrast CT images, encompassing 30 male patients and 12 female patients, with an average age of 60.0 ± 11.4 years. These images were annotated by five physicians and accompanied by corresponding labelled images (gold standard). The CT images possess matrix dimensions of 512×512 , with a slice count ranging from 116 to 323. The pixel dimensions range from 0.52×0.52 mm to 1.56×1.56 mm, and the inter-slice spacing ranges from 2.0 mm to 3.0 mm. The annotated region encompasses both the common

carotid artery (CCA) and ICA. In this research, 34 images were allocated for training, and 8 images were designated for testing.

The current undertaking is conducted under the approval of the Ethics Committee of Nantong University. It involves the intelligent assessment and research of acquired non-invasive data, including human medical images and vascular imaging.

2.2 Carotid Artery Segmentation

In carotid artery CT images, the vascular background area is intricate, and the boundaries are not clear enough. Therefore, the initial step is the elimination of irrelevant tissues and vascular enhancement to emphasise the features of vessels and reduce segmentation difficulty. Filtering is applied within region of interest (ROI), revealing that the grayscale distribution of carotid artery primarily falls within the range of 0–1000 (HU). Consequently, grayscale values below 0HU and above 1000HU are set to zero to eliminate superfluous tissues and achieve the contrast enhancement in vascular regions. Subsequently, the Hessian-based Frangi filter is employed [11]. Hessian-based Frangi filter is primarily used for enhancing vascular structures. This method exploits the characteristic that vascular grayscale remains relatively constant along the vascular direction, but exhibits abrupt changes in the cross-sectional direction; filtering is applied to the images in various directions to enhance the pixel intensity of blood vessels (Fig. 1).

This study introduces a modified nnU-Net based on parameter shared axial attention, termed parameter shared axial attention (PSAA)–nnUNet. The network architecture is shown in Fig. 2.

The nnU-Net is used as the baseline neural network, which stands as a highly adaptable network capable of autonomous adjustment based on each specific training data set [12]. In this study, network specifications were improved by increasing the convolutional kernel size from 320 to 512, thereby enhancing the performance of feature extraction. Furthermore, in the encoder section of

Fig. 1 Gray-based tissue removal method. (a) Before removal, (b) after removal, (c) after removal (annotated)

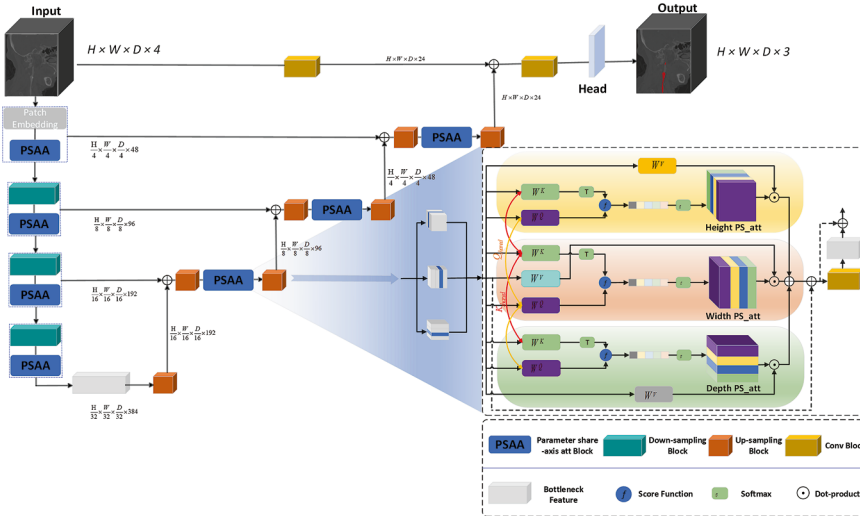
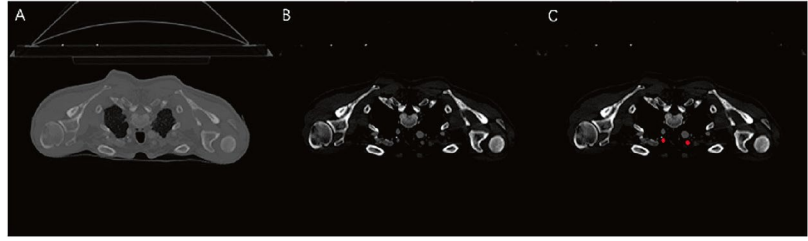


Fig. 2 Network architecture of the PSAA–nnUNet

the neural network, we introduce the parameter shared axial attention (PSAA) module. PSAA accomplishes parameter weight sharing in the calculation of q and k across three dimensions, significantly reducing the model's parameter count and elevating training speed. The PSAA module decomposes attention computation into three independent axial operations, effectively addressing the problem of the inapplicability of self-attention to large-scale data. Specifically, it performs attention calculations separately on the width, height, and depth dimensions, instead of applying global attention across the entire input. This decomposition strategy reduces the computational complexity from quadratic to linear, significantly improving computational efficiency.

To address issues related to over-segmentation during the segmentation process, this study employs a connected component algorithm to eliminate over-segmented tissues. In response to possible vascular discontinuity occurrences

within the segmented vessels, a topological thinning method is utilised to extract the central vessel line. Subsequently, a dilation operation is applied to reconnect the fragmented vessels, thereby enhancing the integrity of the segmentation results.

2.3 Analysis of Carotid Artery Stenosis

This study employs the North American Symptomatic Carotid Endarterectomy Trial (NASCET) formula for CAS evaluation. This formula utilises diameter ratio of the normal vessel in the distal segment to that of the stenotic region as the measure of stenosis severity [13]. The formula is

$$\text{NASCET} = \frac{A - B}{A}, \quad (1)$$

where A is the distal normal vessel diameter, and B is the diameter of the vessel in the stenosis area.

The NASCET formula classifies the degree of arterial stenosis into four categories: stenosis <30% is considered mild, 30–69% is moderate, and 70–99% is severe, and >90% is complete occlusion. Due to the relatively straight and curved nature of carotid artery vessels, the vessel diameter can be deduced from the cross-sectional area.

3 Results

The method proposed in this study introduces a series of optimisation strategies to enhance nnU-Net and achieves an outstanding level of segmentation accuracy in the task of carotid artery vessel segmentation.

This study employs the Dice Similarity Score (DSC) to evaluate the performance of the model. DSC measures the overlap between voxel segmentation predictions and ground truth [14]. The definition of DSC is as follows:

$$\text{DSC}(T, P) = 2 * \frac{|T \cap P|}{|T| \cup |P|}, \quad (2)$$

where T and P represent the ground truth values and predicted values for all voxels, respectively.

Ablation experiments are conducted to assess the individual impact of model components on performance by systematically removing them with each training run comprising 80,000 epochs. The DSC for the experimental results is presented in Table 1, and the comparative segmentation results are depicted in Fig. 3.

As shown in Table 1, compared to unaltered nnU-Net, our PSAA–nnUNet network can effectively enhance segmentation performance.

According to the NASCET formula, the degrees of stenosis for samples 1–3 in Fig. 3 are 23.3%, 39.7%, and 25.4%, respectively, corresponding to mild stenosis, moderate stenosis, and mild stenosis, consistent with the results provided by the physicians.

Table 1 nnUNet ablation experiment

Methods	Left CA	Right CA
nnU-Net (baseline)	0.74	0.73
PSAA–nnUNet	0.75	0.74
PSAA–nnUNet + cropping	0.78	0.79
PSAA–nnUNet + cropping + threshold processing	0.81	0.82

4 Discussion

Considering the characteristic of CT carotid artery images, this study introduces a non-invasive automated assessment method for CAS based on the PSAA–nnUNet framework. Initially, we incorporate a threshold-based preprocessing step using the hessian-based Frangi filter. Subsequently, we made modifications to the network structure, adjusted network parameters, and introduced the PSAA module to enhance the algorithm's network performance. Following these steps, we applied post-processing operations based on connected component analysis and topological thinning method. Finally, we used the NASCET formula to calculate the severity of CAS, quantitatively evaluating the model's performance and confirming the algorithm's superiority.

Although CT imaging can only observe the vascular area and carotid artery calcification, it offers advantages, such as rapid imaging and cost-effectiveness, making them widely applicable in health checkups. Evaluating early CAS from CT images holds potential advantages for preventing ischaemic strokes [15].

Notable innovations in this study: First, it establishes a non-invasive method for CAS assessment based on CT imaging. Second, considering the characteristics of carotid artery CT imaging, the nnU-Net network was improved in various aspects, including CT image preprocessing, network adjustment, and post-processing. Finally, it demonstrates that the PSAA–nnUNet exhibits excellent generalisation performance when confronted with unfamiliar data sets.

Deficiencies in this study: first, the data sample is limited to a mere 42 observations, which

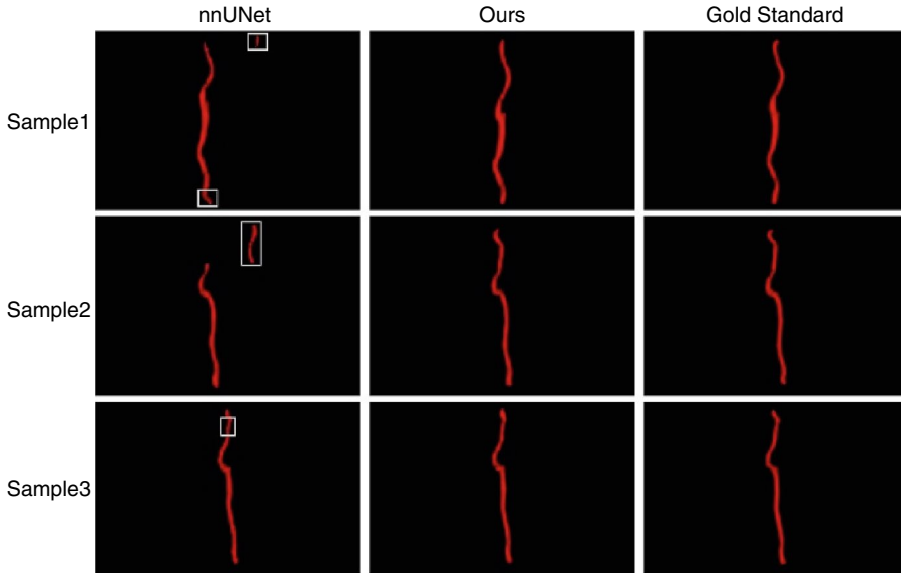


Fig. 3 Segmentation results. “nnUNet” represents the segmentation results of the unmodified baseline model, “ours” represents the segmentation results of PSAA–nnUNet, and “gold standard” represents the annotations

by medical professionals. White rectangles in the images indicate segmentation inaccuracies produced by the baseline model nnUNet

may affect the accuracy of the model. Second, the assessment and prevention of ischaemic cerebrovascular events represent a multifaceted, intricate process, requiring integration with cardiac and cerebral vascular. Third, some parameters in the nnU-Net framework are still uncertain, and future research is needed to explore methods for the automatic calculation of these parameters.

5 Conclusion

This study develops a non-invasive CAS stenosis automatic assessment method based on PSAA–nnUNet, achieving precise segmentation of carotid artery vessels from complex head and neck CT images. This method provides aid for the early detection of ischaemic stroke, exhibiting excellent accuracy and good application potential.

References

1. Xu H, He K, Hu R et al (2022) Identifying key biomarkers and immune infiltration in female patients with ischemic stroke based on weighted gene co-expression network analysis [J]. *Neural Plast* 2022:5379876
2. Li F, Yang L, Yang R et al (2017) Ischemic stroke in young adults of Northern China: characteristics and risk factors for recurrence [J]. *Eur Neurol* 77(3–4):115–122
3. Flaherty ML, Kissela B, Khoury JC et al (2012) Carotid artery stenosis as a cause of stroke [J]. *Neuroepidemiology* 40(1):36–41
4. Wang Y, Yao Y (2023) Application of artificial intelligence methods in carotid artery segmentation: a review [J]. *IEEE Access* 11:13846–13858
5. Zhou T, Tan T, Pan X et al (2021) Fully automatic deep learning trained on limited data for carotid artery segmentation from large image volumes [J]. *Quant Imaging Med Surg* 11(1):67
6. Peng D, Yu X, Peng W et al (2021) DGFAU-net: global feature attention upsampling network for medical image segmentation [J]. *Neural Comput & Applic* 33:1–15

7. Nederkoorn PJ, Majoie CB, Stam J (2007) The role of imaging in diagnosis and management [M]. Cambridge University Press, Cambridge
8. Randoux B, Marro B, Koskas F et al (2001) Carotid artery stenosis: prospective comparison of CT, three-dimensional gadolinium-enhanced MR, and conventional angiography [J]. *Radiology* 220(1):179–185
9. Saba L, Sanches JM, Pedro LM, Suri JS (2013) Multimodality atherosclerosis imaging and diagnosis. Springer Science & Business Media, p 423
10. Naidich DP, Müller NL, Webb WR (2007) Computed tomography and magnetic resonance of the thorax. Lippincott Williams & Wilkins, p 930
11. Longo A, Morscher S, Najafabadi JM et al (2020) Assessment of hessian-based Frangi vesselness filter in optoacoustic imaging [J]. *Photo-Dermatology* 20:100200
12. Isensee F, Jaeger PF, Kohl SAA et al (2021) nnU-Net: a self-configuring method for deep learning-based biomedical image segmentation [J]. *Nat Methods* 18(2):203–211
13. Moneta GL, Edwards JM, Chitwood RW et al (1993) Correlation of North American Symptomatic Carotid Endarterectomy Trial (NASCET) angiographic definition of 70% to 99% internal carotid artery stenosis with duplex scanning [J]. *J Vasc Surg* 17(1):152–159
14. Hatamizadeh A, Tang Y, Nath V et al (2022) Unetr: transformers for 3d medical image segmentation [C]. In: *Proceedings of the IEEE/CVF winter conference on applications of computer vision*, pp 574–584
15. Chen Y, Hu Z, Li M et al (2019) Comparison of non-gated chest CT and dedicated calcium scoring CT for coronary calcium quantification using a 256-detector row CT scanner [J]. *Acad Radiol* 26(10):e267–e274

Relationship Between Dementia and Systemic Metabolic Disorders

Kaoru Sakatani

Abstract

Recent research has linked systemic metabolic disorders to cognitive decline and dementia risk, including Alzheimer's. This is suspected to be due to lifestyle-related vascular impairments from atherosclerosis and other factors, such as malnutrition and anaemia. Applying deep learning using 2897 cases from a rehabilitation hospital and health screenings, we trained a model to predict cognitive function [mini-mental state examination (MMSE) scores] and brain atrophy [Brain Healthcare Quotient (BHQ) scores] from basic blood tests and age. The deep learning model accurately estimated MMSE and BHQ from these inputs, with age, nutritional information, and organ function indicators being top predictors. These findings highlight the relationship of dementia with systemic metabolic disorders and suggest the potential of using routine blood tests for dementia risk assessment. Furthermore, personalised dietary interventions could be tailored based on blood test anomalies. This holistic view mirrors traditional Chinese medicine, which considers

brain disorders systemic, that is related to vital organs but not the brain itself.

Keywords

Cognitive decline · Alzheimer's disease
Atherosclerosis · Deep learning · Blood tests

1 Introduction

The number of patients with dementia is increasing rapidly in both developed and developing countries. However, it is currently considered challenging to develop molecular-level therapeutic medications for Alzheimer's disease. It had been thought that dementia was a brain-localised disease, and dementia drugs were developed based on this idea, but they have not been successful.

In recent years, it has become apparent that systemic metabolic disorders cause the onset of dementia. For example, lifestyle-related diseases significantly contribute to the onset of dementia. Such diseases can lead to atherosclerosis, which in turn can cause vascular cognitive impairment (VCI) [1, 2]. VCI is a leading cause of vascular dementia. Many elderly patients with Alzheimer's disease also have atherosclerosis, suggesting that VCI plays a role in the emergence of Alzheimer's disease in older individuals. Furthermore, non-lifestyle-related metabolic disorders also contrib-

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ute to the onset of dementia. It has been reported that malnutrition [3], anaemia [4], kidney diseases [5], and electrolyte imbalance [6] can lead to cognitive impairment or increase the risk of dementia.

Recently, we developed a deep learning-based approach to assess dementia risk using health examination data, including basic blood test results [7–9]. These results reflect systemic metabolic disorders stemming from both lifestyle-related and non-lifestyle-related diseases. In this review paper, we introduce a deep learning-based approach to assess dementia risk using health examination data and discuss the significance of this method for the early detection and prevention of dementia. Furthermore, based on the relationship between dementia and systemic metabolic disorders, we examine the relationship between the brain and the body (other organs). We highlight the similarity of viewing the brain and body as an integrated whole system with the oriental medical philosophy of perceiving the brain and body as a unified entity.

2 Deep Learning-Based Approach to Estimate Dementia Risk Using Health Examination Data

Figure 1 illustrates the concept of estimating the risk of dementia onset based on systemic metabolic status. While cognitive impairment originates from brain dysfunction, systemic metabolic disorders can influence the brain, potentially leading to cognitive issues and raising the risk of dementia. Health examinations can detect these systemic metabolic disorders. By analysing data from these examinations, including general blood tests, we can assess the potential onset of dementia. This estimation method employs deep learning.

We used a feedforward deep neural network (DNN) model for estimating dementia onset risk (Fig. 2). The input layer of the DNN model includes the subject's age and 23 basic blood data

items. The output layer includes scores from the mini-mental state examination (MMSE), which is a widely used cognitive assessment test [7], and the Brain Health Quotient (BHQ), which quantifies the extent of brain atrophy using voxel-based morphometry [8].

We utilised a data set comprising 2538 cases to train the DNN model, which included data from patients diagnosed with stroke and dementia, as well as data from healthy control subjects. For the validation and application phases, we employed a separate data set of 37,861 cases.

Table 1 shows the blood test items in the input layer of the DNN model. These include a complete blood count and basic metabolic panel, but not the dementia specific biomarkers, such as amyloid beta.

3 Validation of Estimation Accuracy for Cognitive Function and Brain Atrophy in the DNN Model

Figure 3 shows the correlation between actual and estimated MMSE scores using the DNN model. The estimated MMSE scores through leave-one-out cross-validation showed a high correlation with the actual MMSE scores ($r = 0.85$, $p < 0.001$) (A). When estimating without including age in the input layer, the correlation coefficient still exhibited a high correlation ($r = 0.75$, $p < 0.001$) (B). When validating the estimation accuracy for a test group, the correlation coefficient was $r = 0.66$ ($p < 0.001$), indicating a moderate correlation (C).

Figure 4 shows the correlation between actual and estimated BHQ using the DNN model. The subject's age was included in the input layer for (A) and excluded for (B). The correlation coefficient slightly decreased from 0.74 to 0.54 in the DNN model with age excluded (B), but a significant correlation was still observed.

It should be noted that the study examining the relationship between MMSE and BHQ utilised

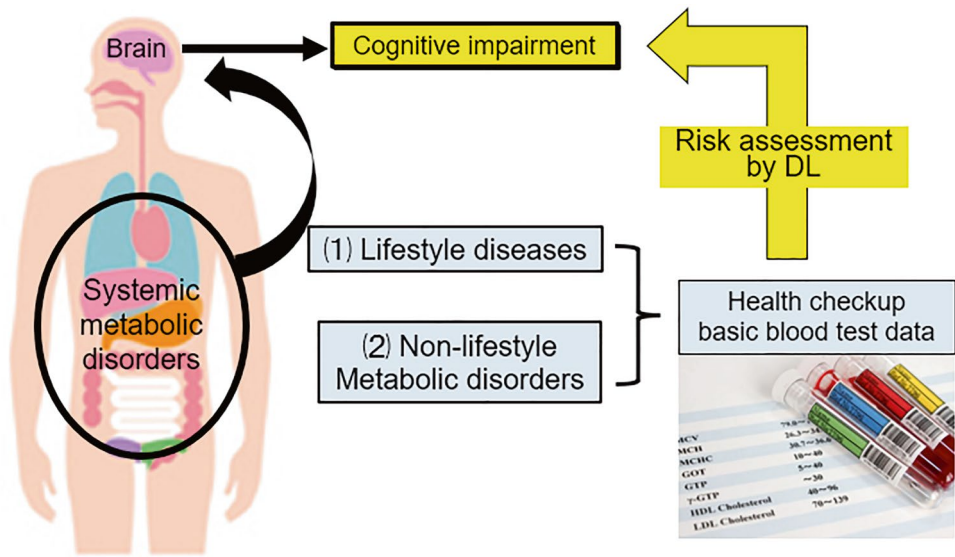


Fig. 1 Basic concept of deep learning-based estimation of dementia onset risk based on systemic metabolic disorders

Fig. 2 Schema of feedforward deep neural network (DNN) model for estimating dementia onset risk

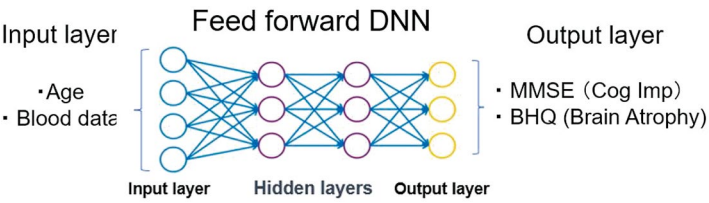


Table 1 Blood test items in the input layer of the DNN model

Complete blood count	General biochemical examination	
WBC count	Total protein	BUN
RBC count	Albumin	Creatinine
Haemoglobin	A/G ratio	Uric acid
Haematocrit	AST	Glucose
MCV	ALT	Na
MCH	r-GTP	K
MCHC	Total cholesterol	Cl
Platelet count	Triglyceride	

MCV mean corpuscular volume; *MCH* mean corpuscular haemoglobin; *MCHC* mean corpuscular haemoglobin concentration; *BUN* blood–urea–nitrogen; *A/G ratio* albumin/globulin ratio; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *r-GTP* γ -glutamyl transpeptidase; *Na* sodium; *Cl* chloride; *K* potassium

brain dock data [8]. Therefore, the participants of the brain dock were predominantly middle-aged, working individuals, and their cognitive functions were mostly normal (with an average MMSE score of 28.6 ± 1.9). However, there was significant individual variation in the degree of brain

atrophy (mean BHQ = 92.13 ± 9.40 ; $57.39 \sim 123.17$ range). There was a weak positive correlation between the GM-BHQ and MMSE scores ($r = 0.23$, $p < 0.001$). This suggests that even when cognitive functions are normal, there are not a few cases that involve brain atrophy.

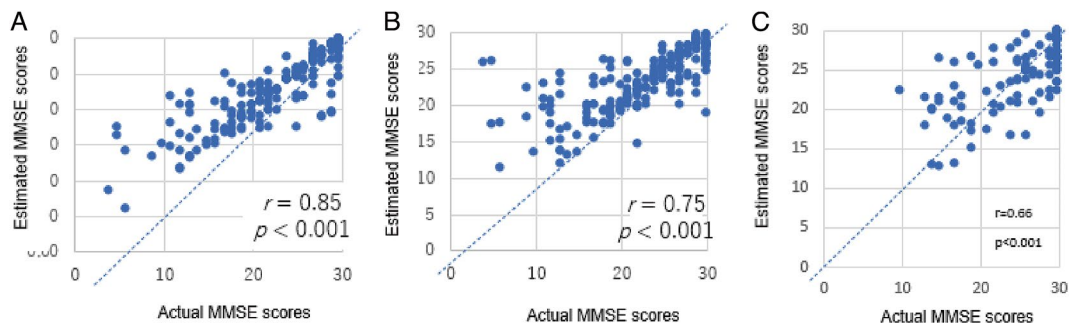


Fig. 3 Correlations between estimated and actual MMSE scores. (a) High correlation in MMSE score estimation ($r = 0.85$). (b) Still high without age ($r = 0.75$). (c) Moderate correlation for test group ($r = 0.66$)

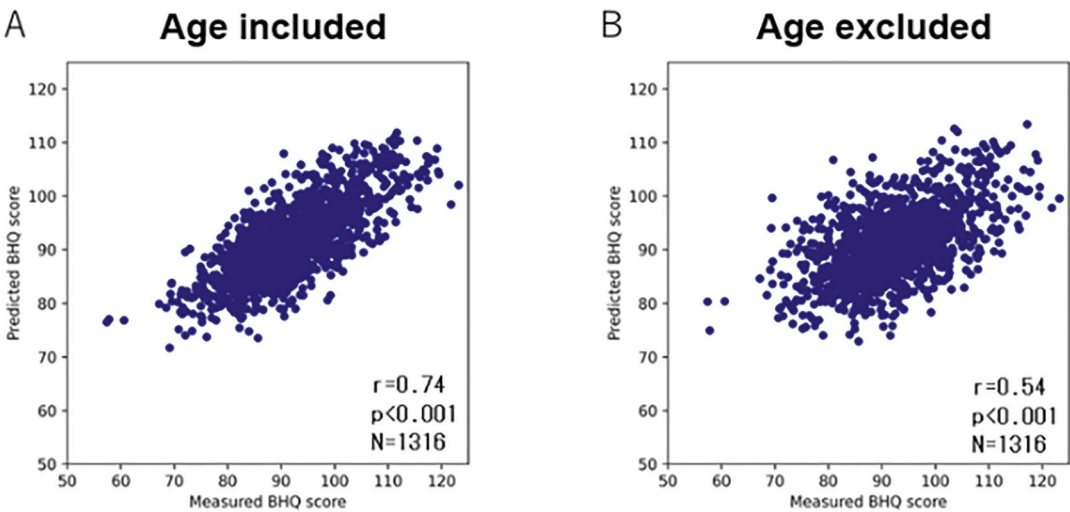


Fig. 4 Correlation between estimated and actual BHQ with (a) and without (b) subject's age

4 Importance of Input Variables for Estimating Cognitive Dysfunction and Brain Atrophy

In the estimation of both cognitive dysfunction and cerebral atrophy, age demonstrated a much higher variable importance compared to blood data (Table 2), suggesting that the effects of ageing on the brain are much higher than the effects of systemic metabolic disorders. It should be noted, however, that cerebral atrophy can be estimated from blood data even if age is removed from the input data of the DNN model, which supports the idea of a close relationship between the visceral organs and the brain.

5 Conclusions and Discussion

Based on the above findings, we hypothesised that ageing and systemic metabolic disorders affect cerebral atrophy (Fig. 5). Ageing affects the brain directly through extrinsic factors, such as lifestyle, and intrinsic genetic factors. In parallel, ageing also affects the internal organs, resulting in various systemic metabolic disorders. Systemic metabolic disorders associated with ageing can affect the brain. For example, the kidneys affect the brain by the kidney–brain axis. Uraemic toxins released as a result of chronic kidney disease directly contribute to brain damage and consequential cognitive decline. In addition, anaemia and undernutrition may reduce

Table 2 Variable importance of subject’s age and blood items

Rank	Items in input layer (variable importance)	
	Cognitive function (MMSE)	Cerebral atrophy (BHQ)
1	Age (1)	AAge (1)
2	Albumin (0.59)	Haematocrit (0.04)
3	Platelet (0.55)	HbA1c (0.03)
4	Haematocrit (0.54)	Glucose (0.03)
5	Haemoglobin (0.53)	RBC (0.03)

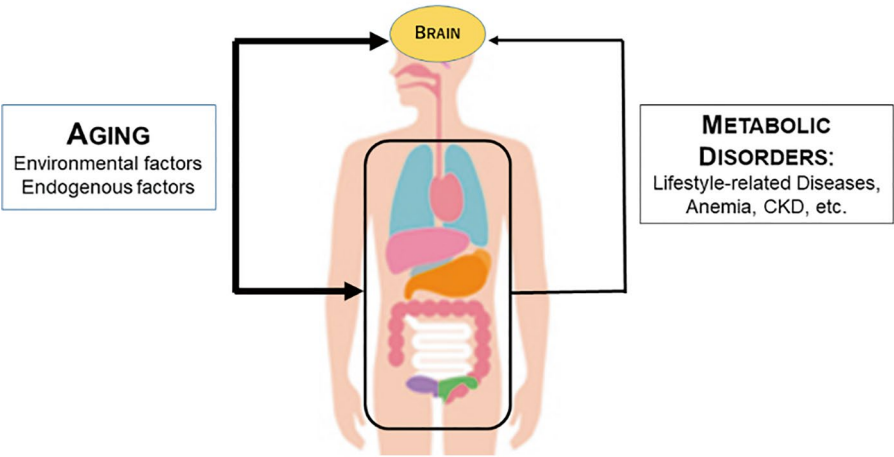


Fig. 5 Mechanism by which ageing and systemic metabolic disorders affect cognitive function. The thickness of the arrow indicates the strength of the influence. The thicker line of age than the line of metabolic disorders

including anaemia indicates that age has a stronger influence on metabolic atrophy than the metabolic disorder line

oxygen metabolism and energy metabolism in the brain.

The advantages of the DNN model as a screening test of cognitive impairment are that it only requires blood data values, does not need special devices to measure biomarkers, offers immediate analysis results in a cost-effective manner, is suitable for digital health monitoring, and can be used as a mass-screening test for dementia. Another advantage of this method is that the patterns in blood test data may indicate individual metabolic abnormalities and contribute to personalised prevention methods for dementia. Table 3 shows the relationship between the main metabolic disorders associated with dementia risk and blood test items. Based on this relationship, personalised dietary guidance is provided to

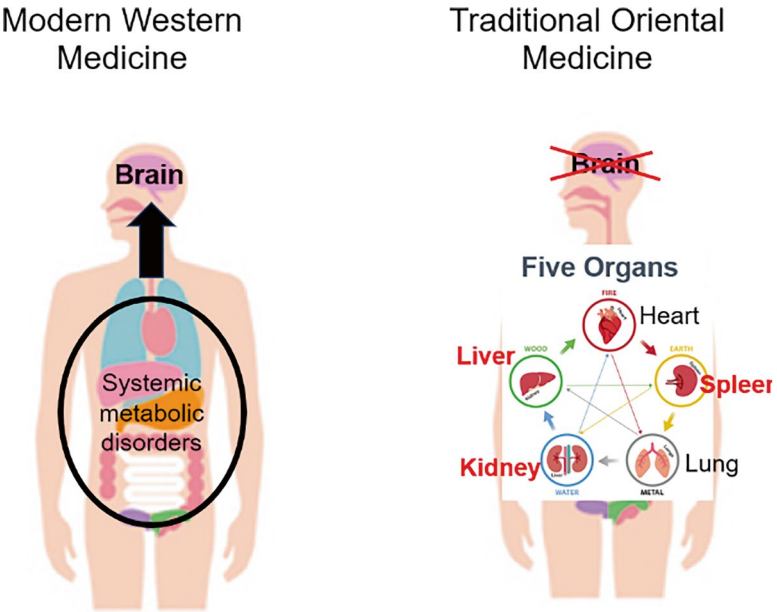
individuals according to their specific metabolic disorders.

Interestingly, the new approach to dementia in modern Western medicine is similar to the concept of dementia in traditional Chinese medicine (TCM) (Fig. 6). In TCM, it is believed that the five organs, consisting of the heart, spleen, lungs, kidneys, and liver, play a role in regulating the body’s functions. It should be noted that there is no mention of the brain among the five organs. Indeed, the brain is not considered one of the organs in traditional oriental medicine. Dementia is thought to be caused by impairment of the five organs, especially the kidneys. The kidneys, in TCM, are described as the “foundation of congenital constitution.” They represent the source of an individual’s vitality or life force. It is

Table 3 Relationship between the main metabolic disorders associated with dementia risk and blood test items

Metabolic disorders associated with dementia risk	Blood data
Anaemia	RBC, haemoglobin, haematocrit
Malnutrition	Total protein, albumin, A/G ratio
Lipid metabolic disorder	Total cholesterol, triglyceride
Kidney dysfunction	Creatinine, BUN
Liver dysfunction	AST, ALT, r-GTP
Diabetes mellitus	Glucose

Fig. 6 Comparison of the new approach to dementia in modern Western medicine and the concept of dementia in traditional Chinese medicine



believed that the kidneys govern the bones and nourish the brain. Therefore, when kidney function declines or becomes imbalanced, it can cause effects on the bones and brain.

In TCM, specific herbal remedies are believed to strengthen kidney function and support cognitive abilities. Herbs categorised as “kidney-tonifying medicines,” targeted at enhancing kidney health, include Huang Jing (*Polygonatum*), He Shou Wu (*Polygonum multiflorum*), and Gou Qi Zi (*Lycium barbarum* fruit). These herbs are believed to restore kidney essence and balance the body’s overall energy, thus positively influencing cognitive functions. TCM is founded on the ancient natural philosophy of Yin-Yang and the Five Elements Theory, distinguishing it from the science-based modern Western medicine. Nevertheless, TCM approaches to treating and

preventing dementia might be potentially effective.

It is important to highlight that the TCM understanding of dementia closely aligns with the perspective of contemporary Western medicine. Notably, TCM’s preventive strategies for dementia have a rich heritage spanning thousands of years, indicating a significant opportunity to derive valuable insights from it.

In conclusion, we have developed a DNN model that predicts cognitive impairment using basic blood data from health checkups. This method is based on the idea that cognitive impairment in the elderly is caused by systemic metabolic disorders that include not only lifestyle diseases but also non-lifestyle diseases, such as malnutrition and anaemia. Hence, this method can be used to perform mass screening tests for demen-

tia. It also has the advantage of being able to provide personalised diet therapy based on individual metabolic disorders that increase the risk of dementia onset. Finally, this method is not limited to dementia; it has the potential to be applied to a wide range of diseases associated with the elderly that are caused by lifestyle factors, such as frailty.

References

1. van der Flier WM, Skoog I, Schneider JA et al (2018) Vascular cognitive impairment. *Nat Rev Dis Primers* 4:18003
2. Gorelick PB, Scuteri A, Black SE et al (2011) Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 42:2672–2713
3. Sugimoto T, Arai H, Sakurai T (2021) An update on cognitive frailty: its definition, impact, associated factors and underlying mechanisms, and interventions. *Geriatr Gerontol Int* 22:99
4. Hong CH, Falvey C, Harris TB, Simonsick EM, Satterfield S, Ferrucci L, Metti AL, Patel KV, Yaffe K (2013) Anemia and risk of dementia in older adults: findings from the Health ABC study. *Neurology* 81(6):528–533
5. Miranda AS, Cordeiro TM, Dos Santos Lacerda Soares TM, Ferreira RN, Simões E Silva AC (2017) Kidney-brain axis inflammatory cross-talk: from bench to bedside. *Clin Sci (Lond)* 131(11):1093–1105
6. Chung MC, Yu TM, Shu KH et al (2017) Hyponatremia and increased risk of dementia: a population-based retrospective cohort study. *PLoS One* 12:e0178977. <https://doi.org/10.1371/journal.pone.0178977>
7. Sakatani K, Oyama K, Hu L (2020) Deep learning-based screening test for cognitive impairment using basic blood test data for health examination. *Front Neurol* 11:588140
8. Sakatani K, Oyama K, Hu L, Warisawa S (2022) Estimation of human cerebral atrophy based on systemic metabolic status using machine learning. *Front Neurol* 13:869915
9. Oyama K, Sakatani K (2022) Machine learning-based assessment of cognitive impairment using time-resolved near-infrared spectroscopy and basic blood test. *Front Neurol* 12:624063

Understanding Oxygen “Buffering” by Caveolae Using Coarse-Grained Molecular Dynamics Simulations

Samaneh Davoudi and An Ghysels

Abstract

The “oxygen paradox” embodies the delicate interplay between two opposing biological processes involving oxygen (O_2). O_2 is indispensable for aerobic metabolism, fuelling oxidative phosphorylation in mitochondria. However, excess O_2 can generate reactive species that harm cells. Thus, maintaining O_2 balance is paramount, requiring the prioritisation of its benefits while minimising potential harm. Previous research hypothesised that caveolae, specialised cholesterol-rich membrane structures with a curved morphology, regulate cellular O_2 levels. Their role in absorbing and controlling O_2 release to mitochondria remains unclear. To address this gap, we aim to explore how the structural features of caveolae, particularly membrane curvature, influence local O_2 levels. Using coarse-grained (CG) molecular dynamics simulations, we simulate a caveola-like curved membrane and select a CG bead as the O_2 model. Comparing a flat bilayer and a liposome of 10 nm diameter, composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), allows us to study changes in the O_2 free energy profile. Our findings reveal that curvature has a con-

trasting effect on the free energy of the outer and inner layers. These findings show the membrane curvature’s impact on O_2 partitioning in the membrane and O_2 permeation barriers, paving the way towards our understanding of the role of caveolae curvature in O_2 homeostasis.

Keywords

Liposome · Martini · Oxygen partitioning · Free energy profile · Membrane curvature

1 Introduction

The “oxygen paradox” refers to the intricate interplay between two contrasting biological processes involving oxygen (O_2) as a reactant. O_2 is vital for aerobic metabolism, acting as a fuel for oxidative phosphorylation within mitochondria. However, an excessive supply of O_2 can lead to the generation of reactive species that harm cellular health. Therefore, maintaining O_2 homeostasis becomes crucial, requiring a delicate balance that prioritises the former process while minimising the latter. In earlier research, a hypothesis was proposed centred around specialised membrane invaginations called caveolae [1]. These unique structures exhibit a curved morphology and are rich in cholesterol. It was postulated that caveolae play a pivotal role in regulating

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O₂ levels within cells by efficiently absorbing O₂ and attenuating its release to the mitochondria [2]. Caveolae would thus act as dynamic reservoirs for O₂ and aid in its regulation and distribution within cells, hence functioning as O₂ buffers. However, the precise mechanism by which caveolae could facilitate O₂ buffering remains unclear, posing an intriguing research question.

To address this knowledge gap, our primary objective is to investigate how specific structural characteristics of caveolae, such as membrane curvature and cholesterol content, influence the local O₂ abundance and membrane permeability. To accurately simulate a curved membrane resembling caveolae, we employed coarse-grained (CG) molecular dynamics (MD) simulations with interactions described by the well-known Martini force field [3]. The curved membranes require a very large simulation box, and an all-atom (AA) description would make the calculations very computationally demanding, taking weeks on a supercomputer. After conducting a comprehensive set of tests, we carefully selected one of the existing CG beads of the Martini force field to serve as the CG O₂ model [4].

In this work, a box containing a flat membrane composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and a number of oxygen molecules is simulated. This membrane with zero curvature serves as the benchmark (Fig. 1). In addition, a liposome with non-zero membrane curvature is modelled to observe the effect of curvature. This approach allows us to examine associated changes in the O₂ concentration across the membrane. With this comparison, we aim to deepen our understanding of the underlying mechanisms governing O₂ homeostasis. Future steps will focus on simulating caveolae at their true size, ranging between 60 nm and 80 nm in diameter. Additionally, the caveolin protein will be incorporated into the membrane to bring the simulations closer to realistic caveolae. This deeper understanding has the potential to unlock insights into O₂-related disorders, potentially catalysing new avenues of research aimed at treating these conditions.

2 Methods

2.1 Simulated Systems

To study the effect of curvature, two model systems were simulated in a CG description, that is a flat membrane and a liposome with radius of about 50 Å (Fig. 1). Table 1 gives an overview of the components, showing that ion pairs were added to represent physiological conditions. Each O₂ molecule is represented by one TC3 Martini bead, which was selected based on its size (tiny, “T”), polarity, and charge (non-polar and neutral, “C”), and interaction with phospholipids (“3”) in earlier work [4]. In this study, the oxygen concentration employed is deliberately elevated beyond physiological levels to enhance the sampling of oxygen crossings across the membrane. Importantly, thorough checks have been conducted throughout the simulations, and no instances of aggregation have been observed. Note that one water CG bead represents four water molecules in the Martini force field [3]. The simulations were carried out at a temperature of $T = 310$ K (body temperature) and pressure of 1 bar.

2.2 Computational Details

The systems were simulated using GROMACS-2021.4 [5]. Using CHARMM-GUI, initial structures of the membranes were prepared [6–9]. The Martini 3 forcefield was used [3]. The reaction-field approach was used to calculate the Coulombic interactions [10]. With the potential-shift-Verlet modifiers, the Coulombic and the van der Waals potentials were shifted to zero at the cutoff of 1.1 nm. The neighbour list was updated using the Verlet neighbour search algorithm with cutoff length 1.1 nm. The equation of motion was integrated using the leap-frog integrator with a time step of 20 fs. A temperature of 310 K and a pressure of 1 bar were set for all the systems. The velocity rescale thermostats coupling constant was set at 1.0 ps [11]. The Parrinello–Rahman barostat was used with a coupling constant of

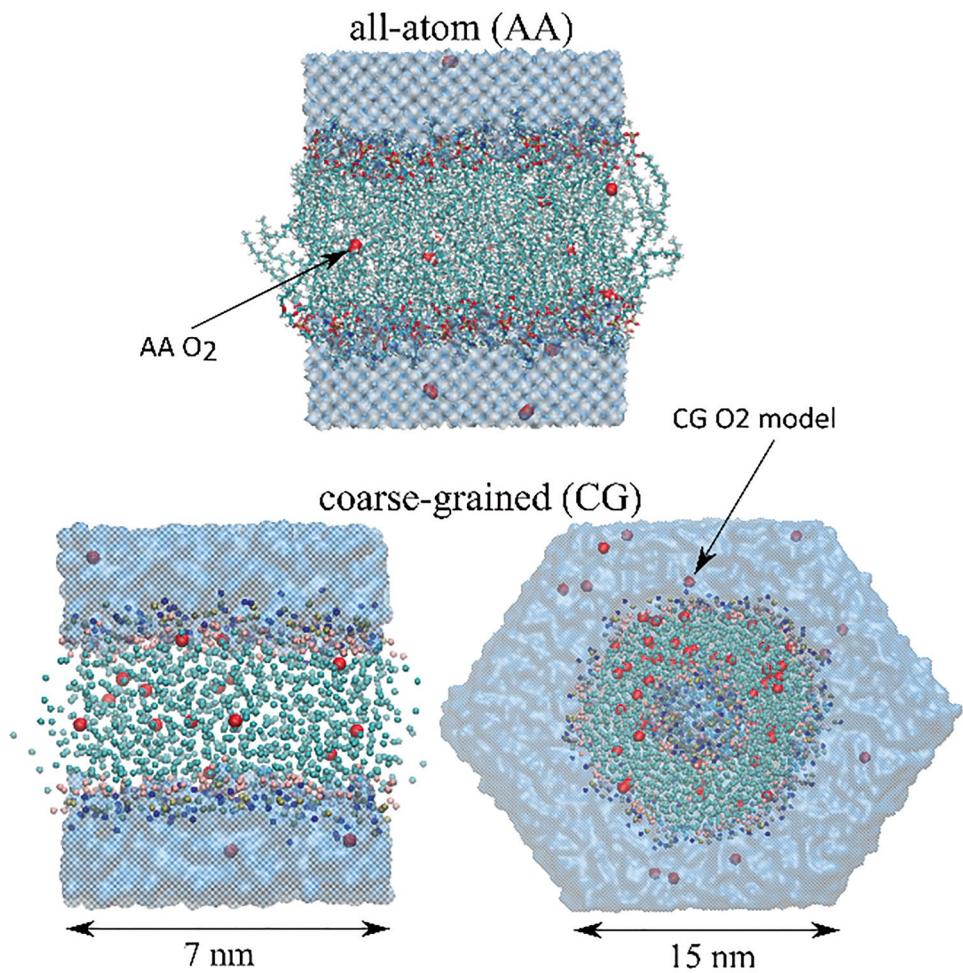


Fig. 1 All-atom (AA) description of a flat membrane and coarse-grained (CG) description of flat membrane (left) and liposome (right). Notice the difference in scale for the liposome. Oxygen (red) is indicated with an arrow in both descriptions. For CG systems: POPC is presented by points, colour-coded by bead type: green for hydrocar-

bons, pink for glycerol, brown for phosphate, and blue for choline beads. For AA system: the same colour scheme is applied, except the glycerol group is red. Water is depicted as transparent light blue. To maintain clarity, ions are not displayed in the visualisation

Table 1 Detailed description of the two simulated systems: a flat membrane and a liposome with radius of about 50 Å

Systems	#POPC	#W	#Na ⁺ /Cl ⁻	#O ₂
POPC-flat	128	1395	14	18
POPC-liposome-50A	394	16,618	192	111

The number of phospholipid molecules (#POPC), water CG beads (#W), ion pairs (#Na⁺/Cl⁻), and oxygen CG beads (#O₂) is indicated

12 ps. For systems with a flat membrane and a liposome, semi-isotropic and isotropic isothermal compressibility of 3×10^{-4} and $4.5 \times 10^{-5} \text{ bar}^{-1}$ was employed, respectively. A dodecahedron box was used to simulate the liposomes. The centre of mass motion of the system was removed every 100 time steps. Systems were first energy minimised and, next, they were equilibrated applying NPT (isothermal–isobaric) ensemble for 100 ns. The production run was 500 ns of NPT simulation.

2.3 Analysis of Results

A histogram was made of the position of the O_2 molecules with respect to the centre of the membrane. In the case of a flat membrane, position is measured along the membrane's normal axis (z -direction). However, for a liposome, position is determined by measuring the radial distance from the centre of the liposome. This centre is defined by the average position of the phosphor CG beads (PO4) located in the head group of the POPC molecules. The logarithm of the histogram of the position q along the membrane normal leads to the free energy profile:

$$F(q) = -k_B T \ln(\text{hist}(q))$$

where k_B is Boltzmann's constant and T is the temperature. At 310 K, $1 k_B T$ is approximately equivalent to 4.28×10^{-21} Joules or 2.58 kJ/mol.

3 Results and Discussion

The free energy profile gives information about the barriers that oxygen must surmount to permeate through the membrane. Figure 2 shows the free energy difference ΔF with respect to the water phase, i.e. $\Delta F(q) = F(q) - F_{\text{waters}}$ where the centre of the two membranes was aligned to the value $q = 0$. The flat membrane (blue line) shows that O_2 needs to overcome a barrier of about $0.7 k_B T$ to enter the POPC membrane based on the CG calculations. More importantly, O_2 experiences a barrier of about $3.7 k_B T$ to escape from

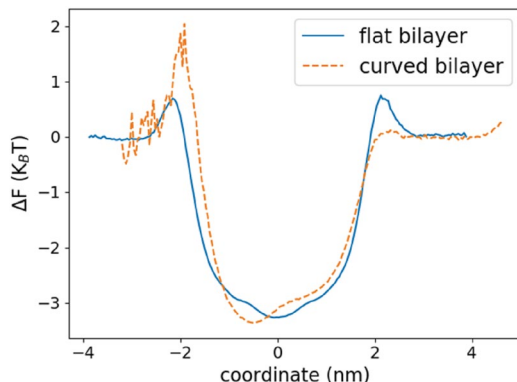


Fig. 2 Oxygen free energy profile as a function of the permeation coordinate q (normal to the membrane)

the membrane. These barrier values are in accordance with the more detailed AA calculations [3]. In practice, this means that the POPC membrane will capture O_2 for a while before it can overcome the barrier and escape. This behaviour of oxygen storage (partitioning) in membranes has now been well-studied in multiple works [12–16].

Next, we turn to the liposome exhibiting a curved membrane (orange line). The positive and negative q ranges correspond to the outer and inner leaflet, respectively. In the inner leaflet, the liposome shows a heightened barrier compared to the flat bilayer, likely because of the packing structure in the inner leaflet of this fairly small liposome with a diameter of only approximately 10 nm. In the outer leaflet, the barrier is reduced compared to the flat bilayer. The curvature not only alters the headgroup region but also the membrane centre, characterised by a shift of the lowest point of the free energy towards the inner layer. This phenomenon is likely attributed to the compact arrangement of lipids within the inner layer. The symmetry between inner and outer bilayer is thus broken, such that, concretely, the O_2 concentration is higher in the inner leaflet than in the outer leaflet. It is possible that this asymmetry could be mitigated in a liposome with a larger radius.

The changes induced by curvature thus show an opposite trend in the inner and outer leaflets, and it remains unclear how these free energy changes will affect the overall oxygen buffering capacity behaviour. To fully understand the effect

on the permeation and storage kinetics, the time aspect should also be included. It should be noted that the diameter of caveolae is significantly larger than the liposomes utilised in our simulations. By focusing on liposomes with the highest curvature, we aimed to explore the limitations of how curvature influences oxygen partitioning within curved membranes. In future work, we aim to investigate the O₂ permeability, O₂ storage capacity, and O₂ residence time.

4 Conclusion

This study employed CG MD simulations to examine the influence of membrane curvature, a structural characteristic of caveolae, on the permeation of oxygen through the membrane. In this investigation, a CG bead was utilised as a representative model for oxygen. By comparing oxygen interaction with a flat membrane and with a liposome membrane, it becomes evident that curvature has a dual effect on oxygen partitioning: It diminishes the free energy barrier in the outer layer while augmenting it in the inner layer. To obtain a more comprehensive understanding of this phenomenon and its impact on oxygen transport, it is essential to compute additional kinetic properties of O₂ in future work.

References

1. Davoudi S et al (2022) Understanding the role of Caveolae in oxygen buffering: the effect of membrane curvature. In: International society on oxygen transport to tissue XLIV. Springer International Publishing, Cham, pp 87–91
2. Zuniga-Hertz JP et al (2019) The evolution of cholesterol-rich membrane in oxygen adaption: the respiratory system as a model. *Front Physiol* 10:1340
3. Souza PCT et al (2021) Martini 3: a general purpose force field for coarse-grained molecular dynamics. *Nat Methods* 18(4):382–388
4. Davoudi S et al. Validation of coarse-grained model for molecular oxygen. To be submitted
5. Abraham MJ et al (2015) GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 1:19–25
6. Jo S et al (2008) CHARMM-GUI: a web-based graphical user interface for CHARMM. *J Comput Chem* 29(11):1859–1865
7. Brooks BR et al (2009) CHARMM: the biomolecular simulation program. *J Comput Chem* 30(10):1545–1614
8. Lee J et al (2016) CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM simulations using the CHARMM36 additive force field. *Biophys J* 110(3):641a
9. Qi Y et al (2015) CHARMM-GUI martini maker for coarse-grained simulations with the martini force field. *J Chem Theory Comput* 11(9):4486–4494
10. Tironi IG et al (1995) A generalized reaction field method for molecular dynamics simulations. *J Chem Phys* 102(13):5451–5459
11. Bussi G, Donadio D, Parrinello M (2007) Canonical sampling through velocity rescaling. *J Chem Phys* 126:1
12. Vervust W, Ghysels A (2022) Oxygen storage in stacked phospholipid membranes under an oxygen gradient as a model for myelin sheaths. In: *Oxygen transport to tissue XLIII*. Springer International Publishing, Cham, pp 301–307
13. Krämer A et al (2020) Membrane permeability of small molecules from unbiased molecular dynamics simulations. *J Chem Phys* 153:12
14. Ghysels A et al (2019) Permeability of membranes in the liquid ordered and liquid disordered phases. *Nat Commun* 10(1):5616
15. De Vos O et al (2018) Membrane permeability: characteristic times and lengths for oxygen and a simulation-based test of the inhomogeneous solubility-diffusion model. *J Chem Theory Comput* 14(7):3811–3824
16. Ghysels A et al (2017) Position-dependent diffusion tensors in anisotropic media from simulation: oxygen transport in and through membranes. *J Chem Theory Comput* 13(6):2962–2976

Part V

Tumor Oxygenation

Analysis of Connexin 43 and Spermine Co-localisation in Glioblastomas

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Abstract

Gap junctions are channels between adjacent cells, contributing to the unhindered exchange of metabolites, second messengers, nucleotides, and other molecules. The functional status of gap junctions in brain tumours is underinvestigated. One avenue of research focuses on exploring the expression of polyamines and their co-localisation with the Connexin 43 (Cx43) in the growth zones of glioblastoma multiforme (GBM). The aim of this work was to analyse the expression of Cx43 and spermine in human GBM to reveal

their roles in neuro-oncogenesis. Human GBM sample sections were used for the immunochemistry [glial fibrillary acidic protein (GFAP), Cx43, and spermine], confocal laser scanning microscopy, and electron immunohistochemistry. Immunofluorescent analysis revealed that the more extensive processes of GBM cells exhibit GFAP. All GBM samples ($n = 10$) exhibited positive Cx43 signals in the form of variously sized dots and lines. Cx43 formed dotted lines around cell bodies with segmented transformed nuclei, which were also present in the gliovascular complexes. Furthermore, spermine was over-expressed in all tumour samples (cytoplasm and large and thin tumour processes), including the areas of Cx43 localisation. Merging the Cx43 and spermine signals showed co-expression in the same regions: the membranes of individual cells and individual points on processes in the tumour tissue. Therefore, we established the staining of the co-localisation of Cx43 and the polyamine spermine within glioblastoma, revealing that tumour processes housing the polyamine indeed form gap junctions, suggesting their potential joint interaction. This finding indicates that glioma cells can integrate into the surrounding neural networks, potentially serving as a mechanism to release glycolysis products, relying on gap junction activity facilitated by spermine. Cx43 exhibits sensitivity to

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polyamines, which play a role in opening gap junctional channels. Furthermore, polyamines have been observed to eliminate the blockades caused by hydrogen ions and calcium, which is crucial for cellular physiology.

Keywords

Proteins of astrocytic gap junctions · Glial tumours · Polyamines · Confocal laser Electron microscopy

1 Introduction

Among glial brain tumours, glioblastoma multiforme (GBM) is the most aggressive and common, representing 50–60% of all gliomas, with peak age incidence at 45–55 years. There is currently no effective treatment to improve survival for patients with GBM, and the median survival is 3 months after diagnosis. Significant efforts are directed towards identifying promising targets, the selective modulation of which can enhance the efficacy of treating GBM. Gap junctions and connexins (Cx) are considered crucial pathogenetic mechanisms of malignant tumour growth and promising targets for targeted therapy [1]. Cx43 is a multifunction protein that forms gap junction channels and hemichannels and thus is suggested to play a key role in oxygen–glucose deprivation induced by neuroinflammation during the process of tumour expansion into healthy tissue. Gap junctions, composed of docked membrane half-channels, form a pore between adjacent cells, contributing to the unhindered exchange of metabolites, second messengers, nucleotides, and other molecules. In previous studies, we did not find these contacts in oligodendroglioma grade II, but we demonstrated the presence of gap junctions in samples of pleomorphic xanthoastrocytoma grade II [2], hemistocytic astrocytoma grade II [3], malignant anaplastic astrocytomas grade III [4], and some samples of glioblastoma grade IV. However, the functional status of gap junction in GBM remains an area of interest requiring investigation. Regulation may occur through small molecules

moving through gap junctions, direct control of connexin-encoding genes, oversight of connexin synthesis and traffic, management of connexin degradation, and control over connexin interactions with cytoplasmic proteins. One of the possible options for the Cx43 behaviour control could be the interaction with polyamines. Polyamines, multifunctional molecules containing several amine groups, play a vital role in the brain [5]. Spermine biosynthesis is activated in tissues with cell proliferation and is suggested as a marker of various malignancies of gliomas [6]. One avenue of research focuses on exploring the expression of polyamines and their co-localisation with the Cx43 protein in the growth zones of glioblastomas. Glial fibrillary acidic protein (GFAP) has proved to be the most specific marker for cells of astrocytic origin under normal and pathological conditions. Gliomas can heterogeneously express GFAP, a signature type III IF protein of glioma cells implicated in tumour migration and invasion. At the same time, biochemical evidence was obtained indicating direct binding of the cytoplasmic domains of integrins to gliofibril proteins at cell–cell contact sites and their substrates. Thus, GFAP may participate in complex cellular processes controlling astrocytoma cell morphology, adhesion, and proliferation. The aim of this work was to analyse the expression of Cx43, GFAP, and spermine in human GBM to reveal the roles of these proteins in neuro-oncogenesis.

2 Methods

Surgically resected human glioblastoma samples were used as material for research. The informed consent was obtained from patients or their relatives. The sample group included tumour fragments from patients with ages ranging from 45 to 66 years (3 women and 7 men) with a pathomorphological diagnosis of glioblastoma grade 4. Samples ($n = 10$) were fixed in 4% paraformaldehyde. Vibratome (VT 1000E, Leica, Germany) was used to cut 40 μm sections from the samples. Sections were cryoprotected in 15% and 30% sucrose and instantly frozen over liquid nitrogen

vapor. For immunohistochemistry, sections were thawed in a phosphate buffer (PBS). The sections were then subjected to a 7-day incubation using the following mixtures: (1) rabbit polyclonal to Cx43 (Elabscience, USA)/mouse monoclonal to GFAP (Sigma-Aldrich, USA), (2) rabbit polyclonal to spermine (Cloud-Clone, USA)/mouse monoclonal to GFAP (Sigma-Aldrich, USA), and (3) rabbit polyclonal to spermine (Cloud-Clone, USA)/mouse monoclonal to Cx43 (Thermo Fisher Scientific, USA). Sections were washed in PBS and incubated for 24 h at 10 °C in a mixture of secondary antibodies conjugated with a fluorescent label: (1) goat anti-rabbit conjugated with Abberior STAR ORANGE (Abberior, Germany), (2) goat anti-mouse conjugated with Abberior STAR RED (Abberior, Germany), and (3) with Sytox green stain (Thermo Fisher Scientific, USA). Finally, the sections were mounted in an anti-fade solution and examined using a confocal laser scanning microscope (Abberior Facility Line, Abberior Instruments GmbH, Germany). After confocal microscopy, the cover glass was carefully removed from a part of the tumour sections ($n = 3$). Sections were incubated in secondary antibodies RTU Envision Flex/HRP (Dako, Denmark) for 24 h at 20 °C. Immune complexes were detected using the EnVision HRP + Peroxidase imaging system (Dako, Denmark). For electron microscopy, the sections were washed in PBS and postfixed in 1% OsO₄, dehydrated, and embedded in epoxy resin. Sections were cut using EM UC 7 ultramicrotome (Leica, Germany) and an ultra-45° diamond knife (Diatome, Switzerland), contrasted with uranyl acetate and lead citrate. Images were taken under a Jem 1011 electron microscope (Jeol, Japan) with an accelerating voltage of 80 kV.

3 Results and Discussion

Confocal microscopy demonstrated that GBM forms clusters of polymorphic cells with branching processes of various diameters and directions, containing GFAP (Fig. 1a). All GBM samples exhibited positive Cx43 signals in the form of dots of different sizes located on the cell

processes. Figure 1b shows the nucleus of a tumour cell and Cx43 points located along its periphery, apparently on the processes of the cells. Co-localisation analysis demonstrated that most Cx43 points are localised in processes containing GFAP in GBM and around microcapillaries. In some cases, the Cx43 signals were observed as narrow lines associated with the cell bodies (Fig. 1c, d, arrow). Serial optical imaging (0.2 µm step size along the Z-axis) unveiled variations in the depth at which Cx43 signals were detectable, indicating the heterogeneous size of Cx43. The Cx43-positive signals within the examined volume were also correlated with GFAP-positive structures in GBM. We observed Cx43+ reaction in the form of dots in all tumour samples. In each sample, we performed a separate count of dots (elements) around tumour cells ($n = 20$). The median of the numerical series was determined to be equal to 11 (7–13). In this case, the results ranged from 5 to 20 elements per sample. Spermine was found to be overexpressed in all tumour samples. Spermine was localised in the cytoplasm, and the large and thin tumour processes were in the intercellular space of GBM.

Moreover, a positive spermine reactivity was identified in regions where GFAP response was weak or negative (Fig. 1e–g). Furthermore, we observed heterogeneity in the expression of polyamines in the specific areas of the glioblastoma. In addition, heterogeneity in the expression of polyamines in some areas of glioblastoma was noted. GBM regions containing the most significant number of spermine were further examined by electron immunohistochemistry.

Electron immunohistochemistry showed that polyamine mainly accumulated in the cytoplasm of GBM cells, located amidst a network of glial filaments and remnants of myelinated fibres of neuron axons (Fig. 1h)—these large bizarre cells with pleomorphic atypical nuclei often contained inclusions. The nuclei were frequently segmented and did not contain spermine. Multiple Golgi complexes and centrioles indicated the process of endomitosis. The polyamine-containing cell processes formed contact with gap junctions (Fig. 1i). They were seen as “stuck together” bilipid membranes with an osmophilic gap between

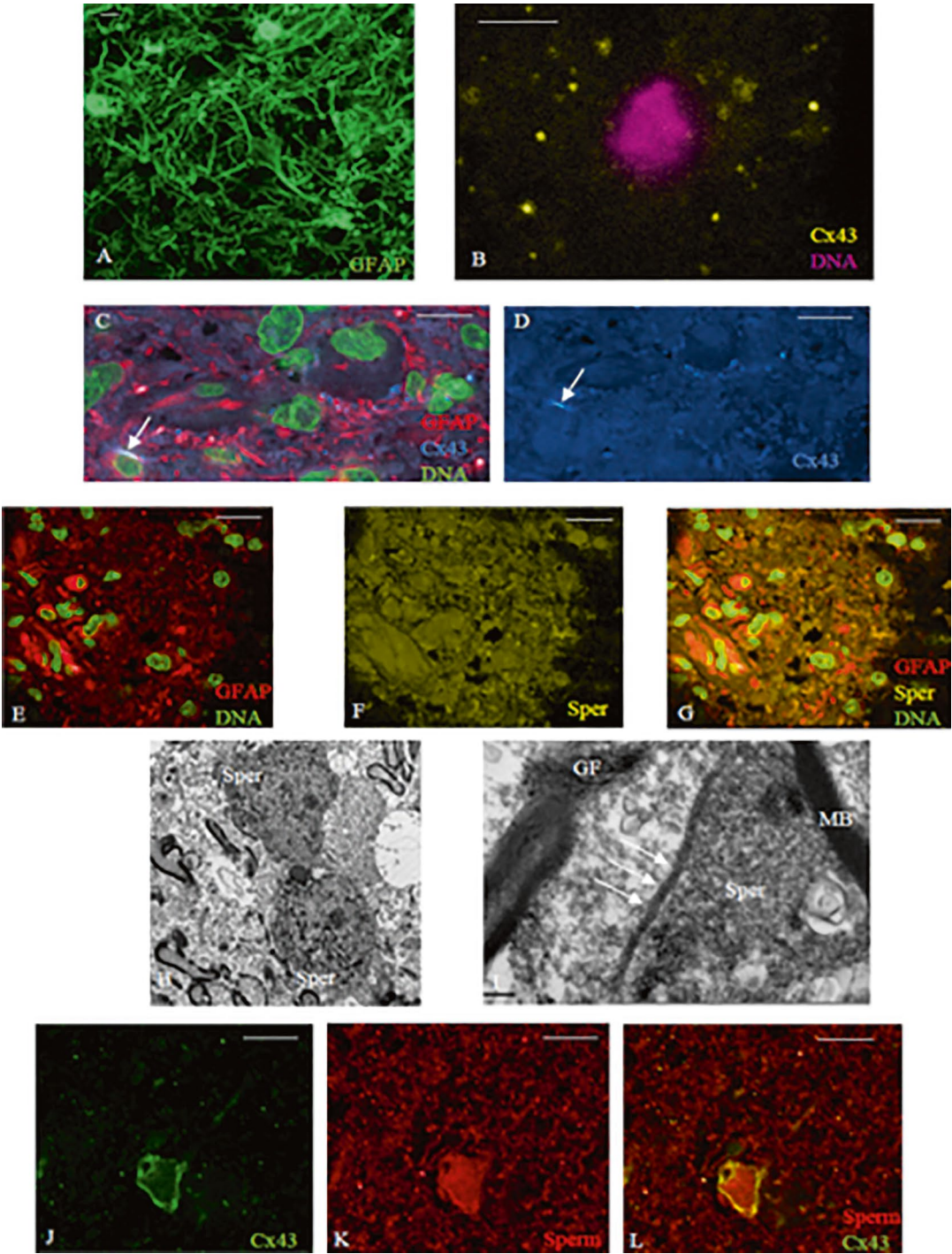


Fig. 1 Immunofluorescence and electron microscopic examination of Cx43 and spermin in glioblastomas. (a) Localisation of GFAP in GBM cells, scale bar 20 µm; (b) localisation of Cx43+ dots around the tumour cell, scale bar 5 µm; (c) Cx43+ dots in the GFAP branches

around the microcapillary and Cx43+ lines on membranes of single cells (arrow), scale bar 10 µm; (d) Cx43+ signal in GBM, scale bar 10 µm; (e) GFAP+ signal, indicate the glial fibrils, Sytox green indicate nuclei, scale bar 10 µm; (f) localisation of spermin in

them. Co-expression of polyamine and Cx43 was done by double fluorescent labelling. The analysis showed that both Cx43 and spermine had the exact intracellular localisation. However, connexin had membrane reactivity, while spermine had both membrane and cytoplasmic reactivity (Fig. 1j, k). Merging the two signals showed co-expression in the same areas: the membranes of individual cells and individual points on processes in the tumour tissue (1 L, yellow colour). Therefore, we established the staining of the co-localisation of Cx43 and the polyamine spermine within glioblastoma, revealing that tumour processes housing the polyamine indeed form gap junctions, suggesting their potential joint interaction. The obtained results show that GBM contains numerous positive fluorescence signals of Cx43 in the form of dots and lines on cell membranes that indicate hemichannels and gap junctions. The multiple gap junctions in GBM may support the hypothesis of forming syncytium for electrotonic and metabolic transmission between glioma cells, promoting its progression. Gap junctions can facilitate the diffuse transfer of lactate and bicarbonate (end-product of glycolytic metabolism) from more hypoxic tumour cells to normoxic ones by providing metabolic cooperation with low resistance. Therefore, it is essential to identify molecules that influence the closed and open states of these contacts.

It has been demonstrated the joint staining of Cx43 and polyamines, which play a role in opening the gap junctional channels. Furthermore, polyamines have been shown to eliminate the blockades caused by hydrogen ions and calcium [7], which is crucial for cellular physiology. The detection of both gap junction networks and spermine overexpression in the same areas of glioblastomas suggests that glioma cells may be able to integrate into the surrounding neural networks of the brain, potentially serving as a mech-

anism to release glycolysis products [8]. Implementing these functions in syncytium relies on the opening of gap junctions, which is facilitated by spermine.

4 Conclusions

We have shown, for the first time, a significant number of Cx43 and polyamines in GBM cells. In glial syncytia, gap junctions are crucial for regulating ion concentrations, neurotransmitters, metabolic processes, and homeostasis. Polyamines, like spermine, facilitate potassium and calcium wave propagation in brain tumour syncytia. Gap junctions and hemichannels can directly control the activity of individual neurons and neural networks surrounding the tumour. To carry out these functions in the syncytium, a necessary condition is opening the gap junctions associated with the presence of polyamines (spermine, spermidine, putrescine, and agmatine).

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References

1. Tabernero A, Gangoso E, Jaraíz-Rodríguez M et al (2016) The role of connexin43-Src interaction in astrocytomas: a molecular puzzle. *Neuroscience* 26:183–194
2. Kirichenko E, Sehweil-Salah M, Goncharova Z et al (2020) Ultrastructural evidence for the presence of gap junctions in the rare case of pleomorphic xanthoastrocytoma. *Ultrastr pathol* 44:227–236
3. Kirichenko E, Savchenko A, Kozachenko D et al (2017) Ultrastructural characteristics of gap junctions in human glial brain tumors. *Arch Pathol* 79:3–11
4. Logvinov A, Kirichenko E, Sehweil S et al (2022) Confocal laser and electron microscopic investigation

Fig. 1 (continued) GBM, scale bar 10 µm; (g) merge of GFAP, Sytox green and spermine, scale bar 10 µm; (h) electron immunohistochemistry with antibodies to spermine. Two cells of GBM accumulated spermine (sper). Magnification—12,000; (i) gap junction formed by the spermine-containing branch. Legend: GF—glial

fibrils, MB—myelinated branch, arrows—gap junction. Magnification—80,000; (j) Cx43+ signals in GBM, scale bar 10 µm; (k) spermine+ signals in GBM, scale bar 10 µm; (l) co-localisation of Cx43 and spermine as yellow dots in tissue and yellow membrane reactivity of cells, scale bar 10 µm

- of gap junctions in anaplastic astrocytomas. *Adv Exp Med Biol* 1395:309–313
5. Makletsova M, Rikhireva G, Kirichenko E et al (2022) The role of polyamines in the mechanisms of cognitive impairment. *Neurochem J* 16:283–294
 6. Ernestus R, Röhn G, Schröder R et al (1996) Polyamine metabolism in gliomas. *J Neuro-Oncol* 2:167–174
 7. Skatchkov N, Bukauskas F, Benedikt J et al (2015) Intracellular spermine prevents acid-induced uncoupling of Cx43 gap junction channels. *Neuroreport* 26:528–532
 8. Venkatesh HS, Morishita W, Geraghty AC et al (2019) Electrical and synaptic integration of glioma into neural circuits. *Nature* 573:539–545

Impact of Acute or Chronic Acidosis and Hypoxia on Gene Expression Patterns in Tumour Cells: Potential Functional Implications

O. Thews, S. Reime, V. Dubourg, and A. Riemann

Abstract

Tumours often exhibit pronounced hypoxia and hereby extracellular acidosis due to intensified glycolysis. Since metabolic parameters can modulate gene expression, the aim of the study was to analyse changes in gene expression patterns induced by acute (24 h) acidosis or hypoxia and also in tumour cells adapted to long-term acidosis (5 weeks). Three tumour cell lines (AT1 prostate carcinoma, MCF-7, and MDA-MB-231 breast carcinoma) were exposed to acidosis (pH 6.6) or hypoxia (pO₂ 1.5 mmHg) for 24 h. For long-term acidosis, AT1 tumour cells were continuously cultured at pH 6.6 for 5 weeks. Gene expression was examined by total RNA-sequencing and the functional significance was assessed by gene set enrichment analysis using the Gene Ontology database. Under short-term acidosis (24 h), AT1 and MCF-7 cells showed comparable changes. 714 genes were acidosis-dependently regulated in AT1 cells (275 up, 439 down), and 221 genes in MCF-7 cells (95 up, 126 down). MDA-MB-231 cells almost did not respond to low pH (13 regulated genes). Hypoxia affected MCF-7 cells the most (1498 regulated genes), whereas fewer

genes were regulated in AT1 and MDA-MB-231 cells. Concerning the function of the regulated genes by short-term acidosis, RNA processing, cell cycle regulation, DNA synthesis, and mitochondrial function were negatively affected. Chronic acidosis showed a different picture. In AT1 cells, 1160 genes were differentially expressed (638 up, 522 down) when cells exposed to low pH for 5 weeks. The putatively acidosis-induced changes in functions included tissue structural development, RNA processing, and mitochondrial activity. This study shows that both acute and chronic acidosis of tumour cells lead to altered gene expression and thus affect cell function. Long-term acidosis leads to fundamentally different changes, indicating an adaptation process of the tumour cells.

Keywords

RNA processing · Cell cycle regulation
DNA synthesis · Mitochondrial function
RNA-sequencing · Gene Set Enrichment Analysis (GSEA)

1 Introduction

Many human tumours have an inadequate vascular network, which leads to an insufficient oxygen supply to the tissue. To meet the energy

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demand, the cell switch to glycolytic metabolism that, in the end, results in an acidification of the tumour tissue with pH values below 6.7 [1]. Low tumour pH has been shown to modulate the expression of genes related to metabolic adaptation to acidosis or to maintain H^+ homeostasis [1]. But also genes to extracellular matrix degradation or tumour cell migration [2, 3] may be affected, which could result in an increased metastatic potential. In addition, genes to cell death as well as inflammatory cytokines (e.g., MCP-1 and iNOS) have been described to be pH-dependent [4, 5].

These findings illustrate that the acidic extracellular environment can affect numerous biological functions of tumour cells by pH-dependent gene expression. However, a comprehensive picture of how acidosis modulates gene expression in tumour cells is presently missing. To distinguish between cell line-specific effects and more general mechanisms, the analyses were performed in three different tumour cell lines. The results were compared to gene expression changes induced by severe hypoxia to clarify whether both environmental factors act via similar or different regulatory mechanisms. In addition, since the tumour pH can be acidotic for various time spans, the impact of pH on gene expression was analysed for short-term (acute, 24 h) and long-term (chronic, 5 weeks) acidosis.

Based on the RNA-sequencing results, possible functional consequences of low pH were derived with bioinformatical methods. Therefore, gene set enrichment analysis (GSEA) was used to access the Gene Ontology database. With this technique, enriched GO terms for biological functions can be identified which may result from the expression data under the different conditions. Finally, to find common GO term patterns, superordinate processes were identified by a graphical cluster analysis of the GSEA results.

2 Methods

2.1 Cell Lines

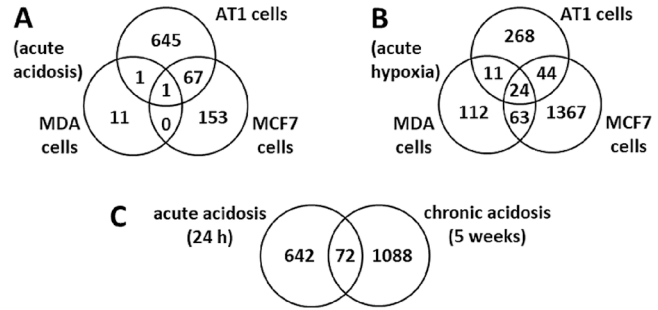
To obtain a comprehensive impression of how acidosis or hypoxia modulates biological behav-

iour of malignancies the impact of moderate acidosis with a pH of 6.6 (as found in many human tumours) or hypoxia with a pO_2 of 1.5 mmHg on gene expression was analysed by whole genome RNA-sequencing. The experiments were performed in the subline AT1 of the rat R-3327 Dunning prostate carcinoma and the human MCF-7 and MDA-MB-231 breast carcinoma cells. All cell lines were grown in RPMI (Roswell Park Memorial Institute). 1640 medium supplemented with 10% foetal calf serum (FCS) at 37 °C under humidified 5% CO_2 atmosphere. For acute acidotic conditions, cells were incubated in FCS lacking medium buffered with 10 mM HEPES/10 mM MES adjusted to pH 7.4 or 6.6 for 24 h. To induce hypoxia, cells were incubated at a pO_2 of 1.5 mmHg in a hypoxia chamber (HypoxyLab, Oxford Optronix, Oxford, UK) for 24 h. After 24 h incubation, the pH changed slightly compared to initial conditions, however, remained stable (AT1 control: 7.14 ± 0.07 , acidosis: 6.59 ± 0.03 , hypoxia: 7.03 ± 0.06 ; MCF-7 control: 7.49 ± 0.05 , acidosis: 6.65 ± 0.07 , and hypoxia: 7.33 ± 0.11). Hypoxia led to a slight acidification which was, however, much less than in the acidosis group. For studying the effects of long-term (chronic) acidosis, AT1 cells were kept for 11 passages (5 weeks) at pH 6.6.

2.2 Experimental Setting

After incubation under different conditions, cells were harvested and lysed and total RNA was extracted with BlueZol reagent according to the manufacturer's instructions. After removing genomic DNA contamination, RNA concentration was determined by NanoDrop (Biochrom, Germany). Whole genome RNA-sequencing was performed by Novogene Co., Ltd. (Cambridge, UK) preparing the sequencing libraries and running the paired-end sequencing (2×150 bp) on a Nova-Seq6000 Illumina system. Adaptor clipping and data quality control was provided by the service company as well. HISAT2 (v. 2.1.0) served for read mapping to the human genome hg38 for MCF-7 and MDA cells and to the rat genome rn7 for AT1 rat cells.

Fig. 1 Venn diagrams for the comparison of differentially expressed genes in the three cell lines by (a) acute acidosis or (b) acute hypoxia and (c) for the comparison of acute acidosis (24 h) and chronic acidosis over 5 weeks



24 genes were uniformly regulated (23 up and 1 down) in all three cell lines (Fig. 1b). Within the list of uniformly up-regulated genes, several genes associated with carbon hydrate metabolism (e.g., HK2, ALDOC, and PGK1) or other HIF-dependent genes (e.g., CA9) were found.

Long-term acidosis (5 weeks at pH 6.6) had also a strong effect on AT1 cells. Under these conditions, 1160 genes were differentially expressed, including 638 up and 522 down (Table 1). Comparing these results to acute acidosis revealed that only 72 of these genes (Fig. 1c) were uniformly regulated under both conditions (43 up, 29 down). This list included up-regulated members of solute carriers (e.g., SLC22A17), intracellular signalling molecules (e.g., CREB3L2 and INPP4A), or ROS metabolism (e.g., CAT).

The functional consequences of the changes in gene expression were assessed by the GSEA. Figure 2a serves as example for the graphical representation of the potential cellular consequences of acute acidosis on AT1 cells. The network highlights that some of the enriched GO terms associate in functionally coherent clusters, suggesting a putative strong impact of the expression changes on the associated processes (indicated in the labels). Acute acidosis predominately down-regulated functions, such as DNA synthesis, cell cycle regulation, mitochondrial function, and RNA processing. These processes were found in all three cell lines analysed (data not shown for the other cell lines).

The functions potentially repressed by hypoxia-induced gene expression regulation included RNA processing, DNA metabolism, and mitochondrial function. However, morphogene-

sis, tissue development, cell adhesion, and intra- and extracellular signalling were found to be potentially promoted by hypoxia (data not shown).

In chronic acidosis, the structural tissue development, cell adhesion, and migration and the response to growth factors were found to be potentially promoted (Fig. 2b), whereas RNA processing and mitochondrial function were predicted to be inhibited.

4 Conclusions

Extracellular acidosis, which is a central parameter of the tumour metabolic microenvironment, can modulate numerous cellular genes and signalling pathways [4, 10]. Most of the analyses on gene regulation focused on the expression of specific genes or only single functional processes (e.g., extracellular matrix turnover [3]). The actual study reveals that low environmental pH modulates a large set of genes in different tumour cell lines. During short-term acidosis (24 h), in AT1 and MCF-7 cells (but not in MDA-MB-231), roughly 60% of these genes were down-regulated, whereas only 40% were increased (Table 1). The functional analysis of the down-regulated genes clearly shows an enrichment in DNA metabolism and repair as well as RNA processing and ribosome function (Fig. 2a). Since the functions were predicted for AT1 as well as MCF-7 cells, it can be concluded that an acidic environment acutely (24 h) may affect the efficacy of treatment modalities that target DNA. Since the results also indicate that acidosis down-regulates that numerous genes involved in cell cycle control low pH could

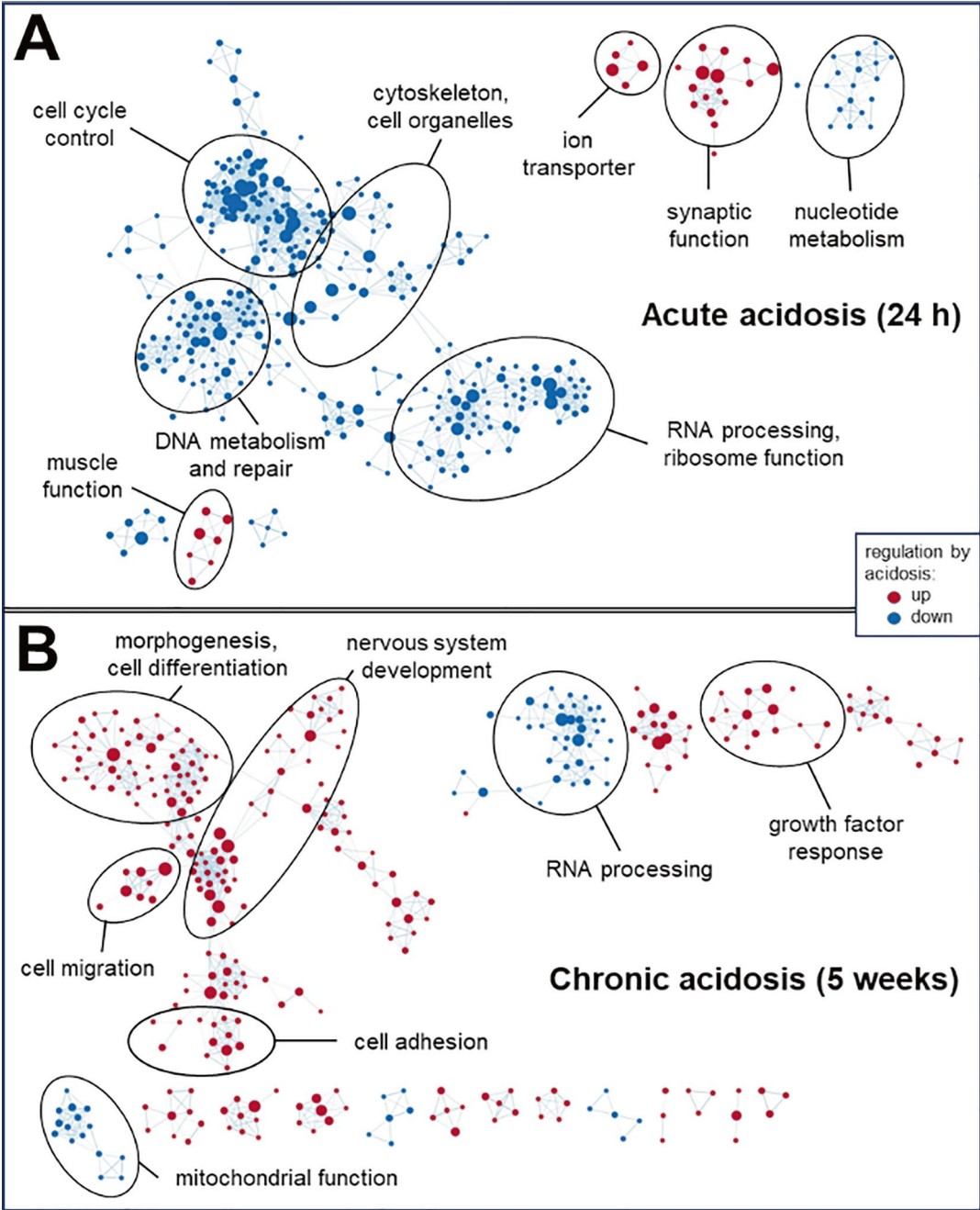


Fig. 2 Result of the GSEA (visualised by Cytoscape) for AT1 tumour cells exposed to an extracellular pH of 6.6 for (a) 24 h (acute acidosis) or (b) continuously for 5 weeks (chronic acidosis). Each dot represents one GO term,

whereas the size of the dot corresponds to the enrichment score of up- or down-regulation. The functions of clusters of GO terms are indicated. $n = 3$ or 4 for each treatment group and respective controls

have an ambivalent effect on tumour progression. On one hand, tumour cell proliferation could be slowed down, but on the other hand, tumour

treatments acting on dividing cells will become less effective. Further studies must show whether the predicted functional alterations by acidosis

have an effect on tumour progression and treatment efficacy.

Under long-term acidosis, a different picture was seen. After 5 weeks of acidosis (chronic), slightly more than half of the genes were up-regulated. This fits to the study of Yao et al. [11] where they described 480 up- and 256 down-regulated genes after 1 month at low pH. Comparing the list of regulated genes after short- and long-term acidosis showed only 72 common genes under both conditions (gene list see Suppl. Mat.). An analysis of these results with GSEA and the software g:Profiler [12] indicated that these genes are associated with a down-regulation of ribosome function and RNA processing. In other words, acidosis per se seems to inhibit the transcriptional process independent from the duration of acidosis. The list of other regulated genes and enriched GO terms (biological processes) (Fig. 2) showed that tumour cells undergo an adaptive process when exposed to acidosis over a longer period. However, since the tumour oxygenation (and, by this, the glycolytic metabolism) may vary quite rapidly, both processes (short- and long-term acidosis) might be relevant for the fate of the tumour tissue.

From the results of chronic acidosis (Fig. 2b), it is of high interest that genes regulated after adaptation to acidosis adaptation associate with increased migration and with tumour cell adhesion. Both processes can influence the metastasis either in the primary tumour where cells detach or in the secondary site where the circulating tumour cells adhere in the new host tissue [13]. Chronic acidosis may lead to an adaptation associated with an increased metastatic potential.

In the present study, MDA-MB-231 cells were almost unaffected by extracellular acidosis. The reason for this remains unclear. However, it is known that MDA-MB-231 cells exhibit high glutathione levels and are less sensitive to rapamycin-induced mTOR inhibition [14]. Since in previous studies, it was demonstrated that ROS play an essential role for the activation MAP kinases by acidosis [15], the increased antioxidant capacity of MDA-MB-231 cells could inhibit MAPK activation. In addition, mTOR signalling is involved in the stress response which is altered in this cell

line and by this could reduce the gene expression response under acidic conditions.

Finally, the comparison between acute acidosis and hypoxia indicated that both environmental parameters affect gene expression, although they induced fundamentally different patterns of gene expression regulation. For instance, in AT1 cells, acute hypoxia promoted the expression of genes related to morphogenesis and hormone or growth factor response, whereas acidosis potentially repressed proliferation and RNS metabolism. Only 50 genes were regulated consistently by both factors in AT1 cells (gene list see Suppl. Mat.). These results show that the central pathophysiological stress factors, hypoxia and acidosis, both modulate the genetic profile of tumours but act on different genes. It should be kept in mind that in the experimental setting, hypoxia also reduced the pH slightly by about 0.13 but much less than in the acidosis group. Therefore, it can be assumed that acidosis and hypoxia act independently in the present study. However, in solid tumours in vivo, both environmental parameters change simultaneously so that mixed effects on gene expression are expected.

In conclusion, the present study shows that tumour acidosis regulates gene expression acutely and chronically, although the expression patterns varied between the two time-scales. The impact of acidosis was also independent from the changes induced by hypoxia. The observed pH-dependent genes may have a strong impact on the biological behaviour of tumours. However, since the present study analysed gene expression by RNA-sequencing only, further functional characterisation needs to clarify the biological role of the reduced pH.

Supplementary Material Supplementary material (gene lists) is available at <https://doi.org/10.6084/m9.figshare.25298773>

References

1. Corbet C, Feron O (2017) Tumour acidosis: from the passenger to the driver's seat. *Nat Rev Cancer* 17:577–593
2. Rofstad EK, Mathiesen B, Kindem K et al (2006) Acidic extracellular pH promotes experimental

- metastasis of human melanoma cells in athymic nude mice. *Cancer Res* 66:6699–6707
3. Nakanishi M, Korechika A, Yamakawa H et al (2021) Acidic microenvironment induction of interleukin-8 expression and matrix metalloproteinase-2/-9 activation via acid-sensing ion channel 1 promotes breast cancer cell progression. *Oncol Rep* 45:1284–1294
 4. Kwon YJ, Seo EB, Jeong AJ et al (2022) The acidic tumor microenvironment enhances PD-L1 expression via activation of STAT3 in MDA-MB-231 breast cancer cells. *BMC Cancer* 22:852
 5. Riemann A, Reime S, Thews O (2017) Tumor acidosis and hypoxia differently modulate the inflammatory program: measurements *in vitro* and *in vivo*. *Neoplasia* 19:1033–1042
 6. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15:550
 7. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139–140
 8. Subramanian A, Tamayo P, Mootha VK et al (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102:15545–15550
 9. Shannon P, Markiel A, Ozier O et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13:2498–2504
 10. Rolver MG, Holland LKK, Ponniah M et al (2023) Chronic acidosis rewires cancer cell metabolism through PPARalpha signaling. *Int J Cancer* 152:1668–1684
 11. Yao J, Czaplinska D, Ialchina R et al (2020) Cancer cell acid adaptation gene expression response is correlated to tumor-specific tissue expression profiles and patient survival. *Cancers (Basel)* 12:2183
 12. Kolberg L, Raudvere U, Kuzmin I et al (2023) g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). *Nucleic Acids Res* 51:W207–W212
 13. Thews O, Riemann A (2019) Tumor pH and metastasis: a malignant process beyond hypoxia. *Cancer Metastasis Rev*:113–129
 14. Cheng SH, Tseng YM, Wu SH et al (2017) Whey protein concentrate renders MDA-MB-231 cells sensitive to rapamycin by altering cellular redox state and activating GSK3beta/mTOR signaling. *Sci Rep* 7:15976
 15. Riemann A, Schneider B, Ihling A et al (2011) Acidic environment leads to ROS-induced MAPK signaling in cancer cells. *PLoS One* 6:e22445

Key Biophysical and Physiological Properties Impacting the Oxygenation Status of Breast Cancers During Thermo-radiotherapy

A.-M. Lüchtenborg, H. Piazena, A. R. Thomsen, and P. Vaupel

Abstract

Mild hyperthermia at 39–43 °C for 30–60 min is applied locoregionally to improve the oxygenation status of recurrent breast cancers, thus enhancing the efficacy of radio-, chemo-, and immunotherapy. In this context, estimated (or even conflicting) data are often used in computational modelling of tumour oxygenation and simulation of O₂ transport. In this chapter, we present information that may help to improve adjuvant thermotherapy delivered immediately prior to radiotherapy of recurrent breast cancers. Data are preferentially derived from clinical investigations; in some cases, measurements in experimental breast cancers are included.

The biophysical properties presented for healthy, mostly postmenopausal, human

breast (composite glandular–adipose–fibrous tissue) measured under normothermic (NT) conditions and in therapeutically heated breast cancers include tissue water content and tissue density. In general, averaged values of parameters reported for NT conditions are higher in breast cancers than in normal breast tissue, i.e., all ratios breast cancer/normal breast are >1. Mean values observed in breast cancers during mild hyperthermia (mHT) are consistently higher than those in NT tumours. Parameters determining convective transports in healthy breast tissue and breast cancer include blood flow rates, blood volume, exchanging water space, arterio-venous shunt flow, interstitial fluid flow rate, interstitial fluid pressure, microvascular permeability, interstitial hydraulic conductivity, and interstitial flow velocity. In general, averaged values of parameters measured under NT conditions are higher in breast cancers than in healthy breast. Except for interstitial fluid pressure, these values increase upon mHT treatment of cancers. Prime factors determining and describing the oxygenation status of the healthy breast, and in NT- versus mHT-treated breast cancers, include: oxygen (O₂) delivery rates, O₂- extractions, O₂- consumption rates, subepidermal microvascular HbO₂, tissue oxygen solubility, oxygen diffusion coeffi-

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cients, mean O_2 partial pressures pO_2 , hypoxic fractions HF <5 mmHg, oxygen enhancement ratio, and mitochondrial ROS production. With the exception of the mean pO_2 , O_2 extraction rate and tissue O_2 saturation all parameters listed are distinctly higher in breast cancers under NT conditions compared to normal breast. Mild hyperthermia results in therapeutically relevant improvements of the oxygenation status of cancers and enhances mitochondrial ROS production, thus improving radiosensitivity. Note: The oxygenation status of the healthy (postmenopausal) breast is very similar to that of the normal human subcutis.

Keywords

Breast cancer · Oxygen delivery · Tumour oxygenation status · Tumour hypoxia
Hyperthermia · Thermo-radiotherapy

1 Introduction

Breast cancer is the most prevalent cancer globally [1]. It is a highly complex disease with heterogeneous inter- and intratumour characteristics and chaotic tissue architecture. The treatment of breast cancer is multifaceted and includes surgery, chemotherapy, radiotherapy, and immunotherapy. Although first-line treatments achieve high cure rates, recurrences are frequent and may comprise locoregional recurrence in the breast or chest wall. These malignancies are particularly challenging to treat due to the effects of prior therapies, including radiotherapy. Re-irradiation with conventional high-dose schemes is contraindicated due to expected toxicities. However, the sensitivity of the tumour tissue to radiotherapy, chemo- and immunotherapies, may be enhanced by localised mild hyperthermia treatment with temperature increases to 39–43 °C (e.g., by water-filtered Infrared A irradiation, wIRA) [2]. Mild hyperthermia modifies the biophysical properties of breast cancer tissue, thereby (a) improving oxygen supply, (b) boosting anti-tumour immunity, and (c) inhibiting DNA repair

enzymes. Hyperthermia allows for administration of significantly lower radiation doses and may be considered a treatment modality for patients with limited options due to previous exposure to high-dose radiotherapy [2, 3]. In this chapter, relevant biophysical and physiological parameters affecting the oxygenation status in breast cancer under NT- and mHT conditions are reviewed.

2 Methods

We have compiled parameters describing the oxygen delivery and the oxygenation status of breast cancers under normothermic (NT) and hyperthermic conditions (39–43 °C), compared to normal breast gland tissue at ambient room temperature. Data are derived mainly from clinical investigations and in some instances from human tumour xenografts to provide a data basis for modelling and therapy optimisation. Ranges are presented whenever reliable and detailed clinical data are available.

3 Results

Key biophysical properties that affect thermal field formation are distinctly different in breast cancer tissue and in normal breast gland. In Table 1, data are presented for breast cancer tissue under normothermic condition (≈ 35 – 37 °C) compared to breast cancer tissue during or immediately after hyperthermic treatment at 39–43 °C and in comparison with normal breast gland at normothermic temperature. The key factor for alterations in parameters describing thermophysical properties, including specific heat capacity, thermal conductivity, and thermal diffusivity, is the significantly increased water content in tumour tissue compared to normal breast [4]. Hyperthermia treatment further increases all parameters listed for breast cancer tissue.

Whole breast is a composite tissue consisting of glandular, fibroglandular, and adipose tissue. In Table 1, only data for gland tissue are pre-

Table 1 Thermophysical properties impacting the thermal field formation in breast cancers during normothermia (NT) and upon oncologic hyperthermia (HT). Normal gland tissue data (normal breast) are additionally presented for comparison. BC/NB: ratio breast cancer/normal breast at NT (37 °C). Values are averaged means and/or ranges

Parameter, [unit]	Normal breast (gland)	Breast cancer NT	Breast cancer HT (39–43 °C)	BC/NB (at NT)	Remarks
Water content (C_w), [wt.%]	53 [4–6]	80 [4, 5]	Increase (exptl.) [11]	≈ 1.5 [5]	Distinct hyperhydration of (breast) cancers [4, 5]
Density (ρ), [kg m ⁻³]	1040–1050 [6, 8]	1050 [7, 8]	Increase	1.0–1.15*	*Depends on individual fat and water contents of whole breast. Strong positive correlation between ρ and C_w [12, 13]
Specific heat capacity (c_p), [J•g ⁻¹ •K ⁻¹]	2.960 [6]	3.610 [5, 7], 3.660 [14], 3.852 [8]	3.780 (43 °C) [14]	≈ 1.2 –1.3 [4]	Linear correlation between c_p and C_w in high water content tissues [5]
Thermal conductivity (κ), [W•m ⁻¹ •K ⁻¹]	0.3345 [6]	0.480 [8], 0.511–0.561 [15], 0.549 [4]	0.572–0.588 [15]	≈ 1.4 –1.7 [4]	Linear correlation between κ and C_w [4]
Thermal diffusivity (α), [mm ² •s ⁻¹]	0.109**	0.1436 [15], 0.117–0.145**	0.1406–0.1426 [15]	≈ 1.1 –1.3 [15]	**Calculation based on listed c_p , κ and ρ values
Metabolic heat generation rate (q_m), [W•m ⁻³]	700 [6]	690–65,400 [7] 5000 [8]		≥ 10 (estimated)	

sented. Corresponding values for fat are: water content (C_w): 22 wt.% [5], specific heat capacity (c_p): 2.220–2.348 J g⁻¹ K⁻¹ [6, 7], density (ρ): 911–930 kg m⁻³ [6, 8], thermal conductivity (κ): 0.171–0.209 W m⁻¹ K⁻¹ [6, 7], thermal diffusivity (α): 0.080–0.103 mm² s⁻¹ (calculation based on c_p , κ , and ρ values), and metabolic heat generation rate (q_m): 400 W m⁻³ [6].

The parameters described in Table 1 are fundamental for the efficient heating of the tissue upon wIRA treatment. In breast cancer tissue, the tumour temperature decreases almost linearly with tissue depth at steady-state HT conditions (Fig. 1). Steady-state surface temperatures of 42 °C are accompanied by temperatures >39 °C at a depth of up to 25 mm as determined by thermometry [10].

Parameters determining the convective transport in breast cancer tissue are compiled in Table 2. Since the efficacy of radiotherapy, as well as the enhanced cytotoxicity of oxygen-dependent drugs greatly depends on oxygen supply to the cells, it is fundamental to consider changes in factors describing convective trans-

port upon oncologic hyperthermia treatment. In general, all parameters listed are elevated in breast cancers tissue compared to normal breast. Except for interstitial fluid pressure, all parameters listed further increase upon oncological hyperthermia treatment.

Table 3 summarises prime factors determining or describing the tissue oxygenation status in breast cancer. Blood flow and oxygen delivery values are considerably higher in tumour tissue compared to the normal gland under normothermic conditions. In contrast, mean O₂ extraction, mean tissue HbO₂ saturation, and mean oxygen partial pressure are substantially reduced in breast cancer tissue. As a result, the oxygen enhancement ratio (OER), a critical indicator for the efficiency of radiotherapy, is ultimately lower. This is consistent with a substantially higher hypoxic fractions found in breast cancer tissue. All parameters are improved upon hyperthermic treatment. Furthermore, hyperthermia enhances mitochondrial ROS production, thus enhancing radiosensitivity and—possibly—activating apoptotic pathways.

Fig. 1 Steady-state temperatures at the tumour surface and at various tissue depths of human recurrent breast cancer (dots) [3] and various human tumours (squares) [9]. Therapeutically relevant hyperthermia levels can be expected up to tissue depths of 25 mm upon wIRA-induced tissue heating

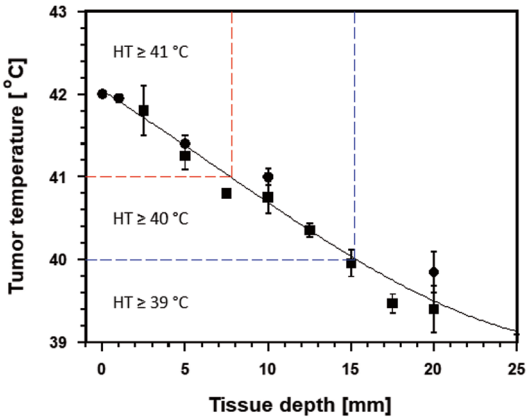


Table 2 Key parameters determining convective transports of oxygen, therapeutic agents, and heat deposition in normal breast and in breast cancers during normothermia (NT) and oncologic hyperthermia (HT). BC/NB: breast cancer/normal breast ratio at NT. Values are averaged means and/or ranges

Parameter, [unit]	Normal breast (gland)	Breast cancer NT	Breast cancer HT (39–43 °C)	BC/ NB (at NT)	Remarks
Blood flow rate (BFR), [mL•g ⁻¹ •min ⁻¹]	0.06 [16–18] (0.03–0.08)	0.24 [7, 16–22], (0.11–0.76)	0.32 [23, 24], (0.25–0.40)	≈4	
Blood volume (BV), [v/v]	0.0336 (0.023–0.042)	0.0432 [21] (0.026–0.065)	Increase	≈1.3	
Exchanging water space, [v/v]	0.14 (0.05–0.26)	0.56 [18] (0.32–0.81)	Increase	4	
Arterio-venous shunt flow, [%]	≈1–2 (estimated)	20–25 [25, 26]	Increase [27]	10–20	
Interstitial fluid flow rate (IFF), [μL•g ⁻¹ •min ⁻¹]	0.6 (1% of BFR)	19 (8% of BFR) [28]	25	≈33	IFF = extravascular fluid flow
Interstitial fluid pressure (IFP), [mmHg]	–1 to 0	+17 [29] (+4 to +33)	Significant drop (Δ = 10 mmHg)	≈20	Experimental data [30, 31]
Microvascular permeability (P), [cm•s ⁻¹]	0.9 •10 ⁻⁷	5.7 •10 ⁻⁷ 0.2 •10 ⁻⁷	8.9 •10 ⁻⁷ [32] 0.6 •10 ⁻⁷ [33]	≈6	HT: 43 °C HT: 42 °C, (exptl. data)
Interstitial hydraulic conductivity (κ), [cm ² •mmHg ⁻¹ •s ⁻¹]	0.85 •10 ⁻⁸	4.13 •10 ⁻⁸ [34]	1.5-fold increase [35]	≈4.85	4–5 times greater interstitial drainage in tumours [36]
Interstitial flow velocity, [μm•s ⁻¹]	0.6 [37]	5–55 [37] 110–170 [38]		8–90	

4 Conclusion

This chapter presents a detailed review of the basic physical and physiological parameters determining the cellular oxygen supply of healthy mammary gland and breast cancer tissue. Additionally, the effects of mild hyperthermia with temperatures of 39–43 °C on these

parameters are summarised. With the exception of the mean pO₂ value, the mean intravascular HbO₂ saturation, the O₂ extraction, and the oxygen enhancement ratio (OER) all parameters listed are distinctly higher in breast cancers under NT conditions compared to normal breast. Mild hyperthermia (39–43 °C)—as used

Table 3 Key parameters determining/describing the oxygenation status of normal breast and breast cancers during normothermia (NT) and oncologic hyperthermia (HT). BC/NB: breast cancer/normal breast ratio. Values are averaged means and/or ranges

Parameter [unit]	Normal breast (gland)	Breast cancer NT	Breast cancer HT (39–43 °C)	BC/NB (at NT)	Remarks
Blood flow rate (BFR), [mL•g ⁻¹ •min ⁻¹]	0.06 [16–18] (0.03–0.08)	0.24 [7, 16–22], (0.11–0.76)	0.32 [23, 24], (0.25–0.40)	≈4	No association between tumour size and BFR
O ₂ delivery rate, [μL•g ⁻¹ •min ⁻¹]	11	43	54	≈4	Delivery = BFR × arterial O ₂ concentration (≈18 mL O ₂ /dL). Syn.: O ₂ supply rate
O ₂ extraction, [%]	72	42	45	0.6	Extraction = consumption/delivery
O ₂ consumption rate, [μL•g ⁻¹ •min ⁻¹]	8	18	24	≈2	
sHbO ₂ , [sat. %]	85 [39]	69 [39]	Increase [40]	1.23 [39]	Normal skin: NT 41% vs. HT 76%
Tissue O ₂ -solubility (αO ₂), [μl•ml ⁻¹ •atm ⁻¹]	3	18	17	6	Increasing αO ₂ with tissue water content [4], decreasing αO ₂ with tissue temperature [41]
FICK's O ₂ diffusion coefficient DO ₂ , [cm ² •s ⁻¹]	0.8 × 10 ⁻⁵	1.75 × 10 ⁻⁵	2.05 × 10 ⁻⁵ [4]	2.2	Linear correlation between DO ₂ and tissue temperature (≈2.5%/1 K) [41], KROGH's diffusion coefficient (K=D•α) also increases although α (= solubility coefficient) decreases during HT [41]
Mean O ₂ partial pressure (pO ₂), [mmHg]	65 (12–100) [16, 42–44]	10 (0–90) [45] 3 [46]	18 (exptl.) [45] 19 (clin.) [46]	0.15	pO ₂ - measurements in hypoxic cancers under ultrasound guidance to avoid invalid readings in necrotic tissues [16]
Hypoxic fraction (HF ≤ 5 mmHg), [%]	0–1	45–50 [46]	≈30	≈45	
Oxygen enhancement ratio (OER) (rel. radiosensitivity)	2.85	2.36 1.85	2.63 (Δ = 0.27) 2.64 (Δ = 0.79)	≈0.8	Exptl. data: [45] Clin. data: [46]
Mitochondrial ROS production, (rel.)		1.0	1.65 [47, 48]		MDA-MB-453 breast cancer cells [47] MCF7 breast cancer cells [48]

in clinical settings—results in therapeutically relevant improvements of the oxygenation status of breast cancers and enhances mitochondrial ROS production, thus improving radiosensitivity. The data presented should allow (a) a detailed modelling of the oxygenation status of breast cancers before and during hyperthermia treatment, (b) the adequate simulation of temperature distributions in breast cancer tissues, and (c) an optimisation of hyperthermic treatment efficacy.

References

1. Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71:209–249. <https://doi.org/10.3322/caac.21660>
2. Notter M, Thomsen AR, Nitsche M et al (2020) Combined wIRA-hyperthermia and hypofractionated re-irradiation in the treatment of locally recurrent breast cancer: evaluation of therapeutic outcome based on a novel size classification. *Cancers (Basel)* 12:606. <https://doi.org/10.3390/cancers12030606>

3. Notter M, Piazena H, Vaupel P (2017) Hypofractionated re-irradiation of large-sized recurrent breast cancer with thermography-controlled, contact-free water-filtered infra-red-A hyperthermia: a retrospective study of 73 patients. *Int J Hyperth* 33:227–236. <https://doi.org/10.1080/02656736.2016.1235731>
4. Vaupel P, Piazena H (2023) Hyperhydration of cancers: a characteristic biophysical trait strongly increasing O₂, CO₂, glucose and lactate diffusivities, and improving thermophysical properties of solid malignancies. *Adv Exp Med Biol* 1438:135–145. https://doi.org/10.1007/978-3-031-42003-0_22
5. Vaupel P, Piazena H (2022) Strong correlation between specific heat capacity and water content in human tissues suggests preferred heat deposition in malignant tumors upon electromagnetic irradiation. *Int J Hyperth* 39:987–997. <https://doi.org/10.1080/02656736.2022.2067596>
6. IT²IS Foundation (2022) Tissue Properties Database V4.1
7. Said Camilleri J, Farrugia L, Curto S et al (2022) Review of thermal and physiological properties of human breast tissue. *Sensors* 22:3894. <https://doi.org/10.3390/s22103894>
8. Channugam A, Hatwar R, Herman C (2012) Thermal analysis of cancerous breast model. *Int Mech Eng Congress Expo 2012*:134–143. <https://doi.org/10.1115/IMECE2012-88244>
9. Seegenschmiedt MH, Klautke G, Walther E et al (1996) Water-filtered infrared-A-hyperthermia combined with radiotherapy in advanced and recurrent tumors. Initial results of a multicenter phase I-II study. *Strahlenther Onkol* 172:475–484
10. Piazena H, Müller W, Vaupel P (2022) Physical and photobiological basics of wIRA-hyperthermia. In: Vaupel P (ed) *Water-filtered Infrared A (wIRA) Irradiation: From Research to Clinical Settings*. Springer, Cham (CH)
11. Gersing E, Kelleher DK, Vaupel P (2003) Tumour tissue monitoring during photodynamic and hyperthermic treatment using bioimpedance spectroscopy. *Physiol Meas* 24:625–637. <https://doi.org/10.1088/0967-3334/24/2/370>
12. Conceição ALC, Meehan K, Antoniassi M et al (2019) The influence of hydration on the architectural rearrangement of normal and neoplastic human breast tissues. *Heliyon* 5:e01219. <https://doi.org/10.1016/j.heliyon.2019.e01219>
13. Graham SJ, Bronskill MJ, Byng JW et al (1996) Quantitative correlation of breast tissue parameters using magnetic resonance and X-ray mammography. *Br J Cancer* 73:162–168. <https://doi.org/10.1038/bjc.1996.30>
14. Bianchi L, Cavarzan F, Ciampitti L et al (2022) Thermophysical and mechanical properties of biological tissues as a function of temperature: a systematic literature review. *Int J Hyperth* 39:297–340. <https://doi.org/10.1080/02656736.2022.2028908>
15. Valvano JW, Cochran JR, Diller KR (1985) Thermal conductivity and diffusivity of biomaterials measured with self-heated thermistors. *Int J Thermophys* 6:301
16. Vaupel P, Mayer A, Briest S, Höckel M (2005) Hypoxia in breast cancer: role of blood flow, oxygen diffusion distances, and anemia in the development of oxygen depletion. *Adv Exp Med Biol* 566:333–342. https://doi.org/10.1007/0-387-26206-7_44
17. Mankoff DA, Dunnwald LK, Gralow JR et al (2002) Blood flow and metabolism in locally advanced breast cancer: relationship to response to therapy. *J Nucl Med* 43:500–509
18. Wilson CB, Lammertsma AA, McKenzie CG et al (1992) Measurements of blood flow and exchanging water space in breast tumors using positron emission tomography: a rapid and noninvasive dynamic method. *Cancer Res* 52:1592–1597
19. Eby PR, Partridge SC, White SW et al (2008) Metabolic and vascular features of dynamic contrast-enhanced breast magnetic resonance imaging and 15O-water positron emission tomography blood flow in breast cancer. *Acad Radiol* 15:1246–1254. <https://doi.org/10.1016/j.acra.2008.03.019>
20. Stevens W, Farrow IM, Georgiou L et al (2021) Breast tumour volume and blood flow measured by MRI after one cycle of epirubicin and cyclophosphamide-based neoadjuvant chemotherapy as predictors of pathological response. *Br J Radiol* 94:20201396. <https://doi.org/10.1259/bjr.20201396>
21. Beaney RP, Lammertsma AA, Jones T et al (1984) Positron emission tomography for in-vivo measurement of regional blood flow, oxygen utilisation, and blood volume in patients with breast carcinoma. *Lancet* 1:131–134. [https://doi.org/10.1016/s0140-6736\(84\)90063-1](https://doi.org/10.1016/s0140-6736(84)90063-1)
22. Lammertsma AA, Wilson CB, Jones T (1991) In vivo physiological studies in human tumors using positron emission tomography. *Funktionsanalyse Biolog Systeme* 20:319–325
23. Waterman FM, Nerlinger RE, Moylan DJ, Leeper DB (1987) Response of human tumor blood flow to local hyperthermia. *Int J Radiat Oncol Biol Phys* 13:75–82. [https://doi.org/10.1016/0360-3016\(87\)90263-x](https://doi.org/10.1016/0360-3016(87)90263-x)
24. Lagendijk JJW, Hofman P, Schipper J (1988) Perfusion analyses in advanced breast carcinoma during hyperthermia. *Int J Hyperth* 4:479–495. <https://doi.org/10.3109/02656738809027693>
25. Groebe K, Vaupel P (1988) Evaluation of oxygen diffusion distances in human breast cancer xenografts using tumor-specific in vivo data: role of various mechanisms in the development of tumor hypoxia. *Int J Radiat Oncol Biol Phys* 15:691–697. [https://doi.org/10.1016/0360-3016\(88\)90313-6](https://doi.org/10.1016/0360-3016(88)90313-6)
26. Vaupel P (2011) Pathophysiology of human tumors. In: Osinsky S, Friess H, Vaupel P (eds) *Tumor hypoxia in the clinical setting*. Akademperiodyka, Kyiv, pp 21–66
27. Vaupel P, Kallinowski F (1987) Physiological effects of hyperthermia. *Recent Results Cancer Res* 104:71–109. https://doi.org/10.1007/978-3-642-82955-0_3

28. Vaupel P, Höckel M (2000) Blood supply, oxygenation status and metabolic microenvironment of breast cancers: characterization and therapeutic relevance. *Int J Oncol* 17:869–879. <https://doi.org/10.3892/ijo.17.5.869>
29. Stylianopoulos T, Munn LL, Jain RK (2018) Reengineering the physical microenvironment of tumors to improve drug delivery and efficacy: from mathematical modeling to bench to bedside. *Trends Cancer* 4:292–319. <https://doi.org/10.1016/j.trecan.2018.02.005>
30. Leunig M, Goetz AE, Dellian M et al (1992) Interstitial fluid pressure in solid tumors following hyperthermia: possible correlation with therapeutic response. *Cancer Res* 52:487–490
31. Winslow TB, Eranki A, Ullas S et al (2015) A pilot study of the effects of mild systemic heating on human head and neck tumour xenografts: analysis of tumour perfusion, interstitial fluid pressure, hypoxia and efficacy of radiation therapy. *Int J Hyperth* 31:693–701. <https://doi.org/10.3109/02656736.2015.1037800>
32. Gerlowski LE, Jain RK (1986) Microvascular permeability of normal and neoplastic tissues. *Microvasc Res* 31:288–305. [https://doi.org/10.1016/0026-2862\(86\)90018-x](https://doi.org/10.1016/0026-2862(86)90018-x)
33. Chen Q, Krol A, Wright A et al (2008) Tumor microvascular permeability is a key determinant for antivasular effects of doxorubicin encapsulated in a temperature sensitive liposome. *Int J Hyperth* 24:475–482. <https://doi.org/10.1080/02656730701854767>
34. Xiaoxiao L, Wangyi W, Gongbi W, Jiaoguo C (1998) Mass transport in solid tumors (I)—Fluid dynamics. *Appl Math Mech* 19:1025–1032. <https://doi.org/10.1007/BF02459190>
35. Zhang XY, Luck J, Dewhirst MW, Yuan F (2000) Interstitial hydraulic conductivity in a fibrosarcoma. *Am J Physiol Heart Circ Physiol* 279:H2726–H2734. <https://doi.org/10.1152/ajpheart.2000.279.6.H2726>
36. Butler TP, Grantham FH, Gullino PM (1975) Bulk transfer of fluid in the interstitial compartment of mammary tumors. *Cancer Res* 35:3084–3088
37. Munson JM, Shieh AC (2014) Interstitial fluid flow in cancer: implications for disease progression and treatment. *Cancer Manag Res* 6:317–328. <https://doi.org/10.2147/CMAR.S65444>
38. Walker-Samuel S, Roberts TA, Ramasawmy R et al (2018) Investigating low-velocity fluid flow in tumors with convection-MRI. *Cancer Res* 78:1859–1872. <https://doi.org/10.1158/0008-5472.CAN-17-1546>
39. Yu Y, Sassaroli A, Chen DK et al (2010) Near-infrared, broad-band spectral imaging of the human breast for quantitative oximetry: applications to healthy and cancerous breasts. *J Innov Opt Health Sci* 03:267–277. <https://doi.org/10.1142/S179354581000112X>
40. Thomsen AR, Saalman MR, Nicolay NH et al (2022) Improved oxygenation of human skin, subcutis and superficial cancers upon mild hyperthermia delivered by WIRA-irradiation. *Adv Exp Med Biol* 1395:255–261. https://doi.org/10.1007/978-3-031-14190-4_42
41. Grote J, Süsskind R, Vaupel P (1977) Oxygen diffusivity in tumor tissue (DS-carcinoma) under temperature conditions within the range of 20–40 °C. *Pflügers Arch* 372:37–42. <https://doi.org/10.1007/BF00582204>
42. Vaupel P, Schlenger K, Knoop C, Höckel M (1991) Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O₂ tension measurements. *Cancer Res* 51:3316–3322
43. Vaupel P, Flood AB, Swartz HM (2021) Oxygenation status of malignant tumors vs. normal tissues: critical evaluation and updated data source based on direct measurements with pO₂ microensors. *Appl Magn Reson* 52:1451–1479. <https://doi.org/10.1007/s00723-021-01383-6>
44. Vaupel P, Briest S, Höckel M (2002) Hypoxia in breast cancer: pathogenesis, characterization and biological/therapeutic implications. *Wien Med Wochenschr* 152:334–342. <https://doi.org/10.1046/j.1563-258x.2002.02032.x>
45. Vaupel P, Frinak S, Mueller-Klieser W, Bicher HI (1982) Impact of localized hyperthermia on the cellular microenvironment in solid tumors. *Natl Cancer Inst Monogr* 61:207–209
46. Vujaskovic Z, Rosen EL, Blackwell KL et al (2003) Ultrasound guided pO₂ measurement of breast cancer reoxygenation after neoadjuvant chemotherapy and hyperthermia treatment. *Int J Hyperth* 19:498–506. <https://doi.org/10.1080/0265673031000121517>
47. Terasaki A, Kurokawa H, Ito H et al (2020) Elevated production of mitochondrial reactive oxygen species via hyperthermia enhanced cytotoxic effect of Doxorubicin in human breast cancer cell lines MDA-MB-453 and MCF-7. *Int J Mol Sci* 21:9522. <https://doi.org/10.3390/ijms21249522>
48. Fu Q, Huang T, Wang X et al (2017) Association of elevated reactive oxygen species and hyperthermia induced radiosensitivity in cancer stem-like cells. *Oncotarget* 8:101560–101571. <https://doi.org/10.18632/oncotarget.21678>

Changes in Blood Volume and Oxygenation in Lower Limb Tissue in Patients Maintaining the Lithotomy Position Under General Anaesthesia

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Abstract

To investigate the effect of the lithotomy position on lower limb circulation under general anaesthesia, near-infrared spectroscopy (NIRS) was used to measure changes in blood volume and oxygenation in thigh tissue in patients ($n = 35$) under general anaesthesia in the lithotomy position. The NIRS measurement items, including total haemoglobin concentration (total-Hb), tissue haemoglobin index (nTHI), and tissue oxygenation index (TOI) in the thigh, were recorded for 60 min, while the patients were in the lithotomy position. The correlation between changes in each measurement item and patient characteristics, elevation of the lower extremities, and cardiorespiratory indi-

ces were evaluated. Data obtained from 24 patients were analysed. The median values (quartile deviation) of changes in total-Hb, nTHI, and TOI during the 60-min period from baseline were $+ 3.09$ (1.99) $\mu\text{mol/L}$, $+0.08$ (0.03) a.u., and $+ 2.25$ (1.75) %, respectively, all of which were significantly increased ($p < 0.05$). Regression analysis showed that no factor was significantly associated with the increase in any measurement item. The present results suggest that circulation in thigh tissue tends to shift towards hyperaemia during 60 min of general anaesthesia in the lithotomy position, regardless of patient background factors or changes in cardiorespiratory conditions.

Keywords

General anaesthesia · Lithotomy position
Thigh circulation · Compartment syndrome
Near-infrared spectroscopy

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1 Introduction

Maintenance of the lithotomy position in patients undergoing surgery under general anaesthesia sometimes causes well-leg compartment syndrome (WLCS) in the lower

extremity. The main reason for WLCS—a condition where there is attenuated haemodynamics due to decrease in blood pressure in the leg, inappropriate leg flexion, and mechanical compression of the leg tissue with a leg supporting device—is likely circulatory failure in the lower extremity for an extended time period [1, 2]. It is well-known that blood flow and oxygenation in the lower legs are decreased immediately after adopting the lithotomy position, although the time-dependent circulatory changes in upper legs maintained in the lithotomy position is unclear. This study measured changes in blood volume and oxygenation in the thigh in patients maintaining the lithotomy position under general anaesthesia, and evaluated their correlation with patient background factors. We hypothesised that the blood volume and oxygenation in the thigh might decrease over time during the lithotomy position. Clarification of the pattern of circulatory changes in the lower extremity in patients in the lithotomy position under general anaesthesia might contribute to appropriate anaesthesia management for the prevention of WLCS.

2 Methods

This prospective observational study was approved by the Hospital Ethics Committee on Human Rights in Clinical Trials and Research of Nihon University Hospital (RK-180508-02), and was registered in the UMIN Clinical Trial Registry (ID: 000033734). Written informed consent was obtained from all patients participating in this prospective observational study. We studied 35 adult patients with ASA (American Society of Anesthesiologists) physical status I or II that were scheduled for elective surgery in the lithotomy position under general anaesthesia. We used NIRS to measure changes in blood volume and oxygenation in thigh tissue in patients maintaining the lithotomy position under general anaesthesia, and evaluated the correlation between the changes and patient background factors.

2.1 Anaesthetic Procedure and Monitoring

All patients received usual general anaesthesia with inhalation anaesthetics under continuous mechanical ventilation (fraction of inspiratory oxygen, $\text{FiO}_2 = 0.4$, tidal volume, $\text{TV} = 8 \text{ mL/kg}$, respiratory rate, and $\text{RR} = 10 \text{ breaths/min}$), with appropriate monitoring and maintenance of their haemodynamics throughout the study. If hypotension (mean blood pressure, $\text{MBP} < 80\%$ of baseline values or systolic blood pressure $< 90 \text{ mmHg}$) was observed, it was treated with injection of ephedrine and/or phenylephrine. An NIRS probe (interoptode distance, 40 mm ; optical path length coefficient, 4.65) (NIRO®-200NX, Hamamatsu Photonics, Hamamatsu City, Japan) was applied to the skin above the middle of the right or left rectus femoris muscle. NIRS measurements, including total haemoglobin concentration (total-Hb), tissue haemoglobin index (nTHI), which is an index of relative variation of tissue blood volume, and tissue oxygenation index (TOI), which is an index of tissue oxygen saturation, and cardiorespiratory parameters, including MBP, heart rate (HR), transcutaneous oxygen saturation (SpO_2), and end tidal carbon dioxide (EtCO_2), were continuously measured throughout the study. All of the values were recorded every 15 min until 60 min after adopting the lithotomy position.

2.2 Data Analysis

Sequential changes in NIRS and cardiorespiratory measurement items during 60 min in the lithotomy position were analysed by either one-way repeated measures analysis of variance or the Friedman test. Simple regression analysis was used to evaluate the correlation between changes in NIRS parameters and age, sex, body mass index, presence of concomitant diseases (hypertension and diabetes mellitus), elevation height of the lower extremities, and changes in cardiorespiratory indices (MBP, HR, SpO_2 , and EtCO_2) during 60 min in the lithotomy position. A P value of < 0.05 was considered statistically significant.

3 Results

We were able to obtain data from 24 patients, after excluding 11 patients, because their surgical procedure time was less than 60 min or their measurement values of poor quality or missing due to instability of the adhesive of the NIRS optical sensor.

The patients' characteristics are shown in Table 1. The changes in total-Hb, nTHI, and TOI in the thigh are shown in Fig. 1. The median values (quartile deviation; QD) of changes in total-Hb, nTHI, and TOI from baseline during the 60-min observation period were + 3.09 (1.99) $\mu\text{mol/L}$, +0.08 (0.03) a.u., and + 2.25 (1.75)%, respectively, all of which were significantly increased (total-Hb: $P < 0.05$, nTHI: $P < 0.05$, TOI: $P < 0.05$). The median values (standard deviation; SD) of changes in cardiorespiratory parameters are shown in Table 2. None of the parameters changed significantly throughout the study. The correlations between changes in NIRS parameters and patient background factors (patient characteristics and changes in cardiorespiratory parameters) are shown in Table 3. Regression analysis showed no significant association between any of the factors and the increases in any of the measurement items.

Table 1 Patient characteristics

Age, years	79.5 (73.5–83)
Male/female (n)	18/6
Body mass index, kg/m^2	23.4 (20.8–25.2)
Operative procedures (n)	
Transurethral bladder tumour resection	16
Transurethral lithotripsy	7
Cervical cerclage	1
Medical history (n)	
Hypertension	14
Diabetes mellitus	6
Lithotomy position duration, min	111 (68.5–140.5)
Height of lower limb elevation, cm	31.5 (29.5–33)

Data are presented mean (SD)

4 Discussion

The present results showed that both the levels of total-Hb and nTHI, as indices of tissue blood volume, and TOI, as an indicator of tissue oxygen saturation, significantly increase in the thigh over time during 60 min in the lithotomy position. In evaluation by NIRS which we used this time, all the total-Hb, nTHI, and TOI increase and decrease depending on the increase and decrease in blood haematocrit. In this study, the total-Hb and nTHI in the thigh significantly increased during the lithotomy position, although the blood haematocrit in patients might decrease slightly with bleeding and transfusion during the maintenance of anaesthesia. Moreover, the cardiopulmonary parameters did not significantly change during the anaesthesia maintenance. Considering the above, contrary to expectations, the present results suggest that the femoral tissue tends to become hyperaemic over time in the lithotomy position under general anaesthesia.

Reportedly, postural change from the supine to the lithotomy position produces significant decreases in blood flow in the lower leg secondary to decrease in both blood pressure and perfusion pressure [3–5]. The present study showed that total-Hb, nTHI, and TOI also decrease immediately after conversion from the supine to the lithotomy position. Subsequently, however, these parameters increase with maintenance of the lithotomy position. Therefore, the hyperaemic trend during maintenance of the lithotomy position in our study was interpreted as a trend towards recovery of femoral tissue circulation over time. The cause of WLCS in the lithotomy position is attributable to local blood flow disturbance caused by the combined effects of compartment pressure in the elevated leg and decrease in blood pressure in the leg [5, 6]. Since WLCS is more likely to occur when the lithotomy position is sustained for more than 4 h [7], longer measurements beyond 60 min might possibly indicate an ischaemic tendency. In the future, additional research should be conducted in this regard.

Fig. 1 Changes in NIRS parameters in thigh tissue while in the lithotomy position under general anaesthesia. Data are expressed as median values (quartile deviation). Total-Hb total haemoglobin concentration, nTHI tissue haemoglobin index, TOI tissue oxygenation index. Time points: every 15 min after adopting the lithotomy position

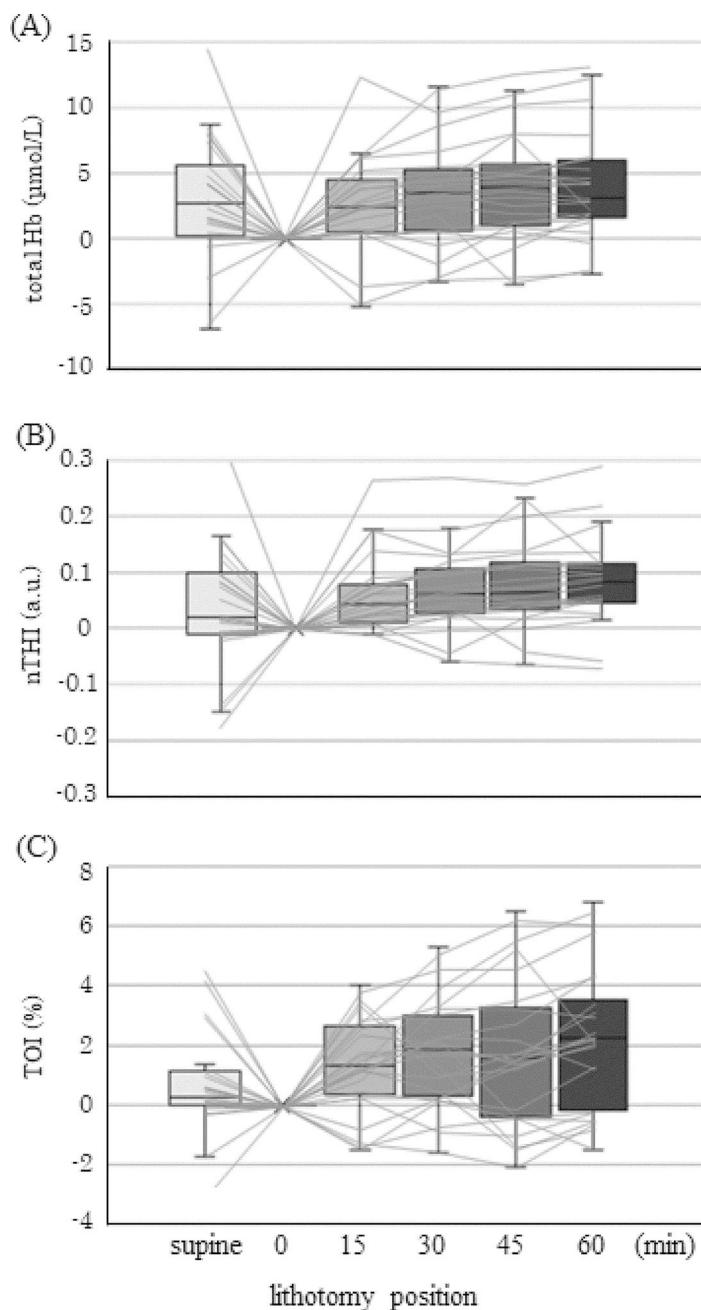


Table 2 Changes in cardiorespiratory parameters during lithotomy position

	Supine	0 min	15 min	30 min	45 min	60 min
MAP (mmHg)	99 (13.0)	73.5 (16.7)	70 (8.2)	72 (7.2)	68.5 (8.3)	70.5 (13.3)
HR (beats/min)	72 (12.5)	66 (10.6)	64 (6.2)	60 (8.8)	58 (8.9)	63 (11.7)
SpO ₂ (%)	98 (1.2)	99 (1.4)	98.5 (1.1)	98.5 (1.3)	98.5 (1.2)	98.5 (1.3)
EtCO ₂ (mmHg)	38 (4.0)	38 (3.2)	39 (2.8)	39 (2.0)	39 (2.3)	39 (2.2)

Data are presented as mean (SD). Supine: immediately before adopting the lithotomy position, 0 min: immediately after adopting the lithotomy position, 15 min to 60 min: every 15 min after adopting the lithotomy position

Table 3 Correlations between changes in NIRS parameters and patient background factors evaluated by regression analysis

Factors	Total-Hb		nTHI		TOI	
	R ²	P	R ²	P	R ²	P
Age	0.02	0.55	0.004	0.75	0.06	0.27
Gender	0.01	0.57	0.03	0.41	0.02	0.53
BMI	0.007	0.7	0.02	0.44	0.09	0.16
Hypertension	0.003	0.78	0.004	0.76	0.000	0.97
Diabetes mellitus	0.12	0.1	0.12	0.09	0.004	0.78
Height of lower limb elevation	0.004	0.75	0.01	0.58	0.007	0.69
ΔMBP	0.12	0.11	0.18	0.04	0.01	0.56
ΔHR	0.000	0.99	0.01	0.55	0.002	0.83
ΔSpO ₂	0.002	0.81	0.06	0.24	0.01	0.65
ΔEtCO ₂	0.08	0.17	0.007	0.69	0.12	0.1
Concentration of inhalation anaesthetics, MAC	0.08	0.17	0.004	0.74	0.04	0.32
Remifentanyl maintenance dose	0.08	0.41	0.04	0.34	0.01	0.62

Data are results of simple regression analysis. *Total-Hb* total haemoglobin concentration; *nTHI* tissue haemoglobin index; *TOI* tissue oxygenation index; *BMI* body mass index; Δ variation during maintenance of the lithotomy position; *MBP* mean blood pressure; *HR* heart rate; *SpO₂* transcutaneous oxygen saturation; *EtCO₂* end tidal carbon dioxide; *MAC* minimum alveolar concentration

Previous literature showed that patient factors that increase the risk of WLCS include peripheral vascular disease, obesity, smoking, and diabetes mellitus, as well as the height of the elevated lower extremity and operative time [8], indicating that many patient factors that increase the risk of WLCS are related to microvascular disease risk. In this study, we did not find any factors closely related to changes in NIRS parameters. One reason for this could be that the trial subjects in this study had their circulation stabilised by anaesthesiologists (e.g., infusion of fluids, depth of anaesthesia, and administration of cardiovascular agents).

One of the most effective means for preventing the development of WLCS is invasive monitoring, such as measurement of intra-compartmental pressure in the lower extremity tissue, with appropriate steps being taken to correct any abnormalities [9, 10]. In contrast, regional tissue oxygen saturation (rSO₂ and TOI) measured by NIRS has also shown non-inferiority compared to direct measurement of compartment perfusion pressure [11]. Therefore, NIRS monitoring in lower extremity tissue, which we used in this study, might be suitable for intra-operative monitoring in clinical settings, because it can measure circulatory changes in the lower extremity easily and non-invasively.

5 Conclusion

The results of this study suggest that circulation in the thigh tends to shift towards hyperaemia within 60 min of adopting the lithotomy position under general anaesthesia, regardless of patient background factors or changes in respiratory and circulatory conditions.

References

1. Khalil IM (1987) Bilateral compartmental syndrome after prolonged surgery in the lithotomy position. *J Vasc Surg* 5:879–881
2. Simms M, Terry T (2005) Well leg compartment syndrome after pelvic and perineal surgery in the lithotomy position. *Postgrad Med J* 81:534–536
3. Turnbull D, Farid A, Hutchinson S et al (2002) Calf compartment pressures in the Lloyd-Davies position: a cause for concern? *Anaesthesia* 57:905–908
4. Chase J, Harford F, Pinzur MS et al (2000) Intraoperative lower extremity compartment pressures in lithotomy-positioned patients. *Dis Colon Rectum* 43:678–680
5. Halliwill JR, Hewitt SA, Joyner MJ et al (1998) Effect of various lithotomy positions on lower-extremity blood pressure. *Anesthesiology* 89:1373–1376
6. Heppenstall B, Tan V (1999) Well leg compartment syndrome. *Lancet* 354:970

7. Martin JT (1992) Compartment syndromes: concepts and perspectives for the anesthesiologist. *Anesth Analg* 75:275–283
8. Hara K, Kuroki T, Kaneko S et al (2021) Prevention of well-leg compartment syndrome following lengthy medical operations in the lithotomy position. *Surg Open Sci* 3:16–21
9. Köstler W, Strohm PC, Südkamp NP (2005) Acute compartment syndrome of the limb. *Injury* 36:992–998
10. Forget P, Ponchon F, Vanhoonacker M et al (2012) In vivo optical spectroscopy monitoring in a new model of muscular compartment syndrome. *Br J Anaesth* 109:561–565
11. Gentilello LM, Sanzone A, Wang L et al (2001) Near-infrared spectroscopy versus compartment pressure for the diagnosis of lower extremity compartmental syndrome using electromyography-determined measurements of neuromuscular function. *J Trauma* 51:1–8

Recognising Potential Ambiguities in Measurements of Oxygen in Tissues

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and Ann Barry Flood

Abstract

Measuring oxygen (O_2) in tissues has been a central theme of the International Society on Oxygen Transport to Tissue (ISOTT) since its founding 50 years ago in 1973. The initial presentations by many distinguished members reflect this focus and demonstrate the importance of the contributions of the members of ISOTT. This paper considers their work and its legacy in the context of the continuing challenges of making meaningful measurements of O_2 in tissue. Because many technical, physiological, and pathophysiological factors are directly or implicitly involved in obtaining any measured value of O_2 in living tissues, interpretations of what the measured value represents and its biological implications need to take these factors into account. The challenges arise from two very simple but painfully true factors that make it challenging to obtain measurements of O_2 in tissues in vivo

that are useful for the understanding of physiological and pathophysiological processes. First, throughout the volume of functioning tissue that is assessed by any technique, there is a complex spatial heterogeneity of O_2 levels. No technique can usually fully represent this complexity in a given measurement, because the heterogeneity extends from the environment in the tissue surrounding cells to variations within the cell. Therefore, the value of the output from a measurement inevitably consists of a complex, averaged summary of O_2 in the tissue. Second, the levels of O_2 are constantly changing in living tissues (variations occur in seconds, minutes, hours, and/or days and differ by location) at rates that are difficult to resolve for available techniques, because they occur faster than data acquisition time and/or cannot be used as frequently as needed to follow the longer-term changes. However, as demonstrated in research reported in the publications from ISOTT, studies of O_2 in tissue, in spite of the potential ambiguities in the measured values, can provide very valuable insights into physiology and pathophysiology. This is most likely to occur if researchers explicitly recognise why and how their measurement does not fully portray the complexity of O_2 . When measurements can be repeated, the resulting change between measurements provides information about the dynamics of the physiology and pathophysiology.

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Assessing change in O_2 levels can also provide evidence about responses to treatments. Similarly, finding evidence of hypoxia, even though it does not capture the heterogeneity and dynamics actually happening in the tissue, can still inform clinical care if the measurement is well-understood.

Keywords

Oxygen in tissues · Direct methods
Heterogeneity of oxygen · Indirect methods
ISOTT · Ambiguities

1 Introduction

In this manuscript, we consider the very important role that members of the International Society on Oxygen Transport to Tissue (ISOTT) have had in making measurements of oxygen (O_2) in tissues (O_2) in tissues and, more importantly, in interpreting their meaning. Within this literature, absolute levels of O_2 have typically been expressed either as the partial pressure of O_2 (pO_2) and/or as the concentration of O_2 ($[O_2]$). These terms, though interrelated (i.e., $pO_2=[O_2]$, adjusted for the partition coefficient for O_2), can have very different biological implications for the physiology or pathophysiology being investigated. Therefore, they should be used precisely, i.e., their different implications for processes in tissue should be explicitly taken into account. However, when the general concept of levels of O_2 within tissues is the topic (as in this manuscript), these terms can sometimes become too precise. Therefore, to avoid the need for unnecessarily complex explanations in support of our major theses and to avoid the ambiguities that can accompany the use of pO_2 or $[O_2]$ *without being precise*, the general terms “levels of O_2 ” or simply “ O_2 ” are used here.

Many very important pathophysiological and physiological processes are strongly influenced by the level of O_2 within cells. These include processes associated with cancer, peripheral vascular disease, stroke, heart ischaemia, and wound healing as well as exercise and the effects of liv-

ing at high altitudes. Therefore, to understand how O_2 impacts these processes, it is useful to accurately measure O_2 in cells in situ, preferably directly within the relevant cells or, at least, more broadly in the tissues.

2 ISOTT's Roles in Developing and Applying Techniques to Measure O_2

The importance of measuring O_2 in tissues was clearly recognised from the beginning of ISOTT. The initial publications in 1973 and the following few years included a remarkable set of well-grounded observations and pioneering development of methodologies to measure O_2 in tissue and its applications. In particular, these pioneering researchers led the development and application of many approaches that remain important to current research. Table 1 summarises these contributions and principal references from early ISOTT proceedings.

Since then, members of ISOTT have further improved these techniques and added new ones. Essentially, every valid technique for measuring O_2 introduced over the past 50 years has been presented at meetings of ISOTT. In many cases, the first introduction of very significant advances in both technologies using measurements of O_2 in tissues and their applications for basic and clinical research occurred at ISOTT meetings. Prominent among these techniques are BOLD MRI, MRI perfusion and diffusion, TOLD MRI, fluorine MRI, NMR spectroscopy, fMRI, fNIRS, EPR spectroscopy, EPR imaging, NIRS, and in vivo magnetic resonance spectroscopy of NAD redox and bioenergetics.

However, in spite of the technical accomplishments and their many effective uses to solve important basic and clinical problems, critical examination of what has actually been measured when these studies describe levels of O_2 in cells or tissues is still indispensable to scientific progress. As implied by our title, we maintain that many *in vivo measurements of O_2 in tissue do not capture well all of the variations present in tissues*.

Table 1 ISOTT’s early major developments in measuring O₂

Development from pioneers at ISOTT	References from ISOTT proceedings
Develop oxygen electrode	[1, 2]
Use oxygen electrode to measure in tissues	[3–9]
Use oxygen electrode clinically	[10–12]
Use redox sensitive chromophores to characterise patho–/physiological states	[13–16]
Use O ₂ dependent staining of cells	[17, 18]
Measure O ₂ levels in blood with gas analyses or spectrophotometric methods	[19–23]
Develop O ₂ -sensitive optical reagents and methodology to use them	[24]
Measure and characterise hypoxia in tumours	[6–8]
Develop computational modelling of O ₂ in tissues	[25–32]
Develop physical constants and modelling of O ₂ transport to tissue	[33, 34]
Evaluate impact on oxygenation from transfusions and blood substitutes/ Develop substitutes for whole blood transfusions	[35, 36]
Measure muscle oxygen distributions making use of myoglobin saturation	[37, 38]

3 Factors Often Overlooked in Making Measurements of O₂ in Tissues

There are at least three major factors often overlooked. First, measurements of O₂ in tissues are usually confounded by spatial heterogeneity of O₂. Second, they are usually confounded by temporal changes of O₂. Third, many of the most extensively used techniques to measure O₂ in tissues only indirectly assess O₂, that is, they use parameters that do not have a determinant link to the O₂ in tissues. (In contrast, direct measures use parameters in tissues which are directly proportional to the O₂.)

3.1 Measurements of O₂ in Tissues Are Usually Confounded by Spatial Heterogeneity

Consider first why there are gradients in the levels of cellular and tissue O₂. The main force for gradients is the consumption of O₂ within the cells. The continued occurrence of gradients results in the transport of O₂ from the microenvironment into the cells. Furthermore, most of the consumption of O₂ in cells occurs in the mitochondria, which, therefore, leads to O₂ gradients within the cells themselves [39–41]. This process

inevitably results in variation of O₂ levels over space (i.e., throughout the volume of a tissue as well as within each individual cell).

Variation in levels of O₂ is especially significant in the microvasculature, where there are very steep gradients of O₂. While these gradients differ across organs, types of tissues (e.g., amount of lipids and water content), and in the presence of pathology [39, 40], the general trend is for the pO₂ to be 80–100 mmHg in the systemic arterioles, decreasing to ~40 mmHg in the proximal capillaries, further decreasing within the microvasculature and the interstitial space and then again within the cell [42]. This leads to variations among cells depending on the number of other functioning cells between them and the afferent circulation, as the cells closer to the O₂ supply deplete the amount of O₂ available to more distant cells. Even among cells equidistant to the afferent vessels, O₂ levels vary due to several factors including variations in O₂ consumption per cell and the volume of the extracellular space.

As shown extensively in human tumours, using the Eppendorf electrode system, there are significant microscopic variations of O₂ pressure ranging from 0 to >20 torr [39, 40]. This may further increase the variations among cells that depend on the number of other functioning cells between them and the afferent circulation. Pathophysiology can affect the osmotic pressure of the extracellular fluid which can impact the functioning of the

microvasculature. Finally, within each cell, pathophysiology can impact the variation in O_2 levels due to the altered oxidative and non-oxidative metabolism and/or variations in intracellular barriers to the free diffusion of O_2 [39, 40].

The spatial variation of O_2 in tissues impacts the meaning of physical measurements of O_2 in tissues. Even assuming the parameter is directly related to O_2 within a given volume (usually a subset of the whole tissue), the content of O_2 in that measured volume is heterogenous as noted above. For a given type of measurement, either some of the spatial variations remain unresolved and/or the measured O_2 level is actually based on a complex ‘averaging’ of O_2 within the volume that is being probed for the measurement. As an example of how even greater complexity arises, the O_2 -sensing parameters are often biased towards non-representative regions within the volume, e.g., O_2 in lipid areas may be disproportionately sampled by some parameters or avoided by others. Of further note, due to specific location of the probe in some techniques, the measured volume may not be fully representative of O_2 levels in the overall tissue.

3.2 Measurements of O_2 in Tissues Are Usually Confounded by Temporal Changes

Well-established data verify that there are also very significant temporal variations in the levels of O_2 in tissue oxygenation levels [43–45]. Detailed measurements and observations of the microvasculature show both that the microcirculation is continually changing, for example, shunting, and that the flow within capillaries is typically irregular. There are also longer-term variations of minutes to weeks in blood flow and perfusion due to normal changes in physiology such as during eating or after sustained exercise training [46]. Consequently, these factors lead to variations in O_2 levels over time, ranging from changes occurring within milliseconds to over several minutes to days [47].

These heterogeneities occur in normal tissues, and they are frequently exacerbated in the pres-

ence of pathophysiology [39, 40]. For example, tumours, due to regional variations in blood flow and perfusion associated with the disorderly growth of their vasculature, usually have very pronounced and extensive macroscopic temporal variations that can occur across tissue volumes from mm^3 to cm^3 and times that range from minutes to days.

Treatment also could change the heterogeneity of O_2 levels. For example, the level of O_2 affects how the cells respond to ionising radiation [48]. In particular, tumour cells that have higher levels of O_2 are preferentially damaged by radiation. Consequently, over a period of several radiation treatments, tumour cells that had been relatively well-oxygenated during each treatment would die off, leaving a different distribution of the remaining cells in regard to their distance to O_2 supply. Therefore, treatments, both during radiation and over time, would change the pattern of heterogeneity of O_2 in the tumour. Additionally, treatments could impact the functionality of the microvascular system, but these effects occur months to years after the treatment [49].

Changes in temperature also can increase the heterogeneity of O_2 in tissues via several mechanisms [40]. Overall, the effect of temperature on O_2 levels in tissue depends on a combination of factors, including the O_2 affinity of haemoglobin, metabolic rate, blood flow regulation, and O_2 solubility. In most cases, higher temperatures tend to enhance O_2 delivery to tissues, while lower temperatures may decrease it. However, the specific effects can vary depending on the tissue type, overall metabolic demand, and other physiological factors. There can also be changes in the sensitivity of physical methods to measure O_2 . For example, relaxation times in magnetic resonance can be misinterpreted if temperature changes are not taken into account.

3.3 Many Techniques Only Indirectly Assess O_2 in Tissues

In addition to sharing the challenges of accurately reflecting the spatial and temporal heterogeneity of O_2 in tissues, many commonly used

methods are indirect in that they do not actually probe O_2 in tissue. Instead, they use parameters that can potentially influence and/or affect O_2 in tissues but do not have a determinant relationship to O_2 in tissues.

Indirect techniques include those that measure parameters that assess O_2 in the vascular system only (e.g., NIRS and BOLD MRI), blood flow (e.g., Doppler ultrasound and MRI angiography), perfusion, and/or diffusion with MRI or assess substances whose retention in tissues is qualitatively related to hypoxia (e.g., MISO-PET), whose metabolism is impacted by hypoxia (e.g., PET of glucose analogues and MRI spectroscopy) or which assess metabolic intermediates that are indicators of redox state (e.g., NAD/NADH and MRI spectroscopy) or which measure genes whose activation is impacted by the level of O_2 (e.g., hypoxia-inducible factors such as HIF-1).

These indirect methods do not provide unambiguous measurements of O_2 in tissues for two reasons:

1. Many factors not measured by indirect methods also impact O_2 levels in tissues.

The challenge when using indirect methods is that many factors influence the relationship between the parameters and the actual O_2 in tissues. For example, consider the relationship between O_2 levels in tissues and some of the most widely used indirect methods such as NIRS and BOLD MRI which are based on measurements of O_2 in the vascular system. While blood-based indirect methods can provide unambiguous data on O_2 levels within the vascular system (e.g., by measuring blood gases or by assessing both oxyhaemoglobin and deoxyhaemoglobin), the relationship between levels of O_2 in the vascular system and those in surrounding tissues is complex and impacted by many factors as noted above, including the balance between consumption and supply of O_2 .

2. Parameters that are the basis of the indirect measures can be influenced by factors other than O_2 .

Indirect parameters can reflect other unrelated processes. For example, the amount of HIF-1 and related genes can change because of mechanisms independent of O_2 levels in tissues [50]. Similar non- O_2 -dependent factors can impact the parameters used for measuring O_2 for most other indirect techniques.

Parallel concerns also occur with direct methods for measuring O_2 in tissues. For example, parameters used by many magnetic resonance techniques (both NMR and EPR) are based on the effects of O_2 on relaxation rates. Other factors also can impact the relaxation rate, for example, the presence of other paramagnetic ions and concentration-dependent interactions of the molecular probe used.

4 Obtaining Useful Measurements of O_2 in Tissues

4.1 The Problem and Possible Solutions

The “problem” with the use of measurements of O_2 in tissues arises when researchers assume that the output of their measurements represents the tissue’s absolute O_2 level and then make incorrect inferences about O_2 in tissue. The “solution” does not require abandoning current techniques (although improving techniques to better capture some or all of the complexities would also be an appropriate goal). To obtain useful measurements (i.e., data that bear on the goals of the study), it is necessary to decide what data are needed to achieve the purpose of the study. This goal should be the overarching rationale for determining the appropriate technique to use. To optimise this decision, it is necessary to understand the volume that any potential technique measures, the time frame used to obtain a given data point and/or its summary measurement, and how the measured parameter relates to O_2 in tissues. For clinical applications, it also is important to consider the clinical applicability including whether measurements could be made under anticipated clinical conditions.

4.2 Specific Circumstances Where Currently Available O₂ Measurements Have Been Very Useful

One can readily find very productive uses of measurements of O₂ in tissues in spite of their inability to fully resolve spatial and temporal heterogeneity. Some examples from research by Swartz, Flood, and colleagues include using measurements of O₂ to optimise the administration of a hypoxia-modifying drug, determining whether a tumour responds to interventions to increase O₂ levels [51–57], and optimising evaluation and therapy for peripheral vascular disease based on changes of O₂ in subcutaneous tissue [56–62].

Using the pO₂ histography system, the Vaupel/Höckel Lab [63, 64] was the first to describe that hypoxia is a hallmark in primary and recurrent cancers, triggers malignant progression based on hypoxia-induced changes in the genome and proteome, leads to poor prognosis of patients with primary cancers due to the development of lethal cancer phenotypes, is associated with resistances to existing therapeutic modalities, and leads to poor patient prognosis (see [40] for data summary).

These successful applications have several important characteristics in common. They do *not* require knowledge of absolute levels of O₂. They *do* require rigorous delineation of the analysed area. They especially look at *relative changes* over time and responses (i.e., changes) due to interventions. They also usually assume that changes in some regions are pertinent to changes in other regions; this assumption may sometimes need more supportive evidence.

5 Conclusions

It is important to recognise the underlying heterogeneity of O₂ in tissues, over dimensions of both time and space, that make measurements fully resolving O₂ in tissues very challenging.

It is unlikely that technical advances in measurement techniques will fully overcome

these challenges. Nonetheless, it is important to recognise these limitations when describing what is/is not being portrayed and whether they limit the interpretations and implications of the measurements. Equally important, if these limitations are appropriately recognised and taken into account, important information can still be derived from measurements of O₂ in tissue, especially when interpreting changes between repeated measurements. When existing techniques are used to follow changes, they can be used to assess response to therapy, including interventions designed to alter O₂ in tissues, or monitor the impact of pathophysiology over time and the impact of interventions designed to alter O₂ in tissues. Key to achieving practical uses of measurements of O₂ is to determine what data could provide the best test of the hypothesis of interest and then decide which technique(s) can best provide that data.

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Conflict of Interest The authors have no conflicts of interest for this manuscript.

References

1. Silver IA (1973) The oxygen micro-electrode. *Adv Exp Med Biol* 37A:7–16
2. Whalen WJ (1973) Intracellular oxygen microelectrodes. *Adv Exp Med Biol* 37A:17–22
3. Vaupel P, Braunbeck W, Thews G (1973) Respiratory gas exchange and pO₂-distribution in splenic tissue. *Adv Exp Med Biol* 37A:401–406
4. Kunze K, Lübbers DW (1973) Absolute pO₂-measurements with Pt-electrodes applying polarizing voltage pulsing. *Adv Exp Med Biol* 37A:35–43
5. Kunze K (1973) Local pO₂ and O₂-consumption in the isolated sciatic nerve. *Adv Exp Med Biol* 37A:271–276
6. Thews G (1976) Pathophysiological aspects of glucose uptake by the tumor tissue under various conditions of oxygen and glucose supply. *Adv Exp Med Biol* 75:547–553
7. Thews G, Vaupel P (1976) O₂ supply conditions in tumor tissue in vivo. *Adv Exp Med Biol* 75:537–546
8. Vaupel P (1977) Hypoxia in neoplastic tissue (presented at ISOTT, April 11–16, 1976). *Microvasc Res* 13:399–408

9. Vaupel P, Grunewald WA, Manz R, Sowa W (1978) Intracapillary HbO₂ saturation in tumor tissue of DS-carcinoma during normoxia. *Adv Exp Med Biol* 94:367–375
10. Bicher HI, Rubin JW, Adams RJ (1973) Clinical use of a new intra-arterial catheter electrode system. *Adv Exp Med Biol* 37A:107–113
11. Erdmann W, Kunke S, Heidenreich J, Dempsey W, Gunther H, Schafer H (1973) Clinical experiences with clamp electrodes in fetal scalp for simultaneous pO₂ and ECG-registration. *Adv Exp Med Biol* 37B:1129–1134
12. Flower RW (1975) A system for in vivo measurement of oxygen in intraocular tissue. *Adv Exp Med Biol* 75:417–424
13. Chance B, Oshino N, Sugano T, Mayevsky A (1973) Basic principles of tissue oxygen determination from mitochondrial signals. *Adv Exp Med Biol* 37A:277–292
14. Lübbers DW (1973) Spectrophotometric examination of tissue oxygenation. *Adv Exp Med Biol* 37A:35–44
15. Mandel LJ, Riddle TG, LaManna LC (1977) A rapid scanning spectrophotometer and fluorometer for in vivo monitoring of steady-state and kinetic optical properties of respiratory enzymes (presented at ISOTT). In: Jöbsis FF (ed) *Oxygen and physiological function*. Prof Inform Lib, Dallas, pp 79–89
16. Lübbers DW (1973) Spectrophotometric examination of tissue oxygenation. *Adv Exp Med Biol* 37A:45–54
17. Longmuir IS, Knopp JA (1973) A new histochemical stain for intracellular oxygen. *Adv Exp Med Biol* 37A:55–57
18. Weigelt H, Seidl E, Acker H, Lübbers DW (1976) The application of histological analysis for the localization of tissue pO₂ and local blood flow with the example of the glomus caroticum of the rabbit (*Oryctolagus cuniculus*). *Adv Exp Med Biol* 75:17–24
19. Wilson GJ, MacGregor DC, Holness DE, Lixfeld W, Yasui H (1973) Mass spectrometry for measuring changes in intramyocardial pO₂ and pCO₂. *Adv Exp Med Biol* 37A:547–552
20. Lübbers DW (1977) Measuring methods for the analysis of tissue oxygen supply (presented at ISOTT). In: Jöbsis FF (ed) *Oxygen and physiological function*. Prof Inform Lib, Dallas, pp 62–71
21. Lübbers DW, Opitz N (1976) Quantitative fluorescence photometry with biological fluids and gases. *Adv Exp Med Biol* 75:65–68
22. Kimmich HP, Kreuzer F, Spaan JG, Jank K, de Hemptinne J, Demeester M (1976) Monitoring of pO₂ in human blood. *Adv Exp Med Biol* 75:33–40
23. Grunewald WA, Lübbers DW (1975) Kryomicrophotometry as a method for analyzing the intracapillary HbO₂ saturation of organs under different O₂ supply conditions. *Adv Exp Med Biol* 75:55–64
24. Jöbsis FF, Mitnick MH, Snow TR (1975) Pyrene butyric acid: a non-invasive probe for in situ intracellular oxygen concentration. *Adv Exp Med Biol* 75:47–54
25. Honig CR, Bourdeau-Martini J (1973) O₂ and the number and arrangement of coronary capillaries: effect on calculated tissue pO₂. *Adv Exp Med Biol* 37A:519–524
26. Oomens JMM, Spaan JAE (1975) A generalized advancing front model describing the oxygen transfer in flowing blood. *Adv Exp Med Biol* 75:233–240
27. Meldon JH, Garby L (1975) A theoretical model of the respiratory function of blood. *Adv Exp Med Biol* 75:241–250
28. Fletcher JE (1975) Some model results on hemoglobin kinetics and its relationship to oxygen transport in blood. *Adv Exp Med Biol* 75:251–260
29. Bruley DF, Groome LJ, Eicher H, Knisely MH (1975) A stochastic model for the transport of oxygen to brain tissue. *Adv Exp Med Biol* 75:267–278
30. Dorson WJ Jr, Voorhees ME (1975) Models for O₂ and CO₂ transfer in macroscopic flow channels and their physiological implications. *Adv Exp Med Biol* 75:279–284
31. Wilson DF, Owen CS, Erecinska M (1978) Regulation of mitochondrial respiration in intact tissues: a mathematical model. *Adv Exp Med Biol* 94:279–287
32. Mochizuki M, Kagawa T (1978) The effect of deoxygenation rate of the erythrocyte on oxygen transport to the cardiac muscle. *Adv Exp Med Biol* 94:169–174
33. Grote J, Suesskind R, Vaupel P (1978) Oxygen diffusion constants D and K of tumor tissue (DS-carcinoma) and their temperature dependence. *Adv Exp Med Biol* 94:361–365
34. Roetman EL, Barr RE (1975) The mechanical basis for Fick's law and its generalizations. *Adv Exp Med Biol* 75:261–266
35. Longmuir IS (1977) Search for alternative cellular oxygen carriers (presented at ISOTT). Pg 247–53. In: Jöbsis FF (ed) *Oxygen and physiological function*. Prof Inform Lib, Dallas
36. Jesch F, Endrich B, Messmer K (1976) Oxygen transport and hemodynamics of stroma-free hemoglobin solutions. *Adv Exp Med Biol* 75:105–112
37. Gageski TEJ, Honig CR (1983) Direct measurement of intracellular O₂ gradients; Role of convection and myoglobin. *Adv Exp Med Biol* 159:613–621. https://doi.org/10.1007/978-1-4684-7790-0_54
38. Gageski TE, Federspiel WJ, Honig CR (1988) A graphical analysis of the influence of red cell transit time, carrier-free layer thickness, and intracellular pO₂ on blood-tissue O₂ transport. *Adv Exp Med Biol* 222:25–35. https://doi.org/10.1007/978-1-4615-9510-6_3
39. Harrison D, Vaupel P (2014) Heterogeneity in tissue oxygenation: from physiological variability in normal tissues to pathophysiological chaos in malignant tumors. *Adv Exp Med Biol* 812:25–31
40. Vaupel P, Flood AB, Swartz HM (2021) Oxygenation status of malignant tumors vs. normal tissues: critical evaluation and updated data source based on direct measurements with pO₂ microsensors. *Appl Magn Reson* 51:1481–1487

41. Khan N, Shen J, Chang TY, Chang CC, Fung PC, Grinberg O, Demidenko E, Swartz H (2003) Plasma membrane cholesterol: a possible barrier to intracellular oxygen in normal and mutant CHO cells defective in cholesterol metabolism. *Biochemistry* 42:23–29. <https://doi.org/10.1021/bi026039t>
42. Levick JR, Michel CC (2010) Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 87:198–210
43. Braun RD, Lanzen JL, Dewhirst MW (1999) Fourier analysis of fluctuations of oxygen tension and blood flow in R3230Ac tumors and muscle in rats. *Am J Physiol* 277(2 Pt 2):H551–H568
44. Griffith TM (1996) Temporal chaos in the microcirculation. *Cardiovasc Res* 3:342–358
45. Hu D, Cai D, Rangan AV (2012) Blood vessel adaptation with fluctuations in capillary flow distribution. *PLoS One* 7(9):e45444. <https://doi.org/10.1371/journal.pone.0045444>
46. Bhujwalla ZM, Artemov D, Ballesteros P, Cerdan S, Gillies RJ, Solaiyappan M (2002) Combined vascular and extracellular pH imaging of solid tumors. *NMR Biomed* 15:114–119
47. Kimura H, Braun RD, Ong ET et al (1996) Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. *Cancer Res* 56:5522–5528
48. Hall EJ, Giaccia AJ (2018) *Radiobiology for the radiologist*, 8th edn. Wolters Kluwer Health Inc, Alphen aan den Rijn
49. Venkatesulu BP, Mahadevan LS, Aliru ML et al (2018) Radiation-induced endothelial vascular injury: a review of possible mechanisms. *JACC Basic Transl Sci* 3(4):563–572
50. Masoud GN, Li W (2015) HIF-1 α pathway: role, regulation and intervention for cancer therapy. *Acta Pharm Sin B* 5(5):378–389. PMID: PMC4629436 PMID: 26579469
51. Schaner PE, Pettus JR, Flood AB, Williams BB, Jarvis LA, Chen EY, Pastel DA, Zurbier RA, diFlorio-Alexander RM, Swartz HM, Kuppusamy P (2020) OxyChip implantation and subsequent electron paramagnetic resonance oximetry in human tumors is safe and feasible: first experience in 24 patients. *Front Oncol* 10:572060
52. Schaner PE, Williams BB, Chen EY, Pettus JR, Schreiber WA, Kmiec MM, Jarvis LA, Pastel DA, Zurbier RA, DiFlorio-Alexander RM, Paydarfar JA, Gosselin BJ, Barth RJ, Rosenkranz KM, Petryakov SV, Hou H, Tse D, Pletnev A, Flood AB, Wood VA, Hebert KA, Mosher RE, Demidenko E, Swartz HM, Kuppusamy P (2021) First-in-human study in cancer patients establishing the feasibility of oxygen measurements in tumors using electron paramagnetic resonance with the OxyChip. *Front Oncol* 11:1–16
53. Caston RW, Schreiber W, Hou H, Williams BB, Chen EY, Schaner PE, Jarvis LA, Flood AB, Petryakov SV, Kmiec MM, Kuppusamy P, Swartz HM (2017) Development of the implantable resonator system for clinical EPR oximetry. *Cell Biochem Biophys* 75:275–283
54. Chen EY, Tse D, Hou H, Schreiber WA, Schaner PE, Kmiec MM, Hebert KA, Kuppusamy P, Swartz HM, Williams BB (2021) Evaluation of a refined implantable resonator for deep-tissue EPR oximetry in the clinic. *Appl Magn Reson* 52:1321–1342
55. Hou H, Khan N, Du G, Hodge S, Swartz HM (2016) Temporal variation in the response of tumors to hyperoxia with breathing carbogen and oxygen. *Med Gas Res* 6:138–146
56. Swartz HM, Williams BB, Hou H, Khan N, Jarvis LA, Chen EY, Schaner PE, Ali A, Gallez B, Kuppusamy P, Flood AB (2016) Direct and repeated clinical measurements of pO₂ for enhancing cancer therapy and other applications. *Adv Exp Med Biol* 923:95–104
57. Jeong JJ, Liu T, Yang X, Torres M, Lin J, Schreiber W, Flood AB, Kuppusamy P, Swartz HM, Williams BB, Schaner PE, Ali A (2019) Measurements of normal tissue oxygenation via electron paramagnetic resonance (EPR) oximetry during and after breast radiation therapy: baseline evaluations and response to hyperoxygenation. *Radiol Radiat Ther* 7(1082):1–6
58. Swartz HM, Williams BB, Zaki B, Hartford AC, Jarvis LA, Chen EY, Comi RJ, Ernstoff MS, Hou H, Khan N, Swartz SG, Flood AB, Kuppusamy P (2014) Clinical EPR: unique opportunities and some challenges. *Acad Radiol* 21:197–206
59. Swartz HM, Khan N, Buckey J, Comi R, Gould L, Grinberg O, Hartford A, Hopf H, Hou H, Hug E, Iwasaki A, Lesniewski P, Salikhov I, Walczak T (2004) Clinical applications of EPR: overview and perspectives. *NMR Biomed* 17:335–351
60. Khan N, Hou H, Hein P, Comi RJ, Buckey JC, Grinberg O, Salikhov I, Lu SY, Wallach H, Swartz HM (2005) Black magic and EPR oximetry: from lab to initial clinical trials. *Adv Exp Med Biol* 566:119–125
61. Khan N, Williams BB, Hou H, Li H, Swartz HM (2007) Repetitive tissue pO₂ measurements by electron paramagnetic resonance oximetry: current status and future potential for experimental and clinical studies. *Antioxid Redox Signal* 9:1169–1182
62. Williams BB, Khan N, Zaki B, Hartford A, Ernstoff MS, Swartz HM (2010) Clinical electron paramagnetic resonance (EPR) oximetry using India ink. *Adv Exp Med Biol* 662:149–156
63. Höckel M, Knoop C, Schlenger K, Vordran B, Baußmann E, Mitze M, Knapstein PG, Vaupel P (1993) Intratumoral pO₂ predicts survival in advanced cancer of the uterine cervix. *Radiother Oncol* 26:45–50
64. Höckel M, Schlenger K, Aral A, Mitze M, Schäffer U, Vaupel P (1996) Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res* 56:4509–4515

Tissue Oxygenation in Individuals with Spinal Cord Injury: A Pilot Study

Tarcisi Cantieni, O. da Silva-Kress, and U. Wolf

Abstract

Pressure injuries (PI) are debilitating tissue lesions characterised by slow healing and a high risk of severe infection. These injuries result from continued tissue hypoxia, or low tissue oxygen saturation (StO_2), which is a consequence of impaired blood circulation caused by continued pressure against tissue. PI result in significant suffering and a substantial financial burden. Therefore, we aim to develop an effective preventive measure. Persons with spinal cord injury (SCI) are at a high risk for PI due to their impaired mobility and lack of sensation to alert them to danger. The aim was to test the feasibility of an alert system based on StO_2 monitoring in persons with SCI using a novel textile-based near-infrared spectroscopy (NIRS) sensor.

Sensors consisted of two emission and two detection points, each point comprising eight fibres each with three light-coupling bends. Textile integration of fibres was achieved by stitching on a mass-production stitching machine. Emission fibres were connected to

LEDs of 740 nm and 810 nm. Detection fibres were connected to avalanche photodiodes. Sensors were attached to the skin in the high-PI-risk buttocks area covering the bony prominences of the ischial tuberosity. StO_2 was measured in three healthy subjects and two subjects with SCI during repeated sitting and pressure recovery phases.

Our experiments showed decreases in StO_2 in both groups during sitting phases: during low-pressure sitting phases, StO_2 decreased on average $-0.32\%/min$ in the healthy subjects and at $-1.24\%/min$ in the SCI subjects. During high-pressure sitting, these rates increased to $-1.16\%/min$ for the healthy subjects and $-2.16\%/min$ for the SCI subjects. During recovery phases, StO_2 returned towards normal values, but did not always reach the initial baseline. No discomfort or skin irritation was observed.

These first measurements show physiologically reasonable StO_2 values and demonstrate sufficient comfort while sitting on a sensor with no skin irritation or other adverse effects of sitting on the sensor for over 30 min. The nurses involved were satisfied with the easy-of-use, materials used, and patient safety demonstrated during the study. These results show that it is feasible to develop an alert system for PI prevention using a safe, wearable, textile-based NIRS sensor.

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Keywords

Near-infrared spectroscopy · Pressure injury Prevention · Spinal cord injury · Health monitoring

1 Introduction

Pressure-induced ischemia and the resulting prolonged tissue hypoxia play the key role in the development of pressure injuries (PI) [1]. The high PI prevalence rate of 26.4% [2] in spinal cord injury (SCI) persons poses significant challenges for both the affected individuals and the healthcare system: Treatment costs for PI as high as \$70,000 [3] for a single occurrence highlight the necessity for better PI prevention.

A promising approach is near-infrared spectroscopy (NIRS), which can monitor tissue oxygen saturation (StO_2) directly, safely, non-invasively, and continuously. StO_2 is an important parameter that represents the balance between local oxygen consumption and supply. Hypoxia (low StO_2) can lead to tissue necrosis. By monitoring StO_2 of tissues, where PIs are known to occur, the user (either the patient or the caregiver) can be alerted of critically low StO_2 long before tissue necrosis (PI onset) occurs. So far, conventional NIRS sensors have introduced pressure points [4], which would cause PI themselves if placed at high-pressure regions of the body. Therefore, an NIRS sensor for monitoring high-PI-risk regions needs to be thin, comfortable, wearable, and pose no risk to the skin or underlying tissue when prolonged pressure is applied. We have designed, built, and tested the world's first textile-based NIRS sensor, which can safely provide continuous deep-tissue StO_2 monitoring at high-pressure, high-PI-risk regions.

The aim of this study is to demonstrate initial clinical feasibility by testing sensor performance and usability in healthy subjects and SCI patients.

2 Materials and Methods

Light coupling between the textile-based sensors and human tissue was accomplished by optimally bending polymer optical fibres (diameter 175 μm , DB-175, Asahi Kasei, Japan), so that light emission and detection occurred at pre-defined discrete locations, here called emission and detection points, respectively (see Fig. 1). Each sensor consisted of two emission and two detection points, each point comprising eight fibres each with three light-coupling bends. Source-detector distances were 18 and 28 mm in symmetric configuration (for sensor layout, see Fig. 14.1a in [5]; emission points EP1 and EP3 and detection points DP1 and DP3 were used in this study). Half of the emission fibres at each emission point were connected to LEDs of wavelength 740 nm (SMB1N-740D, Roithner LaserTechnik, Austria; 930 mW optical power output in pulsed operation), and the other half to LEDs of wavelength 810 nm (SMB1N-810D, Roithner LaserTechnik, Austria; 1270 mW optical power output in pulsed operation). Emission fibres were thus illuminated from both ends. Both ends of all detection fibres were connected to avalanche photodiodes (one APD per detection point) S2384 (Hamamatsu, Japan).

Calibration factors for all sensors used in the study were determined by measuring silicone phantoms with known properties. This was done before and after measurements in study participants to ensure optimal calibration.

Five subjects participated in this pilot study: three healthy subjects and two subjects with SCI.

Fig. 1 Sensor used in measurements. Four SMA connectors are connectors to LED light sources and two are connected to the APDs



For the measurements, the sensors were placed on the area covering the ischial tuberosity (IT) on one side of the body. The IT was palpated to ensure correct placement.

The sensors were attached to the skin using biocompatible adhesives (2477P and 9834, 3M, USA). Adhesives were applied to the skin, such that no adhesive was covering the emission and detection points to avoid optical interference and potential undesired effects, such as light piping.

The experimental protocol for the healthy subjects was as follows: symmetric sitting (6 min), standing (1 min), symmetric sitting (5 min), sitting with increased pressure on the sensor side (5 min), standing (1 min), and symmetric sitting (5 min).

The protocol for the subjects with SCI was: symmetric sitting (5 min), push-up pressure relief (1 min), symmetric sitting (5 min), push-up pressure relief (1 min), sitting with increased pressure on the sensor side (5 min), push-up pressure relief (1 min), and symmetric sitting (10 min).

All subjects were repeatedly asked about their comfort during the measurements.

For the phase “increased pressure on sensor side”, the subjects were asked to move as much of their body weight onto the sensor by tilting their bodies. The subjects with SCI were assisted by caregivers.

During the measurement, light intensities were recorded. Following the principles of multi-distance spectroscopy [6] and self-calibration [7], StO_2 curves were calculated.

After the removal of the sensor, the skin which had been in contact with the sensor was checked for signs of irritation.

3 Results and Discussion

Table 1 shows the mean change rates during the sitting phases. These numbers stem from linear regressions for each phase.

Figure 2 shows the StO_2 curves in healthy subjects. In all three measurements, there is a decrease in StO_2 during the first symmetric-sitting phase. In the first recovery phase, all three measurements show a rapid increase in StO_2 to

Table 1 Mean change rates ($\pm$ SE) during sitting phases for the two groups

Phase	Pressure	Healthy participants	SCI participants
1	Low	$-0.56 \pm 0.10\%$ / min	$-0.67 \pm 0.51\%$ / min
2	Low	$-0.30 \pm 0.22\%$ / min	$-2.42 \pm 1.22\%$ / min
3	High	$-1.16 \pm 0.06\%$ / min	$-2.16 \pm 0.03\%$ / min
4	Low	$-0.10 \pm 0.12\%$ / min	$-0.65 \pm 0.64\%$ / min

different levels depending on the subject. StO_2 then falls rapidly in the second symmetric-sitting phase. Here, the average decrease in oxygenation is lower than in the first one. This could potentially be explained by the autoregulatory response of muscle tissue. At the start of the second sitting phase, this response was likely still active due to the first phase.

During the weight shift onto the sensor in the third phase (high pressure), all three measurements exhibit the highest decrease (see Table 1).

The second recovery phase shows a similar pattern as the first one. The final sitting phase again shows a less pronounced linear decrease in StO_2 . This may be explained by the strong reperfusion seen during the second recovery phase (as a result of the very low StO_2 during the high-pressure phase) suggesting a strong autoregulatory response similar to that seen after the first recovery phase.

The curves recorded from the individuals with SCI (Fig. 3) look similar in principle, with decreases in StO_2 during the sitting phases and rapid StO_2 increase during the recovery phases. The two groups cannot be directly compared, because the individuals with SCI were sitting on a special wheelchair cushion during the measurement, while the healthy subjects were sitting on a lightly padded wooden chair. It can be noted that in Fig. 3, StO_2 reaches a lower minimum value during each sitting phase and a lower maximum value during each recovery phase. It is too early to come to a definite conclusion, but these patterns suggest a compromised autoregulatory response [8].

More subjects are needed to confirm the behaviour during the recovery phases, since such

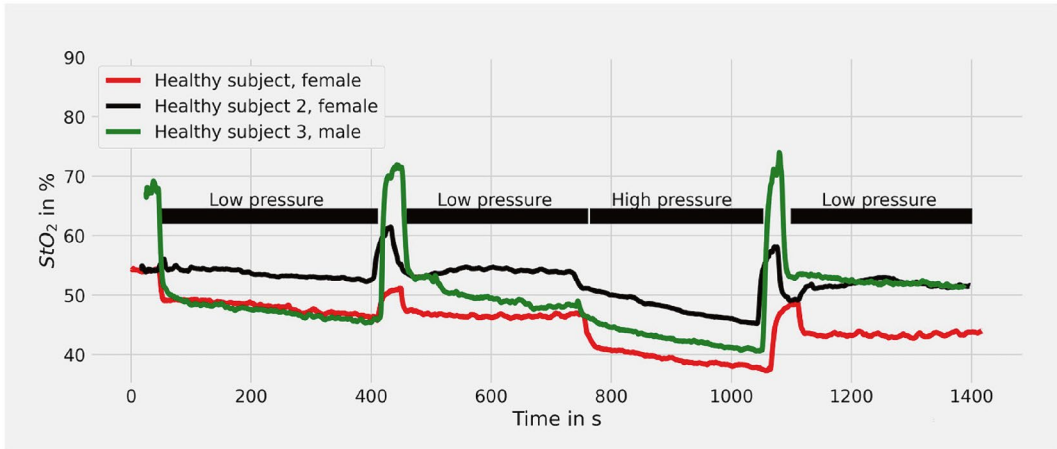


Fig. 2 Three StO₂ measurements performed in the ischial tuberosity region of healthy subjects

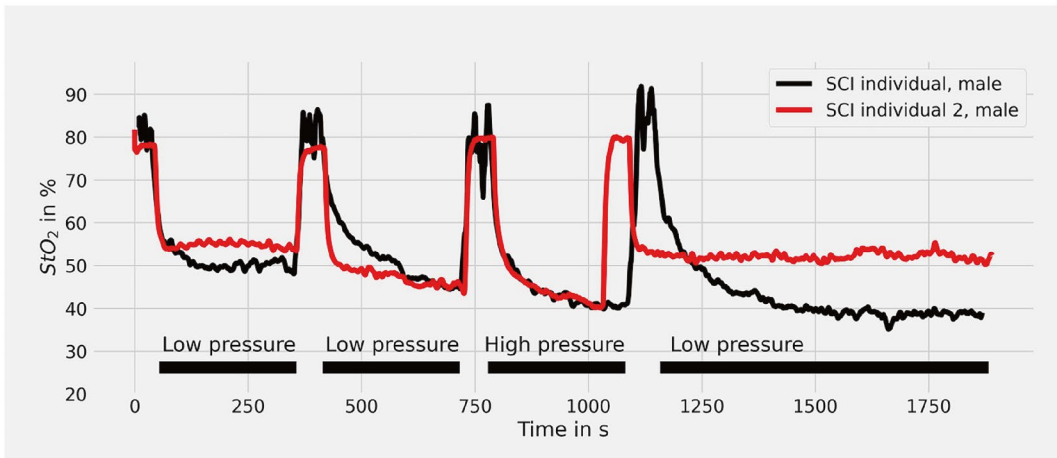


Fig. 3 StO₂ measurements performed in the ischial tuberosity region of individuals with SCI

non-invasive measurements of StO₂ have never been done before without the sensor causing a pressure point itself.

Importantly, the variability of the changes in StO₂ due to pressure within subjects emphasises the need for continuous monitoring of StO₂, i.e. the drop in StO₂ clearly in one period depends on the pressure exerted in a previous period: in sitting period 2, StO₂ reaches a different value after 5 min compared to period 1.

Further analysis of the signals in Fig. 3 reveals higher-than-expected rates of reperfusion at the start of each recovery phase. These almost instant

changes in StO₂ suggest mechanisms that need to be studied further. They are likely related to the ejection of blood from the monitored tissue region during the sitting motion. Other physiological responses specific to paraplegic physiology may play a role in this behaviour.

The signals from the SCI subjects also exhibit exponential behaviour at the beginning of the sitting phases, which may be partially due to the type of seat cushion, the subjects were using. The pressure response of future seating surfaces needs to be accounted for to compare data between SCI subjects and health subjects.

Follow-up studies will monitor pressure at the sensor/subject interface to properly account for pressure when assessing and comparing physiological responses. Additionally, a new protocol with longer sitting times will be implemented to confirm this hypothesis by allowing StO_2 curves to exhibit asymptotic behaviour.

Based on Fig. 3, a simple algorithm for an alert system for PI prevention could be based on recognising a decreasing StO_2 profile followed by a constant level significantly lower than baseline values without the need for analysing the behaviour during the recovering phases.

A final key result of this study is the positive response of the medical staff involved in applying the sensor and evaluating its safety. The sensor's thin profile, soft materials, and gentle adhesives successfully prevented any adverse effects of wear at the high-pressure ischial tuberosity region (which typically experiences pressure of 200 mmHg) for a duration of at least 30 min. Feedback was received that correct application of the sensor was simple and effective.

4 Conclusion

To the best of our knowledge, this is the first-time NIRS measured the oxygenation during sitting in healthy subjects and individuals with SCI with a sensor not influencing the pressure distribution of the tissue. Despite the small number of subjects, a simple first algorithm to detect the danger of PI development is suggested. Importantly, there were no signs of irritation or discomfort caused by the sensor.

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References

1. Stekelenburg A, Strijkers GJ, Parusel H, Bader DL, Nicolay K, Oomens CW (2007) Role of ischemia and deformation in the onset of compression-induced deep tissue injury: MRI-based studies in a rat model. *J Appl Physiol* 102:2002
2. Kovindha A, Kammuang-lue P, Prakongsai P, Wongphan T (2015) Prevalence of pressure ulcers in Thai wheelchair users with chronic spinal cord injuries. *Spinal Cord* 53:767
3. Young DL, Shen JJ, Estocado N, Landers MR (2012) Financial impact of improved pressure ulcer staging in the acute hospital with use of a new tool, the NE1 wound assessment tool. *Adv Skin Wound Care* 25:158
4. Ceylan B, Yapucu Gunes U, Uyar M (2018) Examination of sacral tissue oxygen saturation among immobile patients. *J Clin Nurs* 27:3641
5. Cantieni T, da Silva-Kress O, Wolf U (2023) Accuracy of Tissue Oxygen Saturation Measurements of a Textile-Based NIRS Sensor. *Adv Exp Med Biol* 1438:83–86
6. Matcher SJ, Kirkpatrick PJ, Nahid K, Cope M, Delpy DT (1995) Absolute quantification methods in tissue near-infrared spectroscopy. In: Optical tomography, photon migration, and spectroscopy of tissue and model media: theory, human studies, and instrumentation. International Society for Optics and Photonics
7. Hueber DM, Fantini S, Cerussi AE, Barbieri BB (1999) In: Chance B, Alfano RR, Tromberg BJ (eds) New optical probe designs for absolute (self-calibrating) NIR tissue hemoglobin measurements, San Jose
8. Bader DL (1990) The recovery characteristics of soft tissues following repeated loading. *J Rehabil Res Dev* 27:141

Part VI

Muscle

Changes in Cerebral Oxygenation in the Prefrontal Cortex During Cardiopulmonary Exercise Testing in Patients After Cardiovascular Surgery

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Abstract

Oxygenated haemoglobin (O₂Hb) and total haemoglobin (THb) concentrations rise with increasing exercise load during the cardiopulmonary exercise test (CPX); however, this elevated response is impaired in patients with chronic heart failure. Furthermore, the changes occurring in patients during the acute phase of cardiac surgery are unknown. This study aimed to measure cerebral oxygenation in the prefrontal cortex (PFC) during CPX in patients

during the acute post-operative phase following cardiovascular surgery. Fourteen patients in the acute phase of post-cardiovascular surgery period were enrolled. CPX was administered between the post-operative period and discharge. The protocol employed the ramp method (10 W/min) after 3-min rest and 3-min warm-up periods. Levels of O₂Hb, deoxygenated haemoglobin (HHb), THb, and the regional cerebral oxygen saturation (rSO₂) in the PFC were measured from the resting state through the end of CPX using near-infrared spectroscopy. The mean values of O₂Hb, HHb, and THb levels and rSO₂ were compared at rest, warm-up, anaerobic threshold, and peak points. At the peak, O₂Hb and rSO₂ declined significantly, and HHb rose significantly compared to the respective values at rest; no sig-

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nificant changes were observed in THb. These findings suggest that the oxygen supply to the PFC is reduced in patients with reduced cardiac function following cardiovascular surgery.

Keywords

Post-cardiovascular surgery
Cardiopulmonary exercise test · Prefrontal cortex · Chronotropic response index
Wearable near-infrared spectroscopy

1 Introduction

Chronic cerebral hypoperfusion caused by blood pressure fluctuations and reduced cardiac output can reportedly damage brain structures, contributing to cognitive decline [1]. Although the physiological parameters such as blood pressure and cardiac output that affect cerebral blood flow change constantly during exercise, no objective indicators have been established to assess cerebral blood flow during exercise. Therefore, we focused on near-infrared spectroscopy (NIRS), which can be performed during exercise. Objective indicators measured by NIRS could find application in risk management of cerebral circulation during exercise.

Previous NIRS-based studies reported that the oxygenated haemoglobin (O_2Hb) and total haemoglobin (THb) concentrations in the prefrontal cortex (PFC) increased depending on the exercise load during cardiopulmonary exercise tests (CPX) in healthy participants [2]. In contrast, the increase in O_2Hb and THb levels during CPX has been reported to be impaired in patients with heart failure and reduced cardiac function [3]. Furthermore, a previous study comparing regional cerebral oxygen saturation (rSO_2) before and after CPX among healthy participants, patients with stroke, and patients with heart failure reported that rSO_2 decreased after exercise only in patients with heart failure [4]. However, to date, no studies have investigated patients in

the acute phase following cardiac surgery. Therefore, the clarification of the changes in cerebral oxygenation during CPX is required to investigate the effects of exercise on the brain. This study aimed to measure cerebral oxygenation in the PFC during CPX using NIRS in patients during the acute post-operative phase following cardiovascular surgery.

2 Methods

2.1 Participants

This prospective, observational study was conducted at the Kobe City Medical Center General Hospital in Japan between July 2022 and January 2023. Overall, 64 patients underwent cardiovascular surgery during the study period. Of these, 16 patients were recruited based on the following inclusion criteria: presence of moderate-to-severe carotid stenosis, type 2 respiratory failure (such as chronic obstructive pulmonary disease or stroke), no history of pacemaker implantation, and performance of CPX during hospitalisation. Two patients, who exhibited a peak respiratory quotient of less than 1.1 during CPX, were excluded from the analysis due to insufficient effort [5]. The other exclusion criteria were set as patients who had difficulty in wearing the NIRS probes, required oxygen therapy, had cognitive decline, or had difficulty understanding instructions. Finally, 14 patients were included in the analysis (1 woman, mean age 70.2 ± 6.9 years, BMI 22.4 ± 3.8 kg/m², haemoglobin 11.3 g/dL, and left ventricular ejection fraction $56.8 \pm 8.6\%$). Thirteen of the 14 patients were on beta-blockers. This study was approved by the Ethics Committee of Niigata University of Health and Welfare (approval number: 18893-220810) and the Kobe City Medical Centre Central Municipal Hospital (approval number: zn220801), and conducted in accordance with the Declaration of Helsinki. The patients provided written informed consent to participate in this study.

2.2 Procedures

All participants performed the CPX using a bicycle ergometer (Strength Ergo8, FUKUDA DENSHI, Japan). The participants undertook a ramp load of 10 W/min after 3 min of rest and 3 min of warm-up. The anaerobic threshold (AT) was determined using the V-slope method. The peak point was defined as follows: (1) a plateau in VO_2 , (2) failure to maintain the cycling cadence at <50 rpm despite strong encouragement, and (3) if the examiner decided to end the test based on the discontinuation criteria [5]. The ventilatory equivalent (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (R), end-tidal oxygen, and end-tidal carbon dioxide tension during exercise were measured using the breath-by-breath method with a breath gas analyser (AE-310S, MINATO, Japan). The heart rate, mean arterial pressure, and SpO_2 were also monitored during exercise.

2.3 NIRS Measurements

Brain activity monitoring (Hb133, Astem, Japan) was used to measure cerebral oxygenation. O_2Hb , deoxygenated haemoglobin (HHb), THb, and rSO_2 were measured over time during exercise in the cortex using near-infrared light at two different wavelengths (770 ± 10 nm and 830 ± 10 nm) according to the modified Lambert–Beer law [6]. Two detectors at different distances of 30 mm and 35 mm from the light source were installed to compensate for the effects of intervening tissue and enable highly accurate blood volume estimation [7]. The regions of interest were set as the left and right PFC, and the probe positions of the two channels were determined based on the international 10–20 method [8], aligning channel 1 with FP1 and channel 2 with FP2.

2.4 Statistical Analysis

The chronotropic response index (CRI) was calculated as follows to assess the heart rate response: $(\text{peak HR} - \text{resting HR}) /$

$(220 - \text{age} - \text{resting HR}) \times 100$ [9]. A CRI of 80% or less was considered as response failure. The timepoints for each NIRS parameter were rest, warm-up, AT, and peak times. For all variables, the mean value at 3 min of rest was used as the baseline and the amount of change was calculated. Warm-up was calculated as the average value over a 3-min period, while AT and peak were calculated as the average values over the preceding 5 s. The Bonferroni multiple comparison test was used to compare each timepoint. Statistical software (Statistical Package for the Social Sciences, SPSS version 27.0, IBM, USA) was used for statistical analysis and the threshold for significance was set at 0.05.

3 Results

Table 1 presents the CPX results. The peak VO_2 was 14.0 ± 3.3 mL/min/kg, the VE/VCO_2 slope was $37.7 \pm 9.3\%$, and the CRI was $33.4 \pm 20.0\%$. The NIRS parameters are shown in Fig. 1. O_2Hb underwent a change at peak (left: $9.62 \mu\text{M}$; right: $13.97 \mu\text{M}$) compared to at rest, with a significant decrease on the right side ($P < 0.05$). HHb (left: $6.67 \mu\text{M}$; right: $9.73 \mu\text{M}$) was significantly increased at peak compared to that at rest on both sides ($P < 0.05$). The change in THb (left: $2.96 \mu\text{M}$; right: $4.12 \mu\text{M}$) showed no significant differences. rSO_2 (left: 1.96 points; right: 2.62 points) was significantly reduced bilaterally ($P < 0.01$).

4 Discussion

This is the first study to measure changes in NIRS parameters of the PFC during CPX in patients who underwent cardiovascular surgery. CPX resulted in reduced ventilatory efficiency, heart rate response, and exercise tolerance. O_2Hb levels and rSO_2 showed a significant decline at the peak compared with the corresponding resting state values.

The findings observed during CPX, particularly the lower peak VO_2 and higher VE/VCO_2 slope, may be attributed to the older average age of this study's participants compared to that of patients

Table 1 CPX data for the entire target population

		Rest	AT	Peak
Gas exchange				
VE	L/min	12.4 ± 1.8	24.7 ± 3.9	47.5 ± 11.3
VO ₂	mL/min	231.6 ± 30.8	557.4 ± 105.4	851.2 ± 218.8
Peak VO ₂	mL/min/kg			14.0 ± 3.3
VCO ₂	mL/min	211.2 ± 33.0	548.3 ± 104.1	1049.2 ± 331.5
R		0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
VE/VCO ₂ slope				37.7 ± 9.3
PETO ₂	%	16.3 ± 0.7	16.1 ± 0.7	17.0 ± 0.5
PETCO ₂	%	4.3 ± 0.6	4.6 ± 0.7	4.5 ± 0.6
Haemodynamics				
HR	Beat/min	79.9 ± 8.1	89.8 ± 10.3	103.1 ± 17.3
MAP	mmHg	85.2 ± 13.3	94.4 ± 14.9	98.7 ± 15.1
SpO ₂	%	97.6 ± 1.0	97.2 ± 1.1	97.7 ± 0.8
CRI	%			33.4 ± 20.0

The values represent the mean ± SD
AT (anaerobic threshold), CPX (cardiopulmonary exercise test), VE (ventilatory equivalent), VO₂ (oxygen consumption), VCO₂ (carbon dioxide production), R (respiratory quotient), PETO₂ (end-tidal oxygen), PETCO₂ (end-tidal carbon dioxide tension), HR (heart rate), MAP (mean arterial pressure), SpO₂ (saturation of percutaneous oxygen), CRI (chronotropic response index)

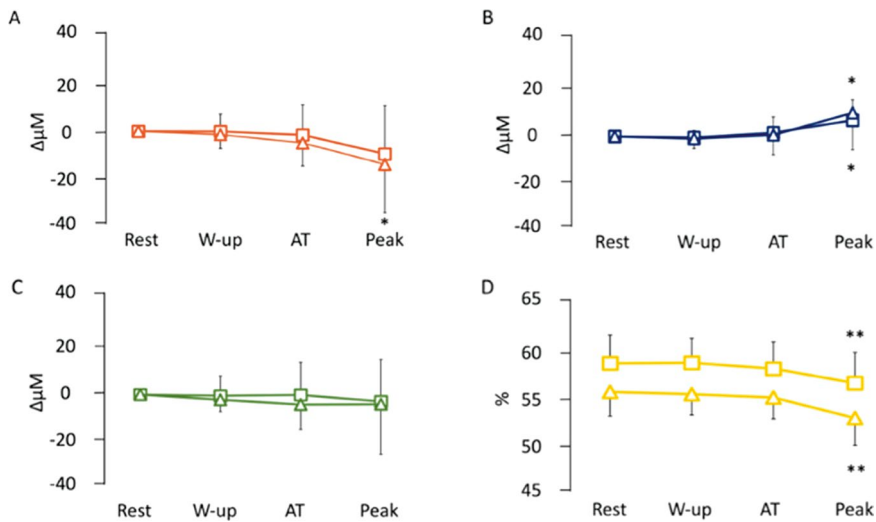


Fig. 1 Changes in the NIRS parameters. Changes in the (a) O₂Hb, (b) HHb, (c) THb, and (d) rSO₂ levels. The values represent the mean ± SD. NIRS (near-infrared spectroscopy), O₂Hb (oxygenated haemoglobin), THb (total haemoglobin), rSO₂ (regional cerebral oxygen saturation), PFC (prefrontal cortex), HHb (deoxygenated haemoglobin), W-up (warm-up), AT (anaerobic threshold). □: PFC on the left, △: PFC on the right. **P* < 0.05 vs rest, ***P* < 0.01 vs rest

enrolled in previous studies of CPX after cardiac surgery [10, 11]. The CRI was 49 ± 23% in the group with peak VO₂ < 16.0 mL/min/kg in a study involving patients with heart failure, which was lower than the corresponding value of the current

patient population [9]. This difference can be attributed to the older mean age of the patients in this study (70.2 ± 6.9 years) compared to previous studies. The decline in the rSO₂ and O₂Hb levels, accompanied by the decline in HHb, and the result-

ing lack of change in the THb suggest a state of cerebral hypoxia, which may be attributed to an insufficient increase in cardiac output during exercise. During exercise, the oxygen demand increases not only in skeletal muscle but also in the PFC, which is involved in executive function and cognitive control [12, 13]. Therefore, cardiac output increases in healthy individuals because of a higher systemic oxygen demand [3]. Post-operative chronotropic incompetence and the use of beta-blockers resulted in a reduced heart rate response during exercise in the patients in this study [14]. Therefore, it is possible that the increase in cardiac output was not proportionate with the increase in the oxygen demand of the PFC during exercise, leading to a state of cerebral hypoxia in this brain region.

This study had several limitations. First, the measurements performed using NIRS devices are influenced by skin blood flow. Furthermore, the study had a small sample size and did not entail comparison with an age-matched control group. Additionally, the methods used in this study did not facilitate direct measurement of cardiac output. Moreover, the impact of the reductions in the rSO_2 and O_2Hb levels on the functional outcomes and life expectancy is unclear. Future studies are warranted to investigate these issues. In conclusion, the results of this study suggest that impaired cardiac function during the acute post-operative phase after cardiovascular surgery may result in an insufficient increase in cardiac output during exercise at the expense of localised prefrontal cortex oxygenation.

References

1. Okonkwo OC, Cohen RA, Gunstad J, Poppas A (2011) Cardiac output, blood pressure variability, and cognitive decline in geriatric cardiac patients. *J Cardiopulm Rehabil Prev* 31:290–297
2. Kojima S, Morishita S, Hotta K et al (2022) Temporal changes in cortical oxygenation in the motor-related areas and bilateral prefrontal cortex based on exercise intensity and respiratory metabolism during incremental exercise in male subjects: a near-infrared spectroscopy study. *Front Physiol* 13:794473
3. Fu T-C, Wang C-H, Hsu C-C et al (2011) Suppression of cerebral hemodynamics is associated with reduced functional capacity in patients with heart failure. *Am J Physiol Heart Circ Physiol* 300:H1545–H1555
4. Chen Y-J, Wang J-S, Hsu C-C et al (2018) Cerebral desaturation in heart failure: potential prognostic value and physiologic basis. *PLoS One* 13:e0196299
5. Agostoni P, Dumitrescu D (2019) How to perform and report a cardiopulmonary exercise test in patients with chronic heart failure. *Int J Cardiol* 288:107–113
6. Yücel MA, Lühmann AV, Scholkmann F et al (2021) Best practices for fNIRS publications. *Neurophotonics* 8:012101
7. Niwayama M (2018) Voxel-based measurement sensitivity of spatially resolved near-infrared spectroscopy in layered tissues. *J Biomed Opt* 23:1–4
8. Gh K, Ho L, Hh J, Elger C (1999) The ten-twenty electrode system of the international federation. *The international federation of clinical neurophysiology. Electroencephalogr Clin Neurophysiol* 52:3–6
9. Vallebona A, Gigli G, Orlandi S, Reggiardo G (2005) Heart rate response to graded exercise correlates with aerobic and ventilatory capacity in patients with heart failure. *Clin Cardiol* 28:25–29
10. Pardaens S, Moerman V, Willems A-M et al (2014) Impact of the preoperative risk and the type of surgery on exercise capacity and training after valvular surgery. *Am J Cardiol* 113:1383–1389
11. Meurin P, Iliou MC, Ben Driss A et al (2005) Early exercise training after mitral valve repair: a multicentric prospective French study. *Chest* 128:1638–1644
12. Tempest GD, Eston RG, Parfitt G (2014) Prefrontal cortex haemodynamics and affective responses during exercise: a multi-channel near infrared spectroscopy study. *PLoS One* 9:e95924
13. Morishita S, Tsubaki A, Hotta K et al (2022) Perceived exertion correlates with multiple physiological parameters during cardiopulmonary exercise testing. *Adv Exp Med Biol* 1395:423–427
14. Seifert T, Rasmussen P, Secher NH, Nielsen HB (2009) Cerebral oxygenation decreases during exercise in humans with beta-adrenergic blockade. *Acta Physiol (Oxf)* 196:295–302

Effect of Gum Chewing Training on Masseter Muscle Oxygen Dynamics

Arata Tsutsui, Tomotaka Takeda, Takahiro Sakaue, Shinji Togo, Susumu Kanno, Misaki Matsui, Kenji Osawa, Kazunori Nakajima, and Kenichi Fukuda

Abstract

This study aimed to clarify the effect of 1 month of gum chewing (GCh) training on masseter muscle oxygen dynamics and determine its effectiveness in improving muscle endurance and fatigue. A total of 16 healthy adult subjects were included in this study. The subjects were randomly assigned to two groups: control and training groups (eight subjects each). GCh training using prototype gum of moderate hardness was performed for 10 min (5 min for each side) three times a day before each meal for 30 days. Clenching effects were evaluated before and after interventions. Masseter muscle oxygen dynamics were measured using spatially resolved near-infrared spectroscopy. Furthermore, masseter muscle fatigue was assessed using the Visual Analog Scale (VAS). After training, a signifi-

cant increase was observed in total haemoglobin and oxygenated haemoglobin during clenching, and the 1/2 recovery time was significantly shortened. The changes in the VAS score showed a decreasing trend after training. In conclusion, 1 month of GCh training changed masseter oxygen dynamics during clenching and recovery and improved muscle aerobic capacity.

Keywords

Gum chewing · Training · Near-infrared spectroscopy · Masseter muscle · Muscle hemodynamics

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1 Introduction

In sustained muscle activity, aerobic metabolism is thought to play an important role in supplying energy during exercise, and the rapid supply of sufficient blood flow to muscle tissue is important for improving muscle endurance and fatigue [1, 2]. In the stomatognathic system, masticatory muscle activity and masticatory muscle fatigability can be reduced due to many factors, such as bruxism, malocclusion, stress, lifestyle, and diet, which can not only reduce occlusal force and masticatory ability but also reduce nutritional intake [3, 4]. Various interventions, such as gum chewing (GCh) training [5–7], using a thermo-

plastic sheet [8], clenching silicone tubes [9], and using hard gummy [3], have been used to strengthen masticatory muscles, improve muscle condition, and increase occlusal force. In addition, these interventions may be useful as GCh routine in older adults increases occlusal force and masticatory ability and prevents the incidence of oral frailty [10]. Previous studies have mainly evaluated maximal occlusal force, masticatory performance, and electromyographic activities. However, no study has examined the effects of masseter muscle training on masseter muscle oxygen dynamics.

Thus, this study aimed to clarify the effect of 1 month of GCh training on masseter muscle oxygen dynamics and determine its effectiveness in improving muscle endurance and fatigue.

2 Methods

A total of 16 healthy adult volunteers were recruited from the Central Laboratory, Lotte Co., Ltd., Saitama, Japan. The mean age of the subjects was 35.7 ± 8.5 years. Of the 16 subjects, 12 were males and 4 were females. The inclusion criteria included general health, no subjective or other abnormalities in the oral cavity, temporomandibular joint, or masticatory muscles, and no history of cardiovascular disease. The participants were randomly assigned to two groups: the training group ($n = 8$, 6 males and 2 females), with a mean age of 33.5 ± 8.6 years, and the control group ($n = 8$, 6 males and 2 females), with a mean age of 37.9 ± 8.5 years. This study was approved by the Ethical Review Committee of the Shiba Palace Clinic (approval date 24 February 2022, approval number 149353_rn-32377). A full explanation of the research plan was provided before conducting the study.

GCh training was performed using prototype gum of moderate hardness ($11 \times 18 \times 5$ mm, 1 g) with little change in hardness from the beginning of chewing. GCh training was performed for 10 min (5 min for each side) using two pieces of gum (one for each side) three times a day before each meal and continued for 30 days. Changes in masseter muscle oxygen dynamics were evalu-

ated by assessing maximum voluntary contraction (maximal clenching) before and after GCh training intervention. Masseter oxygen dynamics were measured using spatially resolved near-infrared spectroscopy (SR-NIRS) (Oxy-Pro tissue oxygen saturation sensor, ASTEM, Kanagawa, Japan) based on previous studies [11]. The thickness of subcutaneous fat, a source of error in muscle oxygen dynamics, was measured and corrected following the manufacturer's instructions. After confirming that the subjects were in a resting state, they underwent 60 s baseline rest, 15 s maximum voluntary contraction, and 120 s recovery rest. The SR-NIRS assessment items were changes in tissue oxygen saturation (StO_2), total haemoglobin (Total-Hb), oxygenated haemoglobin (Oxy-Hb), and deoxygenated haemoglobin (Deoxy-Hb) during clenching and recovery relative to baseline and 1/2 recovery time (1/2 RT). Here, 1/2 RT is an index of the muscle's aerobic capacity and is defined as the time from the end of clenching when StO_2 reaches half of the maximum recovery level [12]. The degree of subjective fatigue of the masseter muscle after the end of clenching was marked using the Visual Analog Scale (VAS), with 0 meaning no fatigue. The higher the value, the greater the fatigue.

Statistical analysis was performed using SPSS Statistics version 25.0 (IBM, Chicago, IL, USA). Changes in StO_2 , Total-Hb, Oxy-Hb, and Deoxy-Hb were calculated from the average of 5 s each of baseline 56–60 s, clenching task 11–15 s, and recovery 16–20 s, respectively. The pre- and post-intervention changes in the training and control groups were analysed using the Wilcoxon signed-rank test. A p value <0.05 was considered to indicate statistical significance.

3 Results

The masseter oxygen dynamics results during clenching before and after interventions in each group are shown in Figs. 1 and 2a. A significant increase in Total-Hb and Oxy-Hb was observed in the training group after training. No significant difference was observed in other items. The mas-

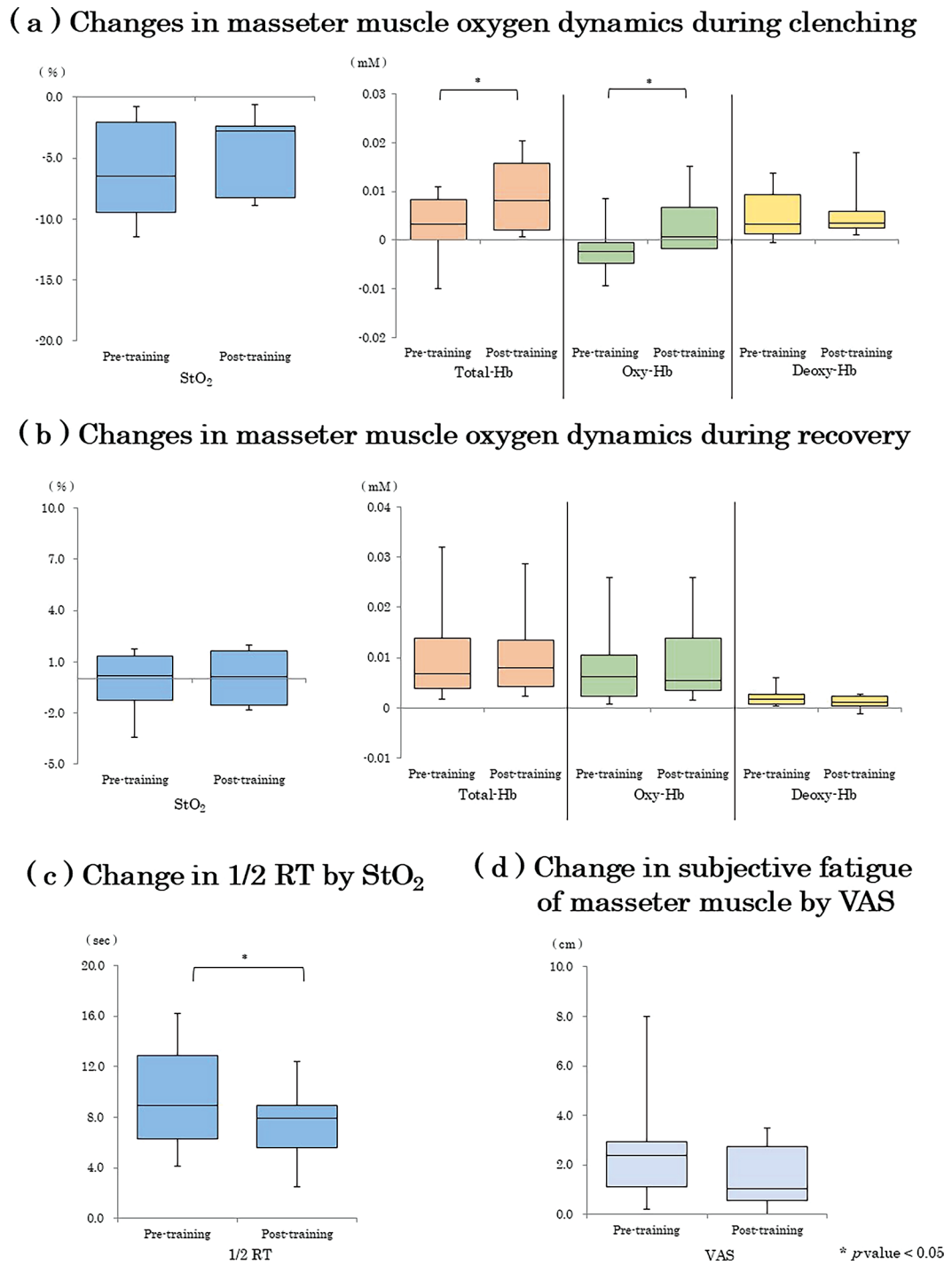
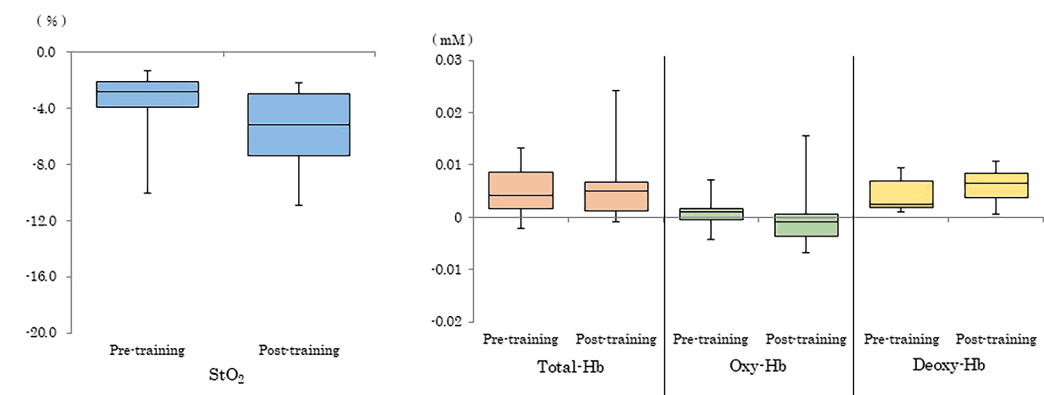
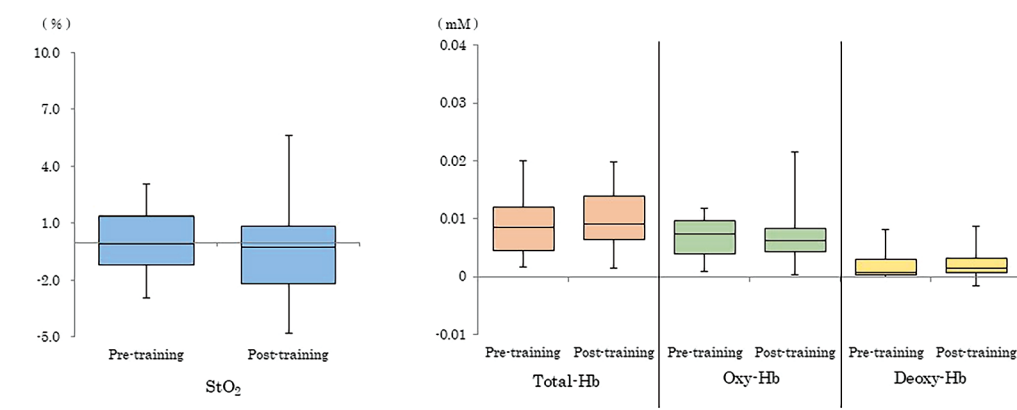


Fig. 1 Changes before and after intervention in the training group

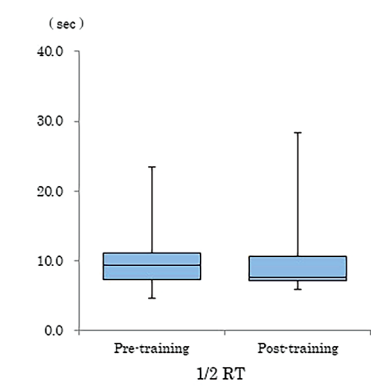
(a) Changes in masseter muscle oxygen dynamics during clenching



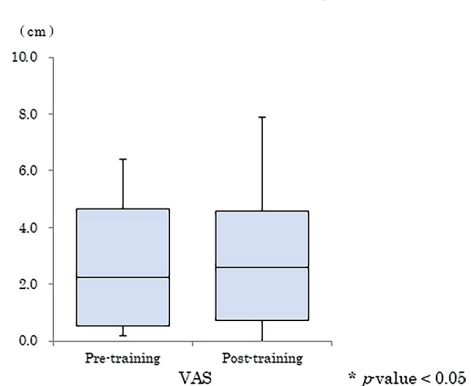
(b) Changes in masseter muscle oxygen dynamics during recovery



(c) Change in 1/2 RT by StO₂



(d) Change in subjective fatigue of masseter muscle by VAS



* *p*value < 0.05

Fig. 2 Changes before and after intervention in the control group

seter oxygen dynamics results during recovery before and after training in each group are shown in Figs. 1 and 2b, c. The 1/2 RT in the training group was significantly shortened after training. No significant difference was observed in other items. Additionally, the masseter VAS results before and after training in each group are shown in Figs. 1 and 2d. No significant difference was observed in any item. However, the VAS results in the training group showed a tendency to decrease after training.

4 Conclusions and Discussion

The study results showed changes in masseter oxygen dynamics during clenching and recovery in the training group. It is known that, in skeletal muscle, increasing the frequency and load of endurance training can induce enhanced mitochondrial adaptive changes and increased muscle oxidative capacity in a relatively short period of time [13, 14]. Additionally, during isometric contraction from mid to late exercise, muscle blood flow during exercise increases because of redistribution of blood by dilation of small arteries and capillaries in active muscles [15]. These findings suggest that endurance training with GCh affects the composition of muscle capillaries and fibres and mitochondrial adaptation in the masseter muscle, causing an increase in Total-Hb and Oxy-Hb during isometric contraction. Ichimura et al. [16] reported that the 1/2 RT of the active group was lower than that of the sedentary group (no regular physical activity) regardless of age. Furthermore, Delcanho et al. [17] observed that the group with chronic muscle pain showed a reduced Hb concentration change in the post-contraction recovery period. These findings are consistent with those of this study, which showed that the aerobic capacity of the masseter muscle was improved, and the 1/2 RT was shortened. Furthermore, in this study, no significant difference in VAS score in the masseter muscle was observed, but a decreasing trend in VAS score was observed after training. Therefore, improvement in the aerobic capacity of the masseter muscle may also affect muscle fatigue.

However, GCh training did not affect the amount of change in NIRS during the recovery period. Because this study analysed a relatively early recovery period, Total-Hb and Oxy-Hb increased from baseline both before and after the intervention, which may have caused exercise hyperaemia after isometric contraction. Satokawa et al. [18] also reported that Deoxy-Hb did not recover to baseline 180 s after low-intensity clenching. Therefore, the amount of change in NIRS may be influenced by the range of analysis during the recovery period.

Takano et al. [8] conducted a study on older adults and reported an improvement in the maximal occlusal force and masseter muscle thickness after 4 weeks of isometric exercises using a mouthpiece. Fujiwara et al. [3] conducted training using hard gummy candy for 3 months in healthy subjects and reported an increase in the maximal occlusal force after 2 months. Therefore, in future research, it is necessary to evaluate muscle thickness and electromyography related to occlusal force to examine the effects of longer-term intervention on masticatory ability and masticatory muscle activity.

In conclusion, 1 month of GCh training changed masseter oxygen dynamics during clenching and recovery and influenced muscle aerobic capacity.

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References

1. Larsson S-E, Bodegård L, Henriksson KG et al (1990) Chronic trapezius myalgia: morphology and blood flow studied in 17 patients. *Acta Orthop Scand* 61:394–398
2. Larsson B, Björk J, Kadi F et al (2004) Blood supply and oxidative metabolism in muscle biopsies of female cleaners with and without myalgia. *Clin J Pain* 20:440–446
3. Fujiwara S, Hori K, Shitara S et al (2021) Effect of hard gummy candy chewing on masticatory function. *J Oral Rehabil* 48:909–915
4. Ohrbach R, Dworkin SF (2016) The evolution of TMD diagnosis: past, present, future. *J Dent Res* 95:1093–1101
5. Nakagawa K, Matsuo K, Takagi D et al (2017) Effects of gum chewing exercises on saliva secretion and

- occlusal force in community-dwelling elderly individuals: a pilot study. *Geriatr Gerontol Int* 17:48–53
6. Ohira A, Ono Y, Yano N et al (2012) The effect of chewing exercise in preschool children on maximum bite force and masticatory performance. *Int J Paediatr Dent* 22:146–153
 7. Kawai N, Shibata M, Watanabe M et al (2020) Effects of functional training after orthognathic surgery on masticatory function in patients with mandibular prognathism. *J Dent Sci* 15:419–425
 8. Takano S, Yamaguchi K, Nakagawa K et al (2021) Effect of isometric exercises on the masseter muscle in older adults with missing dentition: a randomized controlled trial. *Sci Rep* 11:7285
 9. He T, Stavropoulos D, Hagberg C et al (2013) Effects of masticatory muscle training on maximum bite force and muscular endurance. *Acta Odontol Scand* 71:863–869
 10. Kawamura J, Tanaka T, Kanno S et al (2024) Relationship between a gum-chewing routine and oral, physical, and cognitive functions of community-dwelling older adults: a Kashiwa cohort study. *Geriatr Gerontol Int* 24:68–74
 11. Tsutsui A, Nakajima K, Sakaue T et al (2022) Jaw-clenching intensity effects on masseter oxygen dynamics and fatigue: a NIRS oximetry study. *Adv Exp Med Biol* 1395:435–441
 12. McCully KK, Halber C, Posner JD (1994) Exercise-induced changes in oxygen saturation in the calf muscles of elderly subjects with peripheral vascular disease. *J Gerontol* 49:B128–B134
 13. Burgomaster KA, Howarth KR, Phillips SM et al (2008) Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol* 586:151–160
 14. Gibala MJ, Little JP, van Essen M et al (2006) Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* 575:901–911
 15. Hamaoka T (1993) A non-invasive assessment of changes in localized skeletal muscle oxygen content on exercise: application of near infrared spectroscopy. *J Tokyo Med Univ* 51:437–445. In Japanese
 16. Ichimura S, Murase N, Osada T et al (2006) Age and activity status affect muscle reoxygenation time after maximal cycling exercise. *Med Sci Sports Exerc* 38:1277–1281
 17. Delcanho RE, Kim YJ, Clark GT (1996) Haemodynamic changes induced by submaximal isometric contraction in painful and non-painful human masseter using near-infra-red spectroscopy. *Arch Oral Biol* 41:585–596
 18. Satokawa C, Nishiyama A, Suzuki K et al (2020) Evaluation of tissue oxygen saturation of the masseter muscle during standardised teeth clenching. *J Oral Rehabil* 47:19–26

Effect of Resistance Training on Skeletal Muscle Mass and Brown Adipose Tissue Activity

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Abstract

Brown adipose tissue (BAT) may be effective in preventing obesity and type II diabetes; however, there are no established reports on exercise-induced changes in BAT. This study focused on BAT variability in women undergoing resistance training (RT) interventions twice weekly for 10 weeks in cold environments. Fifteen participants were recruited: seven were randomly assigned to an RT group and eight to a control (CT) group. To evaluate BAT density (BAT-d), total haemoglobin concentration was measured using near-infrared time-resolved spectroscopy before and after the intervention. There was no significant difference in BAT-d between the CT and RT groups after the intervention ($p = 0.921$). There was a positive correlation between Δ skeletal muscle (SM) mass and Δ BAT-d in the RT group ($r = 0.615$, $p = 0.142$). Supraclavicular region-specific thermogenesis (SST) had a significant positive correlation with BAT-d in the RT group ($r = 0.889$,

$p = 0.007$). These results suggest that RT may be involved in BAT variability, owing to the trend of increased BAT-d with increased Δ SM mass and SST, although there was no significant increase in BAT-d after the RT intervention.

Keywords

Brown adipose tissue · Exercise · Resistance training · Near-infrared spectroscopy · Thermogenesis

1 Introduction

Adipose tissue is divided into white adipose tissue (WAT) and brown adipose tissue (BAT). The primary function of WAT is energy storage, whereas that of BAT involves energy expenditure and dissipation. When the sympathetic nerve (SN) is stimulated, uncoupling protein 1 (UCP1) expression is induced and thermogenesis using free fatty acids occurs. The key role of BAT is adaptive heat production in cold environments (CE). Other effects include diet-induced and pathological heat production. Recently, the energy consumption mechanism of BAT was confirmed to reduce body weight, decrease plasma triglyceride and cholesterol levels, and improve insulin sensitivity and glucose tolerance. These functions suggest that BAT activation con-

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tributes to its preventive effects against obesity and type II diabetes [1]. Several factors that alter BAT activity have been identified, including CE. However, there are no established reports on its association with exercise.

Animal studies have demonstrated increased UCP1 expression and mitochondrial activity during exercise. In contrast, endurance athletes have been reported to have a lower BAT activity than sedentary individuals [2]. In human resistance training (RT), no significant increase in BAT activity was observed after the intervention [3]; this was a male-only study. Several factors may account for the lack of consistent reports regarding the relationship between exercise and BAT, including differences in exercise type, intervention duration, and sex. Previous studies have suggested that high-intensity exercise produces strong SN stimulation but also causes elevated body temperature [4].

Near-infrared time-resolved spectroscopy (TR-NIRS) can be used to measure total haemoglobin concentration (T-Hb) levels and assess tissue vascular density. Because capillaries are abundantly distributed in BAT, T-Hb can be used as an indicator of BAT [5].

This study focused on BAT variability after 10 weeks of twice-weekly RT in CE in women. We hypothesised that RT in a CE would enhance BAT activity.

2 Materials and Methods

2.1 Participants

This study included 15 healthy adult women (age, 22 years; standard deviation [SD], 2 years). Seven and eight participants were randomly assigned to RT and control (CT) groups, respectively. Body mass index was capped at 30 kg/m², and the participants were not usually in the habit of RT. An intervention experiment was conducted during the winter in Japan (December to March).

2.2 Intervention Protocols

All measurements were performed before and after the intervention. The whole-body skin temperature was measured for each intervention. Room temperature was set at 17 °C (SD: 2 °C).

In the RT group, the participants performed the following RT at 75% intensity twice a week for approximately 1 h for 10 weeks: leg extension, leg curl, chest press, latissimus pull-down, triceps extension, biceps curl, and crunches (all 10 × three sets). Resistance training machines (ATX-225; Tuff Stuff Fitness International, Chino, CA, USA) were used for all the RT sessions.

In the CT group, the participants visited five times during a 10-week period and rested for 1 h in the same environment as the RT group participants. They were instructed to sit in their chairs and move as little as possible.

2.3 Measurements of Skin Temperature and BAT Density (BAT-d)

Skin temperature was measured during each intervention in both groups (ThermoCron SL type; KN Laboratories, Inc., Osaka, Japan) [6]. Skin temperature was measured at seven sites throughout the body (Hardy Dubois Seven Skin Points), in addition to the supraclavicular region (SCR) and chest. We defined the difference between SCR and whole-body average skin temperatures as a measure of SCR-specific thermogenesis (SST).

Brown adipose tissue density was measured using TR-NIRS (Hamamatsu Photonics K.K., Hamamatsu, Japan), as previously described. In biological tissues, TR-NIRS can be used to evaluate T-Hb levels. In contrast to WAT, BAT has a higher capillary density and can be distinguished based on T-Hb levels. Previous studies have confirmed a strong correlation between BAT activity

and fluorodeoxyglucose (FDG)–positron emission tomography (PET), computed tomography (CT) (FDG–PET/CT), and TR-NIRS. Levels of T-Hb were corrected for subcutaneous fat thickness (Vscan Dual Probe; GE Vingmed Ultrasound AS, Horten, Norway) using the following formula: $(\text{Total-Hb-Adj}) = (\text{Total-Hb})/\text{EXP} [-1 \{ \text{SAT (mm)} \times 10/8.0 \}^2]$.

3 Results

There were no significant differences in any of the parameters between the two groups before the intervention. After the RT intervention, the interaction was not significantly different, except for leg extension strength ($p < 0.001$) (Table 1). However, $\Delta\text{BAT-d}$ did not differ significantly between the two groups, although there was a trend towards an increase in both groups. Individual differences were significant in both the groups (Fig. 1).

There was no significant correlation between $\Delta\text{BAT-d}$ and $\Delta\text{skeletal muscle (SM) mass}$ in the RT group ($r = 0.615$, $p = 0.142$). A significant negative correlation was observed in the CT group ($r = -0.785$, $p = 0.021$; Fig. 2).

There was a significant positive correlation between $\Delta\text{BAT-d}$ and SST in the RT group ($r = 0.889$, $p = 0.007$). However, no significant correlation was observed in the CT group ($r = 0.370$, $p = 0.367$; Fig. 3).

4 Discussion

There was no significant increase in BAT-d throughout the 10-week exercise intervention in the women with CE. A previous study in men with a similar RT intervention as the present study also found no significant differences between the two groups [3].

There are two possible explanations for the lack of increase in BAT-d after RT. At first, heat production from exercise suppresses BAT activity, even in CE of approximately 17 °C. Previous studies have reported that acute heat exposure

due to exercise suppresses norepinephrine-dependent non-shivering thermogenesis [7]. Second, the level of SN activity from exercise stimulation may be lower than that from CE. Resistance training stimulation may activate the SN system more strongly than CE stimulation; however, the duration of exposure to RT stimulation was shorter than that of CE. Time integral totals suggested that RT may have been less stimulating than CE [8]. These RT protocols were set up according to the guidelines of the American College of Sports Medicine, which train major muscle groups twice a week; however, more SN RT protocols need to be explored.

We observed a relationship between $\Delta\text{BAT-d}$ and $\Delta\text{SM mass}$. Our previous cross-sectional study revealed a significant negative correlation between BAT-d and SM mass in both men and women. The results for the CT group in the present study were similar to those of our previous cross-sectional study. Similar to BAT, the SM has a balanced non-shivering heat-producing function [9]. This may be one of the reasons for the negative correlation between increased SM mass and BAT activity. In contrast, in this study, the RT group showed a non-significant but positive correlation. Although the number of participants in this study was small, there might have been a significant relationship if the number of participants had been increased. It has been suggested that RT causes changes in SM mass, which may contribute to BAT variability.

A secondary finding was the relationship between $\Delta\text{BAT-d}$ and SST. There was a significant positive correlation between $\Delta\text{BAT-d}$ and SST in the RT group. In addition, SST is a measure of SCR-specific heat production. Previous studies have reported a positive correlation between BAT activity and SCR temperature [10], which is consistent with the results of the present study. This suggests that BAT activity increases heat production at RT. Further studies are required to elucidate the relationship between BAT activity and SST.

In conclusion, RT twice weekly for 10 weeks did not increase BAT-d in a CE. However, changes in SM mass and SCR-specific tempera-

Table 1 Participant characteristics

	Control group <i>n</i> = 8		Training group <i>n</i> = 7		Interaction		Main effects (group)		Main effects (intervention)	
	pre	post	pre	post	<i>p</i>	η^2_p	<i>P</i>	η^2_p	<i>p</i>	η^2_p
Age (years)	22(2)	22(2)	23(2)	23(2)	–	–	–	–	–	–
Height (m)	1.60(0.05)	1.61(0.05)	1.61(0.05)	1.61(0.05)	0.964	<0.001	0.917	<0.001	0.997	<0.001
Body mass index	19.0(2.4)	19.2(2.6)	20.3(1.5)	20.4(1.8)	0.955	<0.001	0.119	0.085	0.882	0.001
Body fat percentage (%)	27.3(5.3)	26.9(5.4)	27.3(3.7)	24.8(4.2)	0.561	0.013	0.538	0.014	0.410	0.024
Skeletal muscle mass (kg)	18.8(2.2)	19.1(2.2)	20.5(1.7)	21.4(1.7)	0.687	0.006	0.010*	0.215	0.454	0.020
Bone mineral mass (kg)	2.14(0.24)	2.15(0.23)	2.33(0.18)	2.35(0.20)	0.973	<0.001	0.019*	0.193	0.798	0.003
Waist circumference (cm)	68.4(5.0)	68.2(5.7)	71.3(4.1)	69.9(2.6)	0.711	0.005	0.163	0.068	0.658	0.007
Visceral fat area (cm ²)	22.6(14.0)	24.3(14.6)	29.1(13.2)	27.1(15.8)	0.734	0.005	0.363	0.030	0.990	<0.001
[Total-Hb] (μM)	63.7(12.4)	68.1(15.5)	72.9(18.2)	78.0(17.5)	0.957	<0.001	0.103	0.092	0.422	0.023

Values are presented as mean ± standard deviation. Two-way analysis of variance was used to assess pre- and post-intervention changes between the two groups. *BMI* body mass index, *[Total-Hb]* supraclavicular region haemoglobin concentration; **p* < 0.05

Fig. 1 Change in brown adipose tissue density (BAT-d) at pre- and postintervention in training and control groups. The circle and diamond plots indicate individual and mean values, respectively. * $p < 0.05$

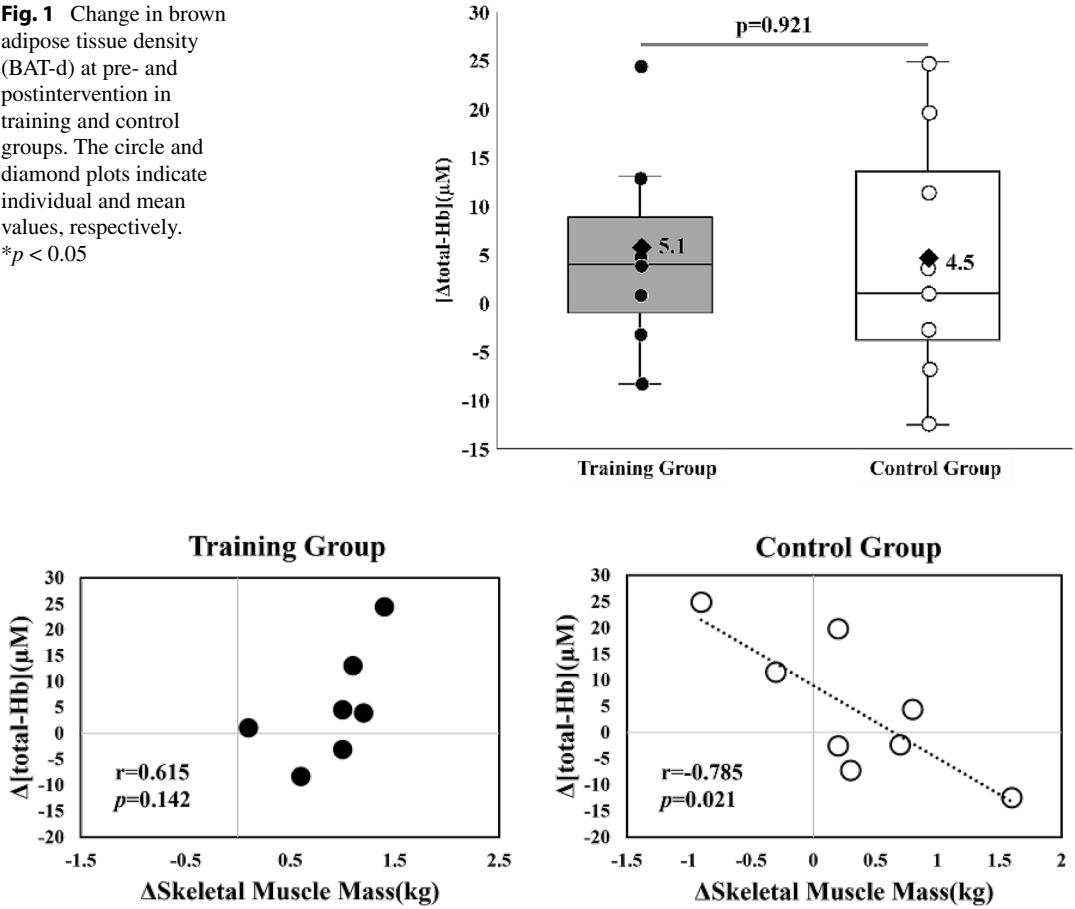


Fig. 2 Correlation between Δ [Total-Hb] (Δ BAT-d) and Δ skeletal muscle mass in training and control groups. Values are presented as mean $\pm$ standard deviation. * $p < 0.05$

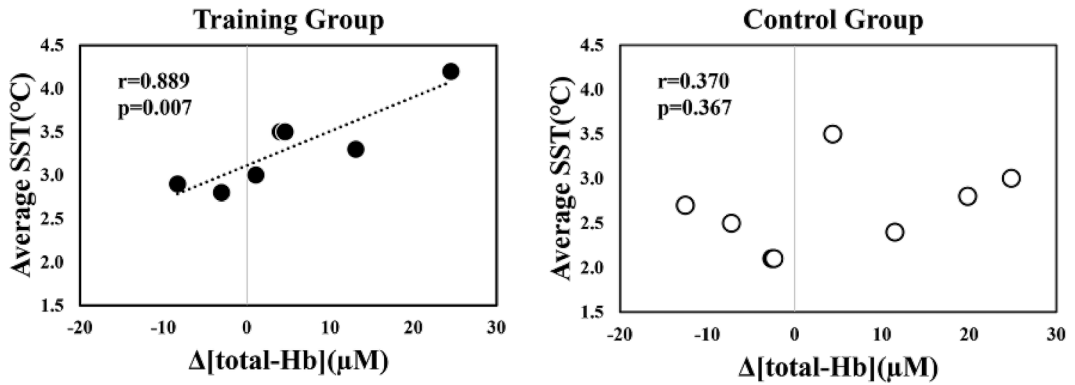


Fig. 3 Correlation between Δ [total-Hb] (Δ BAT-d) and average supraclavicular region-specific thermogenesis in training and control groups. Values are presented as mean $\pm$ standard deviation. * $p < 0.05$

ture increases during RT were found to be involved in BAT variability. The key to this study is the variability in the BAT measurements. Therefore, larger sample sizes are required in future studies.

References

1. Cheng L, Wang J, Dai H, Duan Y, An Y, Shi L, Lv Y, Li H, Wang C, Ma Q, Li Y, Li P, Du H, Zhao B (2021) Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus. *Adipocytes* 10(1):48–65
2. Mendez-Gutierrez A, Osuna-Prieto FJ, Aguilera CM, Ruiz JR, Sanchez-Delgado G (2020) Endocrine mechanisms connecting exercise to brown adipose tissue metabolism: a human perspective. *Curr Diab Rep* 20(9):40
3. Tanaka R, Fuse-Hamaoka S, Kuroiwa M, Kurosawa Y, Endo T, Kime R, Yoneshiro T, Hamaoka T (2022) The effects of 10-week strength training in the winter on brown-like adipose tissue vascular density. *Int J Environ Res Public Health* 19(16):10375
4. Ruivo JA, Alcántara P (2012) Hypertension and exercise. *Rev Port Cardiol* 31(2):151–158
5. Nirengi S, Yoneshiro T, Sugie H, Saito M, Hamaoka T (2015) Human brown adipose tissue assessed by simple, noninvasive near-Infrared time-resolved spectroscopy. *Obesity* 23(5):973–980
6. Hamaoka T, Nirengi S, Fuse S, Amagasa S, Kime R, Kuroiwa M, Endo T, Sakane N, Matsushita M, Saito M, Yoneshiro T, Kurosawa Y (2020) Near-infrared time-resolved spectroscopy for assessing brown adipose tissue density in humans: a review. *Front Endocrinol* 11:261
7. Kuroshima A, Habara Y, Ohno T (1983) Noradrenaline-induced secretions of pancreatic hormones in cold-and heat-acclimated rats. *Jpn J Physiol* 33(5):827–836
8. Kashimura O, Yanagidaira Y, Ueda G (1989) Effect of physical activity training on enhanced nonshivering thermogenesis in cold acclimated rats. *Ann Environ Sci Shinshu Univ* 11:79–84
9. Bal NC, Singh S, Reis FCG, Maurya SK, Pani S, Rowland LA, Periasamy M (2017) Both brown adipose tissue and skeletal muscle thermogenesis processes are activated during mild to severe cold adaptation in mice. *J Biol Chem* 292(40):16616–16625
10. Nirengi S, Wakabayashi H, Matsushita M, Domichi M, Suzuki S, Sukino S, Suganuma A, Kawaguchi Y, Hashimoto T, Saito M, Sakane N (2019) An optimal condition for the evaluation of human brown adipose tissue by infrared thermography. *PLoS One* 14(8):e0220574

Effects of Different Gum Hardness on Masseter Muscle Activity During Gum Chewing: An NIRS Oximetry Study

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Abstract

Gum chewing is used in orofacial therapy to improve oral functions, such as a patient's chewing ability, bite force, tongue pressure, and lip closure strength. However, its effects on masseter muscle oxygen dynamics and muscle activity may vary with the hardness and features of the gum base. Therefore, when considering gum chewing for therapeutic purposes, it is essential to select gum of an appropriate hardness. This study aimed to elucidate the impact of gum hardness differences on masseter muscle tissue oxygen dynamics and muscle activity. We conducted a 120-s gum chewing study using three types of gum with different hardness levels on 11 healthy adult males. Each patient's masseter muscle tissue oxygen dynamics, muscle activity, and heart rate were measured, and the extent of masseter fatigue was assessed using a visual analogue scale (VAS). Per our findings, as gum hard-

ness increased, significant reductions in oxygen saturation (StO₂) and significant increases in deoxyhaemoglobin (Deoxy-Hb) concentrations were observed in masseter muscle tissue oxygen dynamics. Likewise, muscle activity, heart rate, and muscle fatigue (according to VAS) also increased significantly as gum hardness increased. The findings of this study reveal that increasing gum base hardness not only affects masseter muscle tissue oxygen dynamics but also increases muscle activity, masseter fatigue, and heart rate. When selecting gum for orofacial therapy involving gum chewing exercises, it is crucial to choose the appropriate gum.

Keywords

SR-NIRS · Muscle oxygen dynamics · Mastication · Masseter muscle · Electromyography

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1 Introduction

Chewing is the rhythmic isometric contraction movement of the closing and opening muscles controlled by central pattern generators for motor commands. To ensure adequate nutrition, these functions need to be sustained and improved. In recent years, gum chewing has been applied as a therapeutic exercise to

enhance oral functions such as chewing ability, occlusal force, tongue pressure, and lip closure strength, spanning from young individuals to the elderly. Among oral organs, masseter muscles play a significant role in nutrition-related tasks, including food comminution, bolus formation, and swallowing.

Furthermore, aerobic metabolism is critical in the provision of energy to active muscle tissues, a process in which oxygen supply via blood circulation plays a significant role. Factors determining oxygen supply and consumption in muscle tissues include the heart rate, stroke volume, haemoglobin concentration in arterial blood, local muscle tissue vasodilation and vasoconstriction, and mitochondrial oxygen utilisation capacity [1, 2]. The balance between oxygen supply and oxygen consumption can be assessed through the real-time evaluations of muscle tissue oxygen dynamics, with near-infrared spectroscopy (NIRS) offering a non-invasive means of measuring these dynamics. Measurable parameters include changes in tissue oxygen saturation (StO_2), oxygenated haemoglobin (Oxy-Hb) concentration, deoxygenated haemoglobin (Deoxy-Hb) concentration, and total haemoglobin (Total-Hb) concentration [3, 4]. These values are useful in the evaluation of muscle activity [5]. In previous studies featuring the application of NIRS to masseter muscles, it has been revealed that isometric contractions influence the oxygen dynamics of these muscles [6, 7]. Consequently, the hardness of the gum used in exercise therapy might affect its effectiveness, particularly when targeting masseter muscles. Therefore, the choice of gum hardness becomes essential when exercise therapy is used for these purposes.

Hence, this study primarily aims to elucidate the impact of gum hardness on masseter muscles during gum chewing in the areas of muscle oxygen dynamics, muscle activity, fatigue, and heart rate (as an indicator of the patient's overall circulatory dynamics).

2 Methods

A total of 11 consenting adult male volunteers (mean age: 32.8 ± 5.41 years) were selected as participants. Our inclusion criteria were overall good health, the absence of self-reported or observable abnormalities in the oral cavity, temporomandibular joint, and masseter muscles, and no history of cardiovascular diseases. This study was approved by the Ethics Committee of Tokyo Dental University (approval number 928). The experimental block design of this study comprised the following three phases: 60 s of rest, 120 s of gum chewing (task), and 180 s of rest for recovery. Three different hardness levels of gum bases (soft, medium, and hard) measuring $11 \times 18 \times 5$ mm and weighing 1 g were used for gum chewing. Participants chewed two pieces of gum with their habitual chewing side, performing 80 chews per minute. The order of use of each gum hardness level was randomised, and measurements were taken three times for each hardness level. Measurements were performed using SR-NIRS (Oxy-Pro Hb141 Tissue oxygen saturation sensor, Astem, Kanagawa, Japan), electromyography (EMG) device (DPS Wireless EMG Sensor SS-EMGD-HMAG, SPORTS SENSING Co., LTD, Fukuoka, Japan), and a wearable heart rate sensor (myBeat WHS-1, UNION TOOL Co., Tokyo, Japan) to assess masseter muscle tissue oxygen dynamics, surface EMG waveforms, subjective fatigue levels, and heart rates during the task. The SR-NIRS sensor was placed on the lower part of the masseter muscles on the habitual chewing side and measured at a sampling frequency of 5.0 Hz per previous studies [8]. The allocation of measurements to the left and right sides of the tested muscle was randomised. Subcutaneous fat thickness, a potential source of error in muscle oxygen dynamics, was measured and corrected per the manufacturer's instructions [9]. The evaluation parameters for SR-NIRS included changes in StO_2 , Total-Hb, Oxy-Hb, and Deoxy-Hb during the task compared to the rest period. Masseter muscle activity was measured

using EMG, which was attached to the maximum bulging part of the masseter muscles on the same side as the SR-NIRS sensor, and recorded at a sampling frequency of 1000 Hz. Maximum voluntary contraction (MVC) was recorded before measurement, and the percentage of MVC (%MVC) was calculated from the average amplitude of the EMG waveform during the test. In addition, the heart rate was measured using a wearable heart rate sensor fixed to the participant's midsection with an accompanying belt, and the heart rate during the task was recorded at a sampling frequency of 1 Hz. The degree of subjective fatigue of the masseter muscle after the end of the gum-chewing exercise was determined using the visual analogue scale (VAS).

Statistical analyses were performed using the SPSS Statistics 25.0 software package (IBM, Chicago, IL, USA). A significance level of $p < 0.05$ was used for all statistical tests. For the parameters StO₂, Total-Hb, Oxy-Hb, and Deoxy-Hb, Friedman's test was conducted, followed by Scheffé's post hoc pairwise comparisons. For %MVC, HR (heart rate), and the VAS, the one-way analysis of variance was performed, and Bonferroni's multiple comparisons were performed to examine between-group differences.

3 Results

The measurement results by gum hardness level are as follows. For each category of SR-NIRS, significant differences were observed in the StO₂ and Deoxy-Hb categories. Specifically, in StO₂, HARD exhibited a significantly greater decrease compared to SOFT and MEDIUM, while in Deoxy-Hb, HARD showed significantly higher values compared to SOFT and MEDIUM ($p < 0.01$, $p < 0.05$). No significant differences were observed among gum hardness levels in the other categories (Total-Hb, Oxy-Hb) (Table 1). The %MVC and VAS differed significantly among all groups, with HARD having significantly higher values than MEDIUM, and MEDIUM showing significantly higher values than SOFT. Similar results were also obtained for

the VAS ($p < 0.01$). In HR, HARD exhibited significantly higher values than MEDIUM and SOFT ($p < 0.01$, $p < 0.05$) (Table 2).

4 Conclusions and Discussion

In this study, we observed that an increase in gum hardness during gum chewing led not only to a significant increase in masseter muscle activity and self-reported muscle fatigue but also a significant reduction in StO₂ and an increase in Deoxy-Hb as measured by SR-NIRS. This indicates that muscle metabolism increases with gum hardness during short-term gum chewing, suggesting that the hardness of the gum used affects muscle metabolism and not just muscle activity. Masseter muscles, which contain a significant number of type I fibres, frequently rely on mitochondrial oxygen utilisation for muscle metabolism. Therefore, haematogenous oxygen supply plays a crucial role in muscle activity. In the case of sustained muscle activity (such as clenching), the balance between vasodilation [10] and intramuscular pressure [11, 12] is one of the determining factors for local muscle blood flow. However, in intermittent muscle activity (such as the gum chewing performed in this study), it appears that the balance between vasodilation and intramuscular pressure did not culminate in significant differences in Total-Hb between different gum hardness levels. Regarding changes in heart rate, Bakke et al. [13] reported that sustained isometric contractions at $\geq 15\%$ of MVC in skeletal muscles increase this parameter. The increase is more pronounced when the mass of the involved muscle is larger. In this study, even though the muscle activity was limited to the jaw and facial area, chewing HARD gum resulted in activity rates close to 50% of MVC, culminating in a significant increase in heart rate. This increase in heart rate suggests that the required supply of oxygen and energy necessary for chewing hard gum might have been made available.

Considering the above points, when individuals who require gum-chewing exercises or those habitually chewing gum choose their gum,

Table 1 Results of statistical analysis for masseter muscle oxygen dynamics

		Friedman Test	A	B	C	Scheffe pairwise comparison tests
			Soft	Medium	Hard	
SR-NIRS	StO ₂ [%]	Chi-square =9.385 df = [2.0] p = 0.01	−8.302 (6.248)	−8.710 (5.950)	−10.734 (6.738)	A vs B: n.s. A vs C: <i>p</i> < 0.05 B vs C: <i>p</i> < 0.05
	Total-Hb [mM]	Chi-square =6.001 df = [2.0] p = 0.04	0.00965 (0.00729)	0.00997 (0.0084)	0.0146 (0.0117)	A vs B: n.s. A vs C: n.s. B vs C: n.s.
	Oxy-Hb [mM]	Chi-square =1.077 df = [2.0] p = 0.58	−0.000182 (0.00753)	−0.0005 (0.00924)	0.00071 (0.0129)	A vs B: n.s. A vs C: n.s. B vs C: n.s.
	Deoxy-Hb [mM]	Chi-square =14.923 df = [2.0] p = 0.00	0.00984 (0.00765)	0.0108 (0.00732)	0.0141 (0.00806)	A vs B: n.s. A vs C: <i>p</i> < 0.01 B vs C: <i>p</i> < 0.05
					Mean (S.D.)	n.s.: not significant

Table 2 Results of statistical analysis for %MVC, heart rate, and VAS

	One-way ANOVA	A	B	C	Bonferroni multiple comparison tests
		Soft	Medium	Hard	
%MVC [%]	<i>F</i> = 96.59 df = [2.0] <i>p</i> = 0.00	33.543 (12.714)	45.511 (13.214)	63.535 (13.690)	A vs B: <i>p</i> < 0.01 A vs C: <i>p</i> < 0.01 B vs C: <i>p</i> < 0.01
Heart rate [bpm]	<i>F</i> = 7.34 df = [2.0] <i>p</i> = 0.00	72.147 (5.553)	72.569 (6.546)	74.974 (5.880)	A vs B: n.s. A vs C: <i>p</i> < 0.01 B vs C: <i>p</i> < 0.05
VAS	<i>F</i> = 45.03 df = [2.0] <i>p</i> = 0.00	1.287 (1.291)	2.819 (1.955)	4.958 (2.469)	A vs B: <i>p</i> < 0.01 A vs C: <i>p</i> < 0.01 B vs C: <i>p</i> < 0.01
				Mean (S.D.)	n.s.: not significant

it is crucial to consider the purpose of use, minimise potential side effects, and select the appropriate gum hardness to maximise the benefits of gum chewing. In addition, since the study had only male participants, considering that women are believed to have higher muscle endurance compared to men, future research should include sex-based comparisons. Furthermore, there are plans to investigate the relationship between masseter muscle oxygen dynamics, changes in muscle activity, training intensity, training duration, and materials used in future studies.

References

1. Levine BD (2008) VO₂ max: what do we know, and what do we still need to know? *J Physiol* 586:25–34
2. Saltin B, Strange S (1992) Maximal oxygen uptake: “old” and “new” arguments for a cardiovascular limitation. *Med Sci Sports Exerc* 24:30–37
3. Barstow TJ (1985) (2019) understanding near infrared spectroscopy and its application to skeletal muscle research. *J Appl Physiol* 126:1360–1376
4. Jöbsis FF (1977) Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 198:1264–1267
5. Perrey S, Ferrari M (2018) Muscle oximetry in sports science: a systematic review. *Sports Med* 48:597–616

6. Delcanho RE, Kim YJ, Clark GT (1996) Haemodynamic changes induced by submaximal isometric contraction in painful and non-painful human masseter using near-infra-red spectroscopy. *Arch Oral Biol* 41:585–596
7. Kim YJ, Kuboki T, Tsukiyama Y et al (1999) Haemodynamic changes in human masseter and temporalis muscles induced by different levels of isometric contraction. *Arch Oral Biol* 44:641–650
8. Tsutsui A, Nakajima K, Sakaue T et al (2022) Jaw-clenching intensity effects on masseter oxygen dynamics and fatigue: a NIRS oximetry study. *Adv Exp Med Biol* 1395:435–441
9. Kek KJ, Kibe R, Niwayama M et al (2008) Optical imaging instrument for muscle oxygenation based on spatially resolved spectroscopy. *Opt Express* 16:18173–18187
10. Heinonen I (2014) Muscle-specific functional sympatholysis in humans. *Exp Physiol* 99:344–345
11. Hamaoka T, McCully KK, Quaresima V et al (2007) Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *J Biomed Opt* 12:062105
12. Moller E (1979) Mechanism of ischemic pain in human muscles of mastication : intramuscular pressure, EMG, force and blood flow of the temporal and masseter muscles during biting. *Adv Pain Res Ther* 3:271–281
13. Bakke M, Thomsen CE, Vilmann A et al (1996) Ultrasonographic assessment of the swelling of the human masseter muscle after static and dynamic activity. *Arch Oral Biol* 41:133–140

Muscle Oxygen Dynamics Measured by NIRS

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Abstract

Near-infrared spectroscopy (NIRS) has been used to measure skeletal muscle oxidative function for more than 30 years. Several indicators evaluate muscle oxidative function using NIRS during exercise, such as deoxygenation rate at the start of exercise (Deoxy-rate), changes in deoxygenation during exercise (Δ Deoxy), and reoxygenation speed after exercise ($T_{1/2}$ reoxy, reoxy rate). Previous studies have reported that these muscle NIRS indicators are significantly correlated with muscle fibre type, phosphocreatine recovery rate, and peak oxygen uptake. In addition, muscle NIRS indicators have been applied to

the study of a number of chronic health conditions, including patients with ischaemic heart failure. Recently, wearable NIRS devices monitor muscle function continuously and freely in the field, and we predict that NIRS devices will be widely applied to our lifestyles more than ever before. However, there are some critical problems with measuring muscle oxidative function using NIRS devices. We have previously reported that subcutaneous adipose tissue thickness (SATT) greatly influences the light pathlength and makes it difficult to quantify tissue deoxygenation, especially in the measurements of muscle deoxygenation from the skin surface. The effects of SATT need to, therefore, be corrected when using NIRS devices, especially when comparing differences in sex, age, and trainability, as the subjects' SATT could differ significantly. In addition, we have more recently reported that assuming constant mean pathlength (MPL) in NIRS leads to an inaccurate interpretation of muscle deoxygenation, since there are greater changes in MPL during incremental cycling exercise, especially at shorter wavelengths in the NIRS region. In this mini-review, we will summarise the indicators of muscle oxidative function using NIRS and the challenges of using an NIRS apparatus, especially during exercise.

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Keywords

Muscle oxidative function · Muscle NIRS indicator · Wearable NIRS device · Subcutaneous adipose tissue thickness (SATT) · Reoxygenation speed

1 Why We Measure Muscle Oxygen Function During Exercise

Maximal oxygen uptake ($\text{VO}_{2\text{max}}$) is the most important factor for reducing death rate [1]. Oxygen uptake (VO_2) is determined by multiplying QO_2 by arterial O_2 content (CaO_2)–venous O_2 content (CvO_2) (a–v O_2 difference). QO_2 is defined as circulatory function, CaO_2 is defined as respiratory function, and a–v O_2 difference is nearly defined as muscle function. As these parameters are all very significant for VO_2 , which is the most important limiting factor of VO_2 ?

Esposito et al. [2] have reported that there are strongly positive correlations between muscle diffusive O_2 conductance and leg O_2 consumption during knee extension and cycling exercise both in healthy and chronic heart failure (CHF) patients before and after training. These results indicate that leg O_2 consumption is limited by O_2 diffusing capacity in skeletal muscle during local and whole-body exercise both in healthy and CHF patients. In other words, muscle O_2 diffusive function is the most important parameter for limiting muscle O_2 consumption during exercise. As near-infrared spectroscopy (NIRS) can monitor dynamic muscle O_2 balance between muscle O_2 consumption and muscle O_2 supply, muscle O_2 diffusive function can be evaluated by NIRS with cuff occlusion. In addition, there have been many studies evaluating muscle O_2 function using NIRS during and after exercise without cuff occlusion. At the beginning of exercise, muscle tissue O_2 saturation (StO_2) declines by an increase of muscle O_2 consumption compared with muscle O_2 supply. At the recovery phase, StO_2 goes up by an increase of muscle O_2 supply compared with muscle O_2 consumption. This

paper will summarise some our data evaluating muscle oxygenation recovery after exercise and muscle deoxygenation during exercise in relation to muscle oxidative capacity.

2 How to Evaluate Muscle Oxidative Function Using NIRS

2.1 Faster Reoxygenation Rate After Exercise in Higher Oxidative Capacity Muscle

Recovery time of muscle oxygenation following the completion of exercise is used as an index of deficits of O_2 delivery to the muscle in relation to O_2 demand of the working muscle. We have measured $T_{1/2}$ reoxy, which is the time required to reach the value halfway between the Hb/MbO_2 level immediately after exercise and that at peak hyperaemia during recovery. Ichimura et al. [3] reported that $T_{1/2}$ reoxy was prolonged with aging, regardless of habitual physical activity levels. However, habitual physical activity may prevent the age-related prolongation in $T_{1/2}$ reoxy after cycling exercise. In addition, peak O_2 uptake (peak VO_2) showed a significant inverse correlation with $T_{1/2}$ reoxy in both sedentary and active groups. This study suggests that peak VO_2 was related to reoxygenation speed after whole body exercise, and faster reoxygenation was found in higher peak VO_2 .

2.2 Slower Reoxygenation Rate After Exercise in Higher Oxidative Capacity Muscle

As shown previously, reoxygenation speed is defined as the dynamic O_2 balance between muscle O_2 consumption and muscle O_2 supply. When the O_2 supply is limited, such as in short-term isometric exercise, the reoxygenation speed may be more influenced by muscle O_2 consumption. Figure 1 represents the reoxygenation kinetics in the highest oxidative capacity muscle measured by ^{31}P magnetic resonance

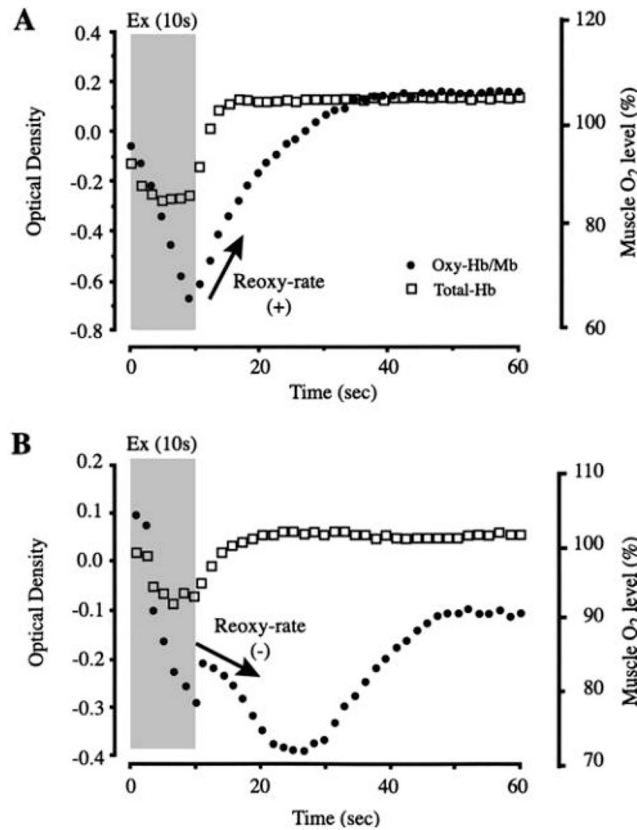


Fig. 1 Changes in Oxy-Hb/Mb and Hbtot during and after maximal isometric handgrip exercise in normal (a) and the highest oxidative capacity muscle determined by phosphocreatine recovery (b). In the highest oxidative

capacity muscle, although total-Hb rose rapidly back to resting level, Oxy-Hb/Mb continued to deoxygenate until 15 s after exercise (Ref. [4])

spectroscopy. After exercise, total-Hb rose back to resting level quickly. However, Oxy-Hb continued to decrease (still deoxygenated) during the recovery period. We found that the reoxygenation rate after 10 s maximal isometric exercise had a significantly positive correlation with phosphocreatine time constant [4]. These results suggest that slower reoxygenation was found in higher oxidative capacity muscle. Surprisingly, the results are the opposite of our previous reoxygenation data. Reoxygenation speed after exercise is strongly affected by the type of exercise, and therefore, researchers have to think carefully about the exercise protocol when evaluating reoxygenation speed as a muscle oxygen function.

2.3 Muscle Deoxygenation Changes During Incremental Exercise

Muscle deoxygenation gradually increased during incremental cycling exercise, and the muscle deoxygenation changes were correlated with peak VO₂. These results suggest that oxygen availability may be enhanced in higher oxidative capacity muscles. In addition, our previous studies have reported that improvement of peak VO₂ by endurance training was negatively related to changes in muscle deoxygenation [5, 6], suggesting that muscle O₂ extraction may be improved in the activating muscle due to endurance training.

Heart disease patients have been characterised by reports of reduced peak aerobic capacity. However, skeletal muscle deoxygenation responses during exercise and their relation to peak aerobic capacity have not been fully established in early post-myocardial infarction (MI) patients. We found that StO_2 in activating muscle was not significantly decreased during incremental cycling exercise in MI patients. In addition, relative change in Deoxy-Hb/Mb concentration from rest to peak exercise was significantly lower in MI than that in controls [7]. These findings indicate that muscle O_2 extraction during incremental exercise was lower in MI patients than that in controls. After aerobic training, muscle deoxygenation was enhanced, and the enhancement was related to increased aerobic capacity in MI patients [8].

2.4 Muscle Oxygenation Changes During Constant Work Rate

Our previous studies have reported that during constant work cycling exercise, both oxygenated Hb (Oxy-Hb) and total Hb (tHb) represented progressive increases until the end of exercise after an initial decrease at the start of exercise [9]. Recently, Endo et al. [10] reported the increased Oxy-Hb using near-infrared time-resolved spectroscopy (NIR_{TRS}) during constant work cycling exercise. We found that increased Oxy-Hb was significantly higher in the endurance training group than controls, and increased Oxy-Hb was significantly positive correlated with peak VO_2 (Fig. 2). These data suggest that higher oxidative capacity muscle represented greater oxygenation during constant work cycling exercise. In summary, muscle O_2 dynamics during exercise are quite different between incremental and constant work cycling exercise.

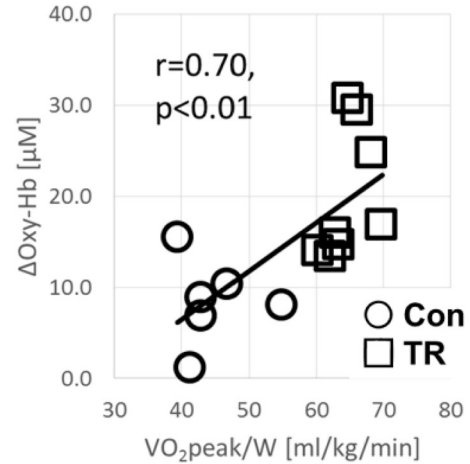


Fig. 2 Relationship between change in muscle oxygenated haemoglobin ($\Delta \text{Oxy-Hb}$) during constant work cycling exercise and peak O_2 uptake. Increased Oxy-Hb was significantly positively correlated with peak O_2 uptake. Open circles indicate control group and open squares indicate endurance training group (submitted for publication)

applied to our lives in the future. However, some critical issues can occur when measuring muscle oxidative function using NIRS devices. Niwayama et al. [11] have previously reported that subcutaneous adipose tissue thickness (SATT) greatly influences the light pathlength and makes it difficult to quantify tissue deoxygenation, especially in the measurements of muscle deoxygenation from the skin surface. The effects of SATT need to, therefore, be corrected when employing NIRS devices, especially when comparing differences in sex, age, and trainability, as the subjects' SATT could vary significantly. In addition, motion artifacts associated with muscle contractions are a major issue in measuring muscle oxygen dynamics using NIRS, since the NIRS apparatus can obtain high sampling rates. Either averaging or smoothing would be needed to evaluate muscle oxygen dynamics using an NIRS apparatus, especially during sprint movements.

In addition, we have recently reported that assuming constant mean pathlength (MPL) in NIRS leads to an inaccurate interpretation of muscle deoxygenation, since there are greater changes in MPL during incremental cycling exercise, especially at shorter wavelengths in

3 Problems of Evaluating Muscle Function Using NIRS

Recently, wearable NIRS devices regularly monitor muscle function in the field, and we predict that NIRS devices will be more extensively

the NIRS region [12]. Moreover, assuming constant PL in NIRS based on the modified Beer–Lambert law significantly underestimated actual muscle oxy/deoxygenation as compared with measurements obtained by real-time PL determination [13].

4 Summary

Muscle O₂ function is one of the most important factors for health promotion, since muscle O₂ diffusive capacity is strongly related to leg O₂ consumption. NIRS has great potential to evaluate muscle O₂ function. As a practical matter, NIRS has some problems, such as wide differences in the results by exercise protocol, the great influence of subcutaneous fat layer thickness, and reduced MPL during incremental exercise. Researchers have to understand how to evaluate muscle oxygen function using the apparatus, what it can tell us, and what the limitations of NIRS are.

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References

1. Myers J, Prakash M, Froelicher V et al (2002) Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* 346(11):793–801
2. Esposito F, Reese V, Shabetai R et al (2011) Isolated quadriceps training increases maximal exercise capacity in chronic heart failure. *J Am Coll Cardiol* 20:1353–1362
3. Ichimura S, Murase N, Osada T et al (2006) Age and activity status affect muscle reoxygenation time after maximal cycling exercise. *Med Sci Sports Exerc* 38(7):1277–1281
4. Kime R, Hamaoka T, Sako T et al (2003) Delayed reoxygenation after maximal isometric handgrip exercise in high oxidative capacity muscle. *Eur J Appl Physiol* 89:34–41
5. Kime R, Niwayama M, Fujioka M et al (2010) Unchanged muscle deoxygenation heterogeneity during bicycle exercise after 6 weeks of endurance training. *Adv Exp Med Biol* 662:353–358
6. Takagi S, Kime R, Murase N et al (2016) Effects of low volume aerobic training on muscle desaturation during exercise in elderly subjects. *Adv Exp Med Biol* 876:63–69
7. Takagi S, Murase N, Kime R et al (2014) Skeletal muscle deoxygenation abnormalities in early post-myocardial infarction. *Med Sci Sports Exerc* 46(11):2062–2069
8. Takagi S, Murase N, Kime R et al (2016) Aerobic training enhances muscle deoxygenation in early post-myocardial infarction. *Eur J Appl Physiol* 116(4):673–685
9. Kime R, Im J, Moser D et al (2009) Noninvasive determination of exercise-induced vasodilation during bicycle exercise using near infrared spectroscopy. *Med Sci Monit* 15(3):CR89–94
10. Endo T, Kime R, Fuse S et al (2018) Evaluation of functional hyperemia using NIR_{TRS} without the influence of fat layer thickness. *Adv Exp Med Biol* 1072:97–101
11. Niwayama M, Suzuki H, Yamashita T et al (2012) Error factors in oxygenation measurement using continuous wave and spatially resolved near-infrared spectroscopy. *J Jpn Coll Angiol* 52:211–215
12. Endo T, Kime R, Watanabe T et al (2020) Reduced optical path length in the vastus lateralis during ramp cycling exercise. *Adv Exp Med Biol* 1232:239–244
13. Endo T, Kime R, Fuse S et al (2021) Changes in optical path length reveal significant potential errors of muscle oxygenation evaluation during exercise in humans. *Med Sci Sports Exerc* 53(4):853–859

Near-Infrared Spectroscopy Analysis of Triceps Brachii Muscle Oxygenation During Sprinting in Wheelchair Basketball Players: A Pilot Study

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Abstract

The purpose of this study was to clarify the characteristics of muscle fatigue by measuring the oxygenation of muscles during wheelchair sprinting in wheelchair basketball players, using near-infrared spectroscopy (NIRS). We believe that this information will be helpful in planning rehabilitation and training programmes.

Five male wheelchair basketball players (mean age 30.0 ± 12.0 years, mean height 166.0 ± 12.1 cm, and mean weight 61.0 ± 15.8 kg) participated in this study.

These participants performed six 20 m sprint tests after warming up. NIRS measurements were obtained at rest before the start of exercise, during sprinting movements, and during the recovery time after sprinting. Measurements were taken using a wireless NIRS device (PortaMon, Artinis, The

Netherlands; sampling rate, 10 Hz) with a single-channel system for tissue oxygenation measurements. The NIRS sensor was applied to the triceps muscle on the participant's dominant arm and fixed with an upper arm supporter. The measurement data were imported into a dedicated computer and the tissue saturation index (TSI) was calculated. Statistical analysis was performed using the Friedman test to compare the TSI before sprinting and at each sprint, and a post hoc Bonferroni test was conducted.

Compared to the pre-sprint measurements, TSI significantly decreased during sprints second, fourth, and fifth.

In the present study, changes in the oxygen status of the triceps muscle during wheelchair driving varied greatly among individuals, and a tendency was observed towards the oxygen saturation being lowest by the fourth sprint, with the decrease being suppressed from this point on.

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Keywords

Wheelchair sprinting · Muscle fatigue ·
Near-infrared spectroscopy (NIRS) · Tissue
saturation index (TSI) · Rehabilitation and
training

1 Introduction

Wheelchair users, including those with spinal cord injuries, need to maximise the use of their remaining trunk and upper limb functions in their daily lives. Therefore, rehabilitation aimed at improving upper limb function is important. For people with disabilities, sports participation is important for improving their quality of life, physical function, and movement abilities. However, there is also a risk of developing or worsening disabilities through sports participation. Upper extremity disorders, mainly of the shoulder, elbow, and wrist joints, occur frequently [1]. In addition, wheelchair athletes need intense strength and skill training for better performance and injury prevention, such as for wheelchair driving and basketball shooting. The triceps brachii and radial carpal flexor muscles play a major role in generating propulsive force during wheelchair driving, and wheelchair sports place a significant load on these muscles [2]. Upper extremity muscle fatigue has been identified as a cause of poor performance and injury in wheelchair basketball players; however, limited research has been conducted on triceps brachii muscle fatigue in such individuals. Therefore, in this study, we aimed to clarify muscle loading by analysing the muscle oxygenation state of the triceps muscle in wheelchair athletes during wheelchair driving. We further aimed to use this as an indicator for setting the load for muscle strengthening training and for preventing injury related to overuse.

Recently, the validity of evaluating muscle fatigue by oxygenated haemoglobin levels using near-infrared spectroscopy (NIRS) has been reported in sports science [3, 4]. Several studies have reported on the role of NIRS in the evaluation of oxidative skeletal muscle performance in sport activities and athletes. Muscle NIRS has been increasingly favoured for monitoring alterations in local muscle metabolism and muscle oxygenation throughout and after physical activity. Muscle oximetry has also been efficacious and dependable in various settings, including research laboratories and practical sports applications. Specific parameters, such as muscle oxygenation levels, deoxygenation rates, and

reoxygenation rates, have been employed to evaluate muscle oxidative capacity in the context of targeted training interventions; however, no prior studies have evaluated wheelchair athletes. The purpose of this study was to clarify the characteristics of muscle fatigue by measuring the oxygenation of muscles during wheelchair sprinting in wheelchair basketball players using NIRS. This will provide useful information for the planning of rehabilitation and training programmes.

2 Methods

2.1 Participants

Five male wheelchair basketball players (mean age 30.0 ± 12.0 years, mean height 166.0 ± 12.1 cm, and mean weight 61.0 ± 15.8 kg) participated in the study. Two patients had spinal cord injuries, one had total hip arthroplasty due to Perthes disease, one had cerebral palsy, and one had scapular–peroneal muscular atrophy. Patients who developed upper limb disability within 3 months were excluded. This study was approved by the Ethics Committee of Fukushima Medical University.

2.2 Experimental Procedures

The participants warmed up adequately for 10 min, after which a 20 m $\times$ 6 sprint test was performed. The participants moved in a straight line for 20 m from the starting point with maximum effort. After sprinting 20 m, the participants rested for 15 s and then sprinted again for 20 m. After six sprints of 20 m, the participants had a 5-min recovery period. The sprint times were measured using a phototube sensor (Dasher Blue, Dasher, USA). NIRS measurements were obtained at rest before sprinting, during sprinting, and during recovery time after sprinting. Participant heart rate (HR) was measured with an HR sensor (Polar H10, Polar, Finland). Muscle and fat masses of the extremities and trunk were measured with a body composition analyser (Inbody S10, Inbody, Korea) using the impedance method.

2.3 NIRS Measurements

NIRS measurements were taken using a wireless NIRS device (PortaMon, Artinis, The Netherlands; sampling rate, 10 Hz) with a single-channel system for tissue oxygenation measurements. The NIRS sensor was applied to the triceps muscle on the participant's dominant arm and fixed with an upper arm supporter. For the rest measurement, the participant was placed in a sitting position on a wheelchair after warming up, and the muscle oxygenation state was measured for 1 min. During sprinting, muscle oxygenation was measured from the start to the finish line. After completing six sets of 20 m, the participants sat in a resting position on a wheelchair, and their muscle oxygenation was measured during the 5-min recovery time. The measurement data were imported into a dedicated computer and the tissue saturation index (TSI) were calculated. The participants stopped taking steroid medications, smoking, alcohol, coffee, and other caffeine-containing substances 12 h prior to the measurement.

2.4 Statistical Analysis

Statistical analysis was performed using the Friedman test to compare TSI before sprinting and at each sprint, and a post hoc Bonferroni test was conducted. The null hypothesis of the Friedman test was that the distribution of TSI at the pre-sprint, sprints 1–6, and post-sprint measurements would be the same. The null hypothesis of the Bonferroni test was that the distributions of the two samples were the same.

In addition, the difference between the maximum and minimum TSI within each sprint time was defined as Δ TSI. To compare the Δ TSI between each sprint, the Friedman test was performed, followed by a Bonferroni test as a post-hoc analysis. The null hypothesis for the Friedman test was that the distribution of Δ TSI% among the pre-sprint, sprints 1–6, and post-sprint measurements is the same. The null hypothesis of the Bonferroni test was that the distributions of the two samples to be compared were the same.

SPSS 28 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and the statistical significance level of this study was 5%.

3 Results

The body composition and TSI of the study participants are presented in Table 1. Compared to the pre-sprint measurements, the TSI significantly decreased during sprints 2, 4, and 5 (Table 1). Figure 1 shows the changes in TSI for each participant over time. Figure 2 shows deoxygenation and re-oxygenation during sprinting and rest periods. Since the null hypothesis could not be rejected by the results of the Friedman test, the Δ TSI% in each sprint was determined to be not significantly different.

4 Discussion

NIRS has been shown to be a valuable method for assessing muscle oxygenation. In the field of sports science, research on muscle oxygenation has predominantly focused on the quadriceps femoris muscle [5, 6], with few studies targeting the upper extremities [7]. Moreover, no existing studies have specifically targeted wheelchair propulsion movements. To the best of our knowledge, this is the first study to investigate athletes with disabilities.

In this study, the TSI was used as an indicator of the oxygenation status of the triceps brachii muscle during wheelchair sprints. The TSI significantly decreased during the second, fourth, and fifth sprints, compared with the measurement taken before sprinting. Previous research with healthy participants has reported decreased TSI in the quadriceps femoris during repeated sprinting [5] and in the forearm flexor muscles during grip exercises [7]. However, as shown in Table 1 and Fig. 1, the changes in TSI were highly individual. Participant E had the lowest TSI at S1, and Participants B and C had the lowest TSI at S2; the timing of the decrease in TSI may differ depending on the wheelchair drive method, muscle activity style, athletic experience, and disability.

Table 1 Body composition and tissue saturation index (TSI) of each participant during sprinting

Participant	BMI	Muscle mass of right upper limb (kg)	TSI									
			Pre	S1	S2	S3	S4	S5	S6	post		
A	23.4	3.2	66.3	52.1	36.4	29.5	24.3	28.6	34.5	66.4		
B	16.6	1.7	73.8	68.8	62.1	65.9	65.3	65.2	62.5	75.8		
C	20.5	2.9	72.6	56.0	49.1	49.5	51.0	56.9	56.5	74.4		
D	26.3	3.5	75.5	62.1	53.9	49.2	50.4	46.6	45.6	74.8		
E	22.9	5.2	67.2	51.6	55.8	58.7	52.2	53.3	57.4	71.6		
Mean ± SD	21.9 ± 3.2	3.3 ± 1.1	71.1 ± 3.7	58.1 ± 6.5	51.4 ± 8.6	50.6 ± 12.2	48.6 ± 13.4	50.1 ± 12.3	51.3 ± 10.0	72.6 ± 3.4		
p-value				0.575	0.037	0.091	0.020	0.037	0.051	1.000		

Compared to the pre-sprint measurement, the TSI significantly decreased during sprints 2, 4, and 5. BMI Body mass index, TSI Tissue saturation index, pre pre-sprinting, SI-6 sprints 1-6, post post sprint for all (recovery)

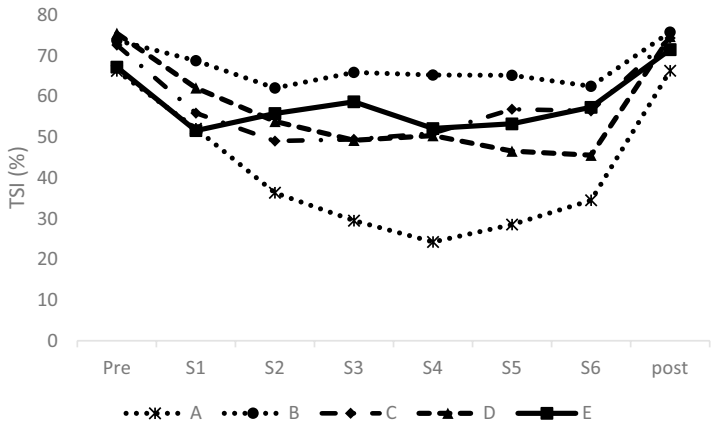


Fig. 1 Tissue saturation index (TSI) during sprinting in each participant. The TSI data are presented for the pre-sprint, sprints 1–6, and post-sprint (after all sprints)

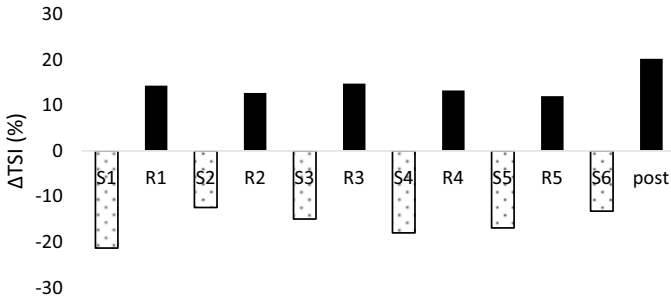


Fig. 2 De-oxygenation and re-oxygenation during sprinting and rest periods. The maximum TSI minus the minimum TSI for each sprint, with the periods between sprints defined as Δ TSI. The dotted bars represent deoxygenation, and the black bars represent reoxygenation. No significant differences were observed in the comparison of Δ TSI% at each sprint. S1–6: sprints 1–6, R1–6: rest 1–6 after sprints, post: post-sprint for all (recovery)

The results of this study suggest that, in the present sample, the change in the oxygen status of the triceps muscle during wheelchair drive varied greatly among individuals, and a tendency was observed for the oxygen saturation to be lowest by the fourth sprint, and the decrease was suppressed from there.

We hypothesised that the fatigue state of the triceps brachii muscle during wheelchair sprinting could be evaluated from the change in TSI using NIRS. When assessing muscle oxygenation status using NIRS, a decrease in TSI is considered a decrease in oxygenation. The results of

the present study suggested a tendency for TSI to decrease due to oxygen consumption with respect to triceps fatigue during wheelchair-driven sprinting, although there were large interindividual differences. In future work, it is necessary to measure oxygenated haemoglobin and deoxygenated haemoglobin as well as to examine changes in exercise physiology by changing the mode of exercise. In addition, the number of participants needs to be increased. This may be a useful indicator for setting training loads, preventing disabilities, and improving the quality of life of individuals in wheelchairs.

References

1. Fairbairn JR, Huxel Bliven KC (2019) Incidence of shoulder injury in elite wheelchair athletes differ between sports: a critically appraised topic. *J Sport Rehabil* 28:294–298
2. Mulroy SJ, Gronley JK, Newsam CJ et al (1996) Electromyographic activity of shoulder muscles during wheelchair propulsion by paraplegic persons. *Arch Phys Med Rehabil* 77:187–193
3. Perrey S, Ferrari M (2018) Muscle oximetry in sports science: a systematic review. *Sports Med* 48:597–616
4. Bhambhani Y (2012) Application of near infrared spectroscopy in evaluating cerebral and muscle haemodynamics during exercise and sport. *J Near Infrared Spectrosc* 20:117–139
5. Kerhervé HA, Samozino P, Descombe F et al (2017) Calf compression sleeves change biomechanics but not performance and physiological responses in trail running. *Front Physiol* 8:247
6. Hopker JG, O'Grady C, Pageaux B (2017) Prolonged constant load cycling exercise is associated with reduced gross efficiency and increased muscle oxygen uptake. *Scand J Med Sci Sports* 27:408–417
7. Fryer SM, Stoner L, Dickson TG et al (2015) Oxygen recovery kinetics in the forearm flexors of multiple ability groups of rock climbers. *J Strength Cond Res* 29(6):1633–1639

Pupil Dynamics, Salivary Alpha-Amylase, and Prefrontal Haemodynamics in Response to Very Light Exercise

Lei Ma, Jiajun Jiang, Diao Xuepeng, Muxing Li, Shinuo Li, and Wenwen Yang

Abstract

This study aimed to investigate the effects of exercise on pupil dynamics, prefrontal haemodynamic, and salivary alpha-amylase (sAA) responses in 45 healthy graduate students. These participants were divided into two groups: a resting control group (CTL) and a very-light-intensity exercise group (EX). Participants in the EX-group engaged in a 10-min exercise at 30% O_2 peak on a cycle ergometer. Salivary samples and pupillometry assessments were collected before the exercise, at the end of the exercise, and 5 min after the completion to evaluate changes over time. Our analysis showed that exercise induced significant changes in the secretion of salivary alpha-amylase, with elevated levels suggesting increased neuroendocrine activity linked to the arousal state triggered by exercise. In addition, functional oxyHb signals indicated greater fluctuations in the mid-left prefrontal cortex among participants in the EX-group compared to those in the CTL group, pointing to altered prefrontal haemodynamic.

Keywords

Pupillometry · Functional near-infrared spectroscopy (fNIRS) · Cognitive performance · Stroop test · Color-Word-Interference-Test

1 Introduction

Regular physical exercise, as an active lifestyle choice, have positive effects on physiological health and cognitive functions of the brain. Studies have suggested that very-light-intensity exercise can influence executive function [1]. Such light exercise can potentially amplify the activation of the prefrontal cortex (PFC) and executive function, but the specific neural mechanisms underlying these effects are not fully understood [2, 3].

Recent research found that pupillometry appears to be a valuable tool for interpreting the beneficial impact of exercise on cognitive enhancement [4–6]. It may be associated with locus coeruleus activation. However, the role of peripheral neuroendocrine substances in this process remains to be further experimentally validated. Therefore, this investigated the effects of a 10-min very-light-intensity exercise (at 30% O_2 Peak) on executive functions. The Stroop task was used to assess executive function before and

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after exercise. Multichannel functional near-infrared spectroscopy (fNIRS) will be utilised to evaluate the activation of the PFC during the task. Finally, salivary alpha-amylase (sAA) will be used to further elucidate the peripheral neuroendocrine mechanisms involved in this process.

2 Methods

2.1 Participants

Participants included in this study were 45 healthy males whose visual acuity was normal. Written informed consent was obtained from all participants after receiving a detailed description of the study. All participants were divided into two groups, resting control (CTL) and a very-light-intensity exercise (EX), at random. Somatic characteristics of the participants are shown in Table 1. There were no significant inter-group differences in age, BMI.

2.1.1 Procedure

All participants engaged in a 10-min exercise at 30% of their individual O₂ peak on a cycle ergometer, as per ACSM’s (2018) classification of “very light” intensity [6]. In the CTL group, participants simply rested on the ergometer. To limit

external influences, participants were advised to avoid exercise, alcohol for 24 h before, and caffeine on the experiment day.

This study included two experimental conditions: a resting control and a very-light-intensity exercise. In the EX-condition, participants performed a 10-min very light exercise on a cycle ergometer at 30% of their individual O₂ peak according to ACSM’s (2018) guidelines. In the CTL condition, participants simply rested on the ergometer. To limit external influences, participants were advised to avoid exercise, alcohol for 24 h before, and caffeine on the experiment day.

In the EX-condition, the Stroop task was completed before and 6.5 min post-exercise. Pupil diameter was assessed at three intervals: pre-, mid-, and post-exercise. During each 5-min exercise half, 3 min were dedicated to pupillometry (Fig. 1).

2.2 Cognitive Inhibition Test

The colour-word-interference test (CWIT) after Stroop (Bäumler, 1985) was used to assess attention and inhibition [7]. The task consists of three parts that have to be performed as fast as possible. (1) Participants need to read aloud a list of colour words (red, yellow, green, and blue). (2)

Table 1 Somatic characteristics of participants (mean ± SD)

Group	N	Age (years)	Weight (kg)	Height (cm)
Exercise	23	21.46 ± 1.16	66.27 ± 13.45	172.83 ± 5.79
Control	22	21.35 ± 1.12	65.88 ± 12.41	172.69 ± 6.33

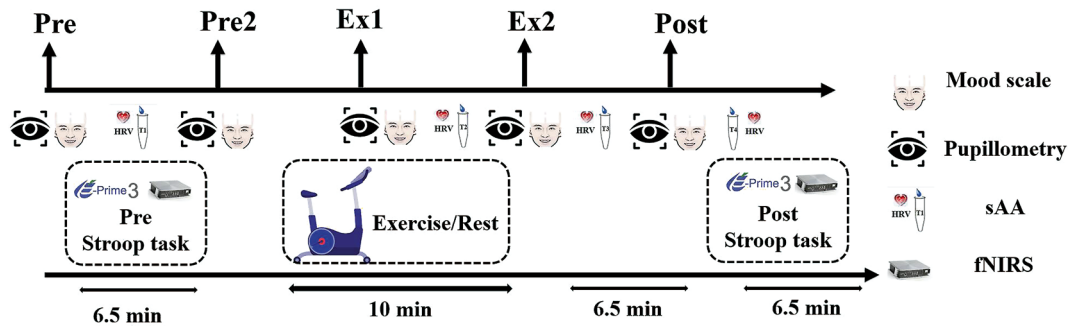


Fig. 1 Scheme of the experimental protocol

They need to name the colour of printed rectangles (also red, yellow, green, and blue). (3) Interference: Participants need to name the colours of colour-printed words. Each colour word is printed in a colour that is not consistent with the word. For example, the word “blue” is printed on red. The participants need to ignore the word “blue” and name the actual colour red. Thus, participants need to pay attention to the actual colour and inhibit the urge to read the word.

2.2.1 Pupil Dynamics Test

Pupil diameters of the participants were measured using a screen-based eye tracker (Tobii pro X3-120, Tobii AB, Danderyd, Sweden). To specifically investigate the sustained pupil dilation during exercise in comparison with the pupil diameter during resting states, continuous eye-tracking data were collected for a duration of 3 min during each experimental session.

2.2.2 Functional Near-Infrared Spectroscopy (fNIRS) System

In this study, we employed the Shimadzu LABNIRS fNIRS brain imaging system to monitor the PFC activity of participants, while they performed the Stroop task. This system is an advanced, non-invasive brain imaging technology that allows for real-time monitoring of cerebral cortex haemoglobin levels. The system features 22 channels, each consisting of an emitter and a detector. The standard distance between the emitter and detector is 30 mm, which is used to accurately capture the haemoglobin changes in the brain activity areas. Dual wavelengths of 780 nm and 830 nm are used to measure the concentration changes of deoxygenated haemoglobin (deoxyHb) and oxygenated haemoglobin (oxyHb). The sampling frequency is set at 6.7 Hz to monitor the haemodynamics of the cortex.

2.2.3 Data Analysis

NIRS-KIT was used to pre-process the raw intensity signals and convert them into haemodynamic changes. The experimentally measured intensity counts at the two wavelengths in each

channel were converted to relative changes in oxyHb and deoxyHb. For interpolation channel 3D visualisation, we used the EasyTopo toolbox provided by Tian et al. [8]. All data analysed in JMP 17.0. 2 × 2 mixed ANOVA, with condition (exercise vs control) as between-subject factors, and time of measurement (pre- vs. post-manipulation) as a within-subjects factor, were conducted for each of the two metrics of the Stroop.

3 Results

3.1 Stroop Test

In the exercise group (EX), there was an improvement in both the accuracy and reaction time after performing the Stroop task. The accuracy increased from 88.72% to 96.18%, and the reaction time decreased from 1104 ms to 936 ms. This may suggest that light exercise can enhance cognitive inhibition and being more focused on the task post-exercise, and it shortens the process information. For the control group (CTL), there was no significant change in performance before and after the Stroop task. The accuracy slightly decreased from 91.63% to 89.33%, while the reaction time remained relatively stable (Table 2).

For total correct [$F(1,43) = 17.63, p < 0.001, \eta^2p = 0.21$], completed categories [$F(1,43) = 5.79, p = 0.02$], and failure to maintain set [$F(1,43) = 21.25, p = 0.05$], participants improved in their performance post exercise. There were significant main effects of sAA concentration or condition.

Table 2 Somatic characteristics of participants (mean ± SD)

		Accuracy rate (%)	Reaction time (mS)
EX group (n = 23)	Pre	88.72 ± 13.20	1104 ± 187
	Post	96.18 ± 14.45*	936 ± 235*
CTL group (n = 22)	Pre	91.63 ± 12.67	1129 ± 195
	Post	89.33 ± 15.01	1095 ± 190

*Indicates significance ($p < 0.05$) between the EX-group and the CTL group

To examine the interaction during exercise, we calculated the difference in haemodynamic responses due to Stroop interference before and after exercise. Figure 2 shows the mean β value of oxy-Hb concentration after exercise. The oxy-haemoglobin components in the left PFC showed significant amplitude fluctuations after exercise. Paired t test showed that the difference of oxy-haemoglobin in the left dorsolateral PFC (DLPFC) was statistically significant [$t(43) = 3.00, P = 0.006$]. Meanwhile, Fig. 3 shows that for changes in deoxy-Hb, paired t test also showed a significant increase in the left DLPFC

[$t(43) = 2.58, P = 0.02$] after exercise (Figs. 2 and 3).

In a repeated measures two-way ANOVA, an interaction effect between condition and time was found to be significant [$F(2.38, 44.12) = 16.86, P < 0.001$]. Post hoc comparisons with Bonferroni correction revealed that pupil diameter was significantly higher in the EX-condition compared to the CTL condition during and after exercise [EX1: $F(1, 43) = 19.22, P < 0.001$; EX2: $F(1, 43) = 28.88, P < 0.001$; Post: $F(1, 43) = 11.67, P = 0.03$] (Fig. 4).

4 Discussion

This study provides potential evidence for the positive impact of exercise on cognitive function, as demonstrated by significant differences in haemodynamic response and the Stroop result observed post-exercise. Notably, the left DLPFC exhibited significant amplitude fluctuations in components of oxyHb, suggesting that exercise may modulate neuronal activity. Stroop result suggest that light exercise may have a positive impact on cognitive inhibition.

Furthermore, exercise has an acute effect on cognitive function, as demonstrated by the significantly higher pupil diameter in the EX-condition compared to the CTL condition during and after exercise. These findings align with the previous research demonstrating the beneficial effects of exercise on cognitive performance [9, 10]. In addition, the deoxy-Hb component also showed significant amplitude fluctuations in the left DLPFC, and we speculate that this phenomenon may be caused by the increase in oxygen consumption exceeding the recruitment of blood flow. This improvement was consistent with increases in sAA concentration, suggesting a possible role for this enzyme in mediating the cognitive benefits of exercise. Our findings highlight the potential of exercise as a non-pharmacological intervention to enhance cognitive function and arousal states [11, 12].

It is important to note that due to the absence of short channels in this fNIRS system, there are

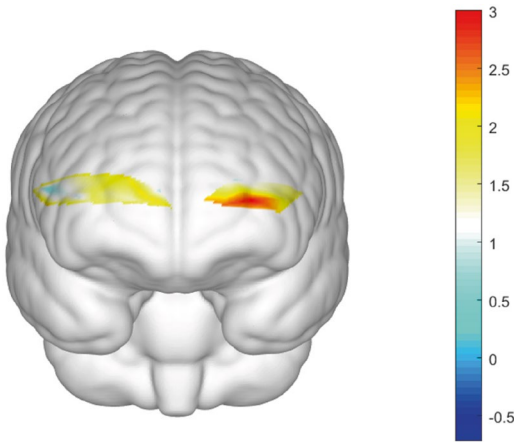


Fig. 2 Averaged β values (t tests) of the oxyHb concentrations after exercise

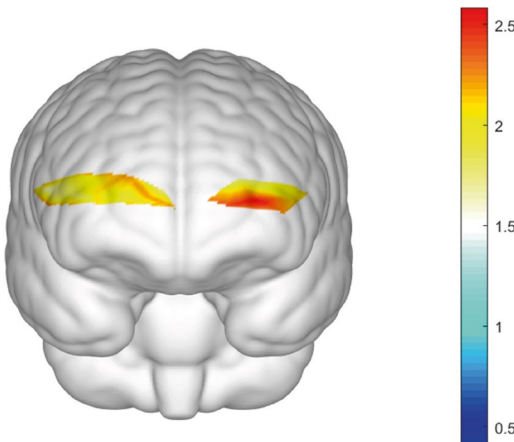


Fig. 3 Averaged β values (t tests) of the deoxy-Hb concentrations after exercise

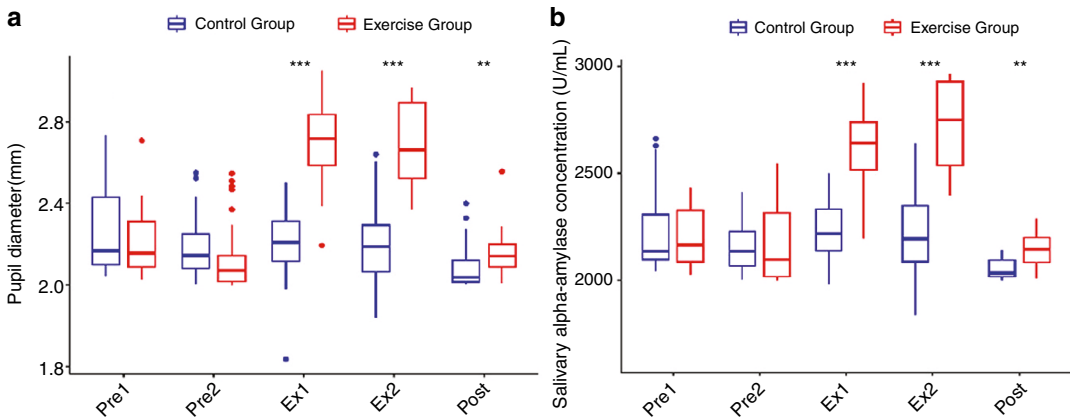


Fig. 4 Mean sAA levels at different stage. **indicates significance ($p < 0.01$) and ***indicates significance ($p < 0.001$) indicates significance ($p < 0.05$) between the EX group and the CTL group

some limitations, particularly the system's increased susceptibility to influences from extracerebral tissues like the scalp and skull. These influences mainly arise, because the fNIRS signals can be easily disturbed by physiological responses when passing through these highly vascularised areas. According to Zhou et al. [13], systems with short channels significantly enhance data quality, especially in reducing systemic responses caused by extracerebral tissues. In addition, when processing data using methods, such as general linear model and principal component analysis, the data from short channels can serve as a reference to help eliminate these interferences, thereby improving the accuracy and reliability of the measurements. Moreover, research by Tachtsidis and Scholkmann [14] also highlights that signal interference from extracerebral layers could lead to false positives and negatives in the data. These interferences are often misidentified as brain activity, affecting the accuracy and reliability of data. Readers should consider these limitations when interpreting the results.

Future research should consider using systems that include short channels to enhance data accuracy and reliability. In addition, efforts should aim to further elucidate the mechanisms underlying the cognitive benefits of exercise and to validate our automated assessment method in larger and more diverse patient populations [8, 15].

5 Conclusion

This study substantiates the positive influence of exercise on cognitive function, as indicated by significant post-exercise changes in haemodynamic response and Stroop test results. The immediate impact of exercise on cognitive performance is further demonstrated by a higher pupil diameter coupled with an increase in sAA concentration in the exercise condition. These findings support the potential of exercise as a non-pharmacological method to enhance cognitive function.

References

1. Loprinzi PD, Frith E, Edwards MK et al (2018) The effects of exercise on memory function among young to middle-aged adults: systematic review and recommendations for future research[J]. Am J Health Promot 32(3):691–704
2. Hyodo K, Jindo T, Suwabe K et al (2019) Acute effects of light-intensity, slow-tempo aerobic dance exercise on mood and executive function in older adults[J]. Bull Phys Fit Res Inst 117:8–16
3. Morris TP, Fried PJ, Macone J et al (2020) Light aerobic exercise modulates executive function and cortical excitability[J]. Eur J Neurosci 51(7):1723–1734
4. Ayala N, Heath M (2021) Pupillometry reveals the role of arousal in a postexercise benefit to executive function[J]. Brain Sci 11(8):1048

5. Yamazaki Y, Suwabe K, Nagano-Saito A et al (2023) A possible contribution of the locus coeruleus to arousal enhancement with mild exercise: evidence from pupillometry and neuromelanin imaging[J]. *Cereb Cortex Commun* 4(2):tgad010
6. Kuwamizu R, Yamazaki Y, Aoike N et al (2023) Pupil dynamics during very light exercise predict benefits to prefrontal cognition[J]. *Neuroimage* 277:120244
7. Xing D, Yoo C, Gonzalez D et al (2022) Effects of acute Ashwagandha ingestion on cognitive function[J]. *Int J Environ Res Public Health* 19(19):11852
8. Tian F, Lin ZJ, Liu H (2013) EasyTopo: a toolbox for rapid diffuse optical topography based on a standard template of brain atlas. In: *Optical tomography and spectroscopy of tissue X*, vol 8578. SPIE, pp 458–467
9. Bayles MP (2023) ACSM's exercise testing and prescription[M]. Lippincott Williams & Wilkins
10. Starcke K, Wiesen C, Trotzke P et al (2016) Effects of acute laboratory stress on executive functions[J]. *Front Psychol* 7:461
11. Byun K, Hyodo K, Suwabe K et al (2014) Positive effect of acute mild exercise on executive function via arousal-related prefrontal activations: an fNIRS study[J]. *Neuroimage* 98:336–345
12. Kuwamizu R, Yamazaki Y, Aoike N et al (2022) Pupil-linked arousal with very light exercise: pattern of pupil dilation during graded exercise[J]. *J Physiol Sci* 72(1):23
13. Zhou X, Sobczak G, McKay CM, Litovsky RY (2020) Comparing fNIRS signal qualities between approaches with and without short channels. *PLoS One* 15(12):e0244186
14. Tachtsidis I, Scholkmann F (2016) False positives and false negatives in functional near-infrared spectroscopy: issues, challenges, and the way forward. *Neurophotonics* 3(3):031405
15. Qi L, Wang GL, Yang YL et al (2024) Positive effects of brisk walking and Tai Chi on cognitive function in older adults: an fNIRS study[J]. *Physiol Behav* 273:114390

Regional Differences in Skeletal Muscle O₂ Dynamics During Body-Weight Resistance Exercise with Slow Movement and Tonic Force Generation

Shun Takagi, Yuya Watanabe, and Ryotaro Kime

Abstract

The aim of this study was to investigate the skeletal muscle O₂ dynamics during body-weight resistance exercise with slow movement and tonic force generation. Thirteen untrained, healthy, young males performed Bulgarian split squats and push-ups until volitional failure. Relative changes from rest in oxygenated haemoglobin (oxy-Hb) and deoxygenated haemoglobin (deoxy-Hb) concentrations were continuously monitored at the vastus lateralis (VL) and rectus femoris (RF) muscles during squats, and pectoralis major (PM) and triceps brachii (TB) muscles during push-ups using spatial resolved near-infrared spectroscopy oximetry. During the squat exercise, deoxy-Hb continuously increased at RF until failure ($10.8 \pm 7.0 \mu\text{mol/L}$), while at VL, deoxy-Hb was

relatively maintained during 25–100% of the number of maximum repetitions ($18.0 \pm 7.4 \mu\text{mol/L}$ at volitional failure). During the push-up exercise, a significant increase in deoxy-Hb was observed during exercise from rest at PM and TB. We found a significant increase in oxy-Hb during exercise at PM ($28.1 \pm 15.8 \mu\text{mol/L}$ at volitional failure), while at TB, no significant difference was observed from rest ($-2.7 \pm 13.7 \mu\text{mol/L}$ at volitional failure). Our findings suggest that the deoxygenation patterns during body-weight squat exercise were heterogeneous within the quadriceps muscles. Moreover, differences in O₂ dynamics between the trunk and extremity muscles may be partly explained by convective O₂ supply during resistance exercise. However, future studies are needed due to inter-individual differences in skills for body-weight resistance exercise.

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Keywords

Multi-joint resistance exercise · Compound resistance exercise · Slow speed training · Muscle oxygenation · Untrained young males

1 Introduction

Skeletal muscle deoxygenation evaluated by near-infrared spectroscopy (NIRS) is heterogeneous between muscles during whole-body aero-

bic exercise, such as cycling [1]. However, little data are available regarding skeletal muscle deoxygenation patterns during body-weight resistance exercise. Skeletal muscle loss is related to mobility and increased risk of several diseases, such as diabetes and heart disease [2, 3]. Muscle hypertrophy may be potentially affected by muscle deoxygenation [4], and therefore, it is important to evaluate regional differences in muscle deoxygenation patterns between muscles, especially during resistance-type exercise using body weight.

Body-weight resistance exercise is a popular type of exercise, because no equipment is required, and it can be performed anywhere. However, due to insufficient exercise intensity, body-weight resistance training at a normal tempo may be ineffective for improving muscle strength and hypertrophy in subjects, except those with a low level of muscle strength [5]. In contrast, similar improvements were observed after low-intensity resistance training with slow movement and tonic force generation, compared to traditional high-intensity resistance training [6]. The aim of this pilot study was to investigate the skeletal muscle O_2 dynamics during body-weight resistance exercise with slow movement and tonic force generation in young volunteers.

2 Methods

2.1 Subjects

Thirteen untrained, healthy, young males (age 20 ± 1 years; height 172.0 ± 5.2 cm; body weight 63.4 ± 8.2 kg, mean $\pm$ SD) participated in the study. None had been involved in any type of resistance training for at least 12 months before enrolment. This study was approved by the Institutional Ethics Committee. All subjects were informed of the purpose of the study and gave written consent.

2.2 Experimental Design

The subjects performed body-weight resistance exercise, specifically Bulgarian split squats and push-ups, until volitional failure. The subjects were asked to exercise under slow movement and tonic force generation [6] (i.e., 5-s eccentric, 5-s concentric, and 1-s isometric actions with no rest between each repetition). For Bulgarian split squats, the subjects were instructed to stand upright with their right foot in front and their left foot placed on a horizontal press bench (42 cm height) behind them, to keep their upper body upright during exercise, and to bend their right knee until the top of the thigh of their forward leg was parallel to the floor, before ascending. The stride distance was normalised to two-thirds of their leg length (measured as the distance from anterior superior iliac spine to the medial malleolus of the tibia). For push-ups, the subjects placed their hands on the floor at 150% of biacromial breadth (shoulder width), and were instructed to exercise so that both hands were at the level of the chest (xiphoid) and the angle of the elbows was approximately 90° , at the lowest position. To be able to transition to exercise immediately, resting posture for squats was defined as standing on the right foot with the knee fully extended and the left foot on the bench. The resting posture for push-ups was to place both hands on the ground at the same width as during exercise, knees bent and touching the ground, and the toes touching the ground in the same position as during exercise.

Muscle O_2 saturation (SmO_2) and relative changes from rest in oxygenated haemoglobin concentration (oxy-Hb), deoxygenated haemoglobin concentration (deoxy-Hb), and total haemoglobin concentration (total-Hb) were evaluated at the vastus lateralis (VL) and rectus femoris (RF) muscles at the right (forward) leg during squats and right pectoralis major (PM) and the lateral head of triceps brachii (TB) muscles during push-ups using spatial resolved NIRS oxim-

etry (Hb12-4, Astem Co., Japan). Fat layer thickness at the measurement site was checked using an ultrasound device (Vscan Dual Probe, GE HealthCare, Japan), and the effects of light scattering in the fat layer on NIRS data were corrected by normalising measurement sensitivity [7].

2.3 Statistical Analysis

All data are given as means $\pm$ standard deviation (SD). The NIRS variables during exercise were averaged over every repetition (i.e., 11 s; 5-s eccentric, 5-s concentric, and 1-s isometric actions). The statistical differences among values during exercise were tested using one-way repeated-measures analysis of variance (ANOVA). Because of the low number of subjects in this pilot study, ANOVA was limited to rest, 25%, 50%, 75%, and 100% of the number of maximum repetitions. When a significant difference was found, multiple comparisons between repetitions were conducted using the Bonferroni post hoc test. For all statistical analyses, significance was accepted at $p < 0.05$.

3 Results

Figure 1 represents the changes in NIRS variables during Bulgarian split squats and push-ups, as a function of percent of the number of maximum repetitions (% of nMR). During squats, at VL, deoxy-Hb was increased during exercise from rest and was relatively maintained at 25–100% of nMR. In contrast, at RF, deoxy-Hb was continuously increased from rest, and consequently, deoxy-Hb at 100% of nMR was higher than at 25% and 50% of nMR. Both at VL and RF, a significant decrease in oxy-Hb and a significant increase in total-Hb were observed. As a result, SmO₂ during exercise was reduced from rest, and no significant change was found during exercise. Deoxy-Hb at VL was significantly positively related to deoxy-Hb at RF ($r = 0.76$,

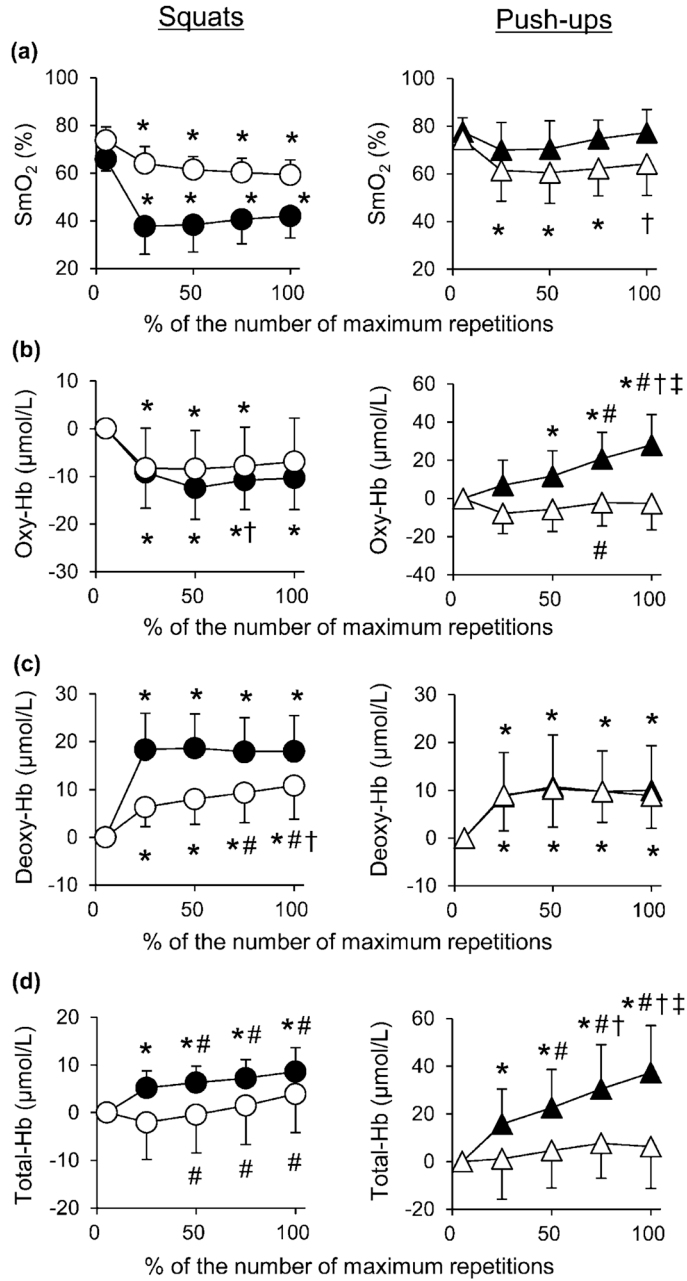
$p < 0.05$) and at volitional failure (Fig. 2). Both at VL and RF, there was no significant relationship between body weight and NIRS variables. During push-ups (Fig. 1), at PM and TB, no significant changes were observed in deoxy-Hb during exercise, though deoxy-Hb significantly increased from rest. Both in oxy-Hb and total-Hb, we found a continuous increase during exercise at PM, while no significant difference was observed from resting value at TB. Consequently, no significant change was observed in SmO₂ at PM, while a significant decrease from rest in SmO₂ was shown during exercise at TB. In contrast to squat exercise at volitional failure, there was a significantly negative relationship between deoxy-Hb at PM and deoxy-Hb at TB ($r = -0.59$, $p < 0.05$) (Fig. 2). Oxy-Hb at volitional failure in PM was unrelated to deoxy-Hb at PM or any NIRS variables at TB. No significant relationships were found between body weight and any NIRS variables at either PM or TB.

The squat exercise was performed at 12 ± 3 repetitions (a range of 8–18). At volitional failure, deoxy-Hb at RF was significantly positively related to repetitions for squats ($r = 0.59$, $p < 0.05$), while deoxy at VL was not significantly associated with repetitions. Push-ups were performed 10 ± 2 repetitions (a range of 7–14). However, both at PM and TB, no significant relationships were observed between oxy-Hb at volitional failure and repetitions for push-ups.

4 Discussion

Resistance exercise with slow movement and sustained force generation partly causes blood flow restriction, secondary to an increase in intramuscular pressure, and may, therefore, potentially enhance muscle deoxygenation during exercise [6]. During the squat exercise, deoxygenation at VL was increased after onset of exercise, and maintained until volitional failure. Similar deoxygenation patterns had been reported during exercise with blood flow restriction [8–10]. At RF, we observed a continuous

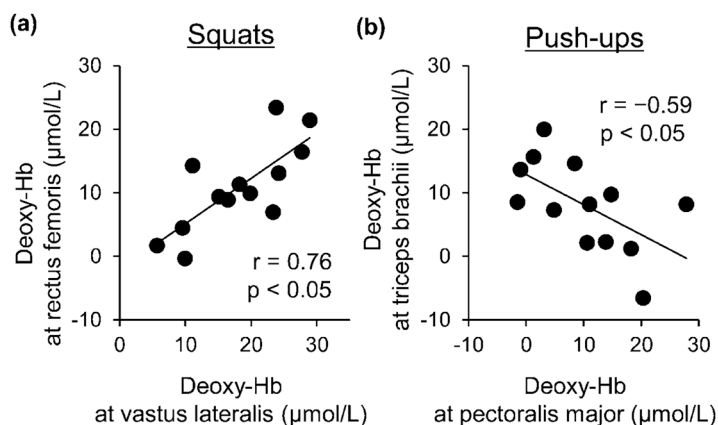
Fig. 1 Change in muscle O₂ saturation (SmO₂: **a**), oxygenated haemoglobin (oxy-Hb: **b**), deoxygenated haemoglobin (deoxy-Hb: **c**), and total haemoglobin (total-Hb: **d**) during Bulgarian split squats (left panel) and push-ups (right panel). The closed circles: NIRS signal at vastus lateralis, the open circles: NIRS signal at rectus femoris, the closed triangles: NIRS signal at pectoralis major, and the open triangles: NIRS signal at triceps brachii muscles. Significant difference from rest (*: $p < 0.05$), 25% (#: $p < 0.05$), 50% (†: $p < 0.05$) and 75% (‡: $p < 0.05$). Values are mean $\pm$ SD



enhancement of deoxygenation in deoxy-Hb. These results suggest that the deoxygenation patterns during body-weight squat exercise with slow movement and tonic force generation were also heterogeneous within quadriceps muscles, similar to aerobic cycling exercise [1]. Though detailed mechanisms are unclear, the difference

in deoxygenation patterns may be potentially affected by intramuscular pressure (i.e., degree of blood flow restriction) [11], blood flow distribution between muscles, change in muscle activities [9], and difference in single vs multi-joint muscles [12]. During push-ups, changes in deoxy-Hb patterns during exercise were similar

Fig. 2 Relationship in deoxygenated haemoglobin concentration (deoxy-Hb) at volitional failure during Bulgarian split squats (a) and push-ups (b)



between PM and TB, though oxy-Hb was continuously increased only at PM. In the leg muscles, deoxygenation during exercise was minor at the proximal site, compared to at the distal site, which can partly be explained by higher O₂ supply [1]. Consequently, higher oxy-Hb at PM may be partly explained by an enhancement of convective O₂ supply to trunk muscles compared with extremity muscles. There is perhaps a possibility that push-up exercise with slow movement is too technically difficult for untrained subjects to sufficiently increase intramuscular pressure. Neither cardiac output nor blood flow to PM muscle was directly monitored in this study; however, to our knowledge, this is the first study to investigate muscle deoxygenation in the chest with correction for the effects of light scattering in the fat layer. Another possible explanation for higher oxy-Hb may be that PM muscle contributed less than TB during push-ups in untrained subjects, as oxy-Hb slightly increases in the forearm (i.e., inactive) muscle during low-to-moderate-intensity cycling exercise in some subjects [13]. In fact, deoxy-Hb at PM was negatively associated with deoxy-Hb at TB in this study, and these results lead us to speculate that there may be inter-individual differences in recruitment of synergist muscles (i.e., PM vs TB muscle). However, because oxy-Hb at PM was not related to deoxy-Hb at PM or NIRS variables at TB in this study, the increase in oxy-Hb cannot be fully explained by degree of recruitment of PM.

Because muscle activities and O₂ demand would be most likely affected by exercise conditions such as hand-width during push-ups [14], we did not directly compare the amplitude of muscle O₂ dynamics between measurement sites. Further investigation for deoxygenation patterns is needed under different exercise conditions. Notably, no significant relationship was observed between body weight and NIRS variables. Exercise intensity in this study was attributed to the force of gravity exerted on the subjects' own body weight. However, during push-ups, there may be some inter-individual difference in vertical ground reaction force and muscle activities, as seen in a previous study [14]. Hence, in future studies, the relationships between more accurately measured exercise intensity (each muscle's activities) and NIRS variables should be evaluated during body-weight resistance exercise, by measuring these factors simultaneously.

In conclusion, our findings suggest that the deoxygenation patterns during body-weight squat exercise were heterogeneous within quadriceps muscles. Moreover, differences in O₂ dynamics between trunk and extremity muscles may be partly explained by convective O₂ supply during resistance exercise. However, future studies are needed due to inter-individual differences in skills for body-weight resistance exercise.

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References

1. Takagi S, Kime R, Niwayama M et al (2013) Muscle oxygen saturation heterogeneity among leg muscles during ramp exercise. *Adv Exp Med Biol* 765:273–278
2. Guillet C, Boirie Y (2005) Insulin resistance: a contributing factor to age-related muscle mass loss? *Diabetes Metab* 31:5S20–5S26
3. Visser M, Kritchevsky SB, Goodpaster BH et al (2002) Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *J Am Geriatr Soc* 50(5):897–904
4. Goto M, Kumada H, Maeda C et al (2020) Temporal increase in muscle cross-sectional area as an acute effect of resistance exercise in resistance-trained and untrained individuals. *J Hum Sport Exerc* 15(2):457–469
5. Yamauchi J, Nakayama S, Ishii N (2009) Effects of bodyweight-based exercise training on muscle functions of leg multi-joint movement in elderly individuals. *Geriatr Gerontol Int* 9(3):262–269
6. Tanimoto M, Ishii N (2006) Effects of low-intensity resistance exercise with slow movement and tonic force generation on muscular function in young men. *J Appl Physiol* (1985) 100(4):1150–1157
7. Niwayama M, Suzuki H, Yamashita T et al (2012) Error factors in oxygenation measurement using continuous wave and spatially resolved near-infrared spectroscopy. *J Jpn Coll Angiol* 52:211–215
8. Bunevicius K, Grunovas A, Vencunas T (2018) Blood flow restriction late in recovery after heavy resistance exercise hampers muscle recuperation. *Eur J Appl Physiol* 118(2):313–320
9. Kacin A, Strazar K (2011) Frequent low-load ischemic resistance exercise to failure enhances muscle oxygen delivery and endurance capacity. *Scand J Med Sci Sports* 21(6):e231–e241
10. Yanagisawa O, Sanomura M (2017) Effects of low-load resistance exercise with blood flow restriction on high-energy phosphate metabolism and oxygenation level in skeletal muscle. *Interv Med Appl Sci* 9(2):67–75
11. Kilgas MA, McDaniel J, Stavres J et al (2019) Limb blood flow and tissue perfusion during exercise with blood flow restriction. *Eur J Appl Physiol* 119(2):377–387
12. de Ruiter CJ, Goudsmit JF, Van Tricht JA et al (2007) The isometric torque at which knee-extensor muscle reoxygenation stops. *Med Sci Sports Exerc* 39(3):443–453
13. Ogata H, Reyihan A, Yano T (2004) Kinetics of oxygenation in inactive forearm muscle during ramp leg cycling. *J Physiol Anthropol Appl Hum Sci* 23(1):7–17
14. Gouvali MK, Boudolos K (2015) Dynamic and electromyographical analysis in variants of push-up exercise. *J Strength Cond Res* 19(1):146–151

Reversal of Rocuronium-Induced Muscle Relaxation with Sugammadex Enhances Oxygen Metabolism in Skeletal Muscle

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Abstract

We measured changes in blood flow and oxygenation in the brachioradialis muscle using near-infrared spectroscopy (NIRS) during reversal of rocuronium-induced muscle relaxation with administration of sugammadex in patients ($n = 25$) under general anaesthesia, to investigate whether reversal of muscle relaxant-induced muscle relaxation increases oxygen metabolism in skeletal muscle under general anaesthesia. NIRS measurements, including oxy-haemoglobin (Hb), deoxyHb, total Hb concentration, tissue oxygen index, and various cardiopulmonary parameters, were recorded at four timepoints: T0 (baseline), 3 min before sugammadex administration; T1,

immediately before sugammadex administration; T2, at complete recovery of muscle contractility; and T3, 3 min after T2. All measured values at each timepoint were compared using multiple comparison tests. The median values (quartile deviation; QD) ($\mu\text{mol/L}$) of oxyHb and deoxyHb at T0, T1, T2, and T3 were 0, -0.01 (0.14), -1.15 (0.54), and -1.52 (0.54), and 0, 0.11 (0.06), 0.86 (0.5), and 1.36 (0.61), respectively. The levels of oxyHb were significantly lower and those of deoxyHb were significantly higher at T2 and T3 when compared to those at T1, respectively ($P < 0.01$). There were no significant changes in other measurements. These results suggest that reversal of rocuronium-induced muscle relaxation by sugammadex slightly increases oxygen metabolism in the brachioradialis muscle. This study might support the clinical finding that administration of neuromuscular blockers decreases whole body oxygen consumption in patients receiving mechanical ventilation under general anaesthesia.

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Keywords

Skeletal muscle · Oxygen metabolism ·
Rocuronium · Sugammadex · Near-infrared
spectroscopy

1 Introduction

Administration of non-depolarising neuromuscular blockers (NMBs), such as rocuronium, might decrease oxygen metabolism in skeletal muscle in patients receiving mechanical ventilation under sedation and general anaesthesia [1–3]. To investigate whether reversal of NMB-induced muscle relaxation increases oxygen metabolism in skeletal muscle, this study measured the changes in blood flow volume and oxygenation in the brachioradialis muscle using near-infrared spectroscopy (NIRS) during the recovery process of rocuronium-induced muscle relaxation with administration of the NMB antagonist, sugammadex, in patients receiving mechanical ventilation with constant settings under general anaesthesia.

2 Methods

This prospective observational study was approved by the Hospital Ethics Committee on Human Rights in Clinical Trials and Research of Nihon University Hospital (RK-150714-4) and registered in the UMIN Clinical Trial Registry (ID: 000022664). Written informed consent was obtained from all patients participating in this prospective observational study. We studied 25 ASA (American Society of Anesthesiologists) physical status I patients scheduled for elective surgery under general anaesthesia. Using NIRS, we measured changes in blood flow volume and oxygenation in the brachioradialis muscle during the recovery process from rocuronium-induced deep muscle relaxation with administration of sugammadex in patients receiving mechanical ventilation with constant settings under general anaesthesia.

2.1 Anaesthetic Procedure and Monitoring

All patients underwent their planned surgical procedure under continuous deep muscle relaxation with general anaesthesia. After the proce-

dure, an NIRS probe (interoptode distance, 40 mm; optical path length coefficient, 3.59) (NIRO®-200NX, Hamamatsu Photonics, Hamamatsu City, Japan) was applied to the skin above the left brachioradialis muscle and an acceleromyograph (TOF-Watch®, Organon Teknika B.V., Boxtel, Holland) was attached to the right forearm. Then, NIRS measurements, including oxyhaemoglobin (Hb), deoxyHb, total Hb concentrations, and tissue oxygen index (TOI), which is an index of tissue oxygen saturation, and muscle relaxation levels were continuously measured every 1 and 20 s, respectively. Patients whose initial muscle relaxation levels were detected as moderate or mild (post-tetanic count >10) were excluded from the study. All the NIRS measurements and cardiopulmonary parameters, including blood pressure (systolic blood pressure; sBP, diastolic blood pressure; dBP), heart rate (HR), percutaneous oxygen saturation (SpO₂), end-tidal carbon dioxide (EtCO₂), and peak inspiratory pressure (PIP) were recorded at four timepoints: T0 (baseline), 3 min before 4 mg/kg sugammadex administration; T1, immediately before sugammadex administration; T2, at complete recovery of muscle contractility [train of four (TOF) ratio = 1.0]; and T3, 3 min after T2; (muscle relaxation state: relaxation, T0 and T1; recovery, T2 and T3). All patients were maintained at a constant anaesthesia depth with sevoflurane (1.5%) and remifentanyl (0.15 µg/kg/min) and were mechanically ventilated under constant settings (fraction of inspiratory oxygen, FiO₂ = 0.3, tidal volume, TV = 8 mL/kg, respiratory rate, RR = 10 breaths/min) throughout the study.

2.2 Data Analysis

All measured values at each timepoint are presented as the mean (SD; standard deviation) or median (QD; quartile deviation). NIRS values other than TOI were expressed as their change from baseline, while TOI and cardiopulmonary parameters were expressed as the actual measured values. All measured values at each timepoint were compared using either the Tukey–

Kramer honestly significant difference test or the Steel–Dwass multiple comparison test. In addition, the difference between changes in deoxyHb and oxyHb (deoxyHb–oxyHb) was calculated at each timepoint in each patient to evaluate the change in muscle oxygen metabolism in individual patients. For this parameter, a higher number indicates greater oxygen metabolism when both the blood gases and haematocrit are constant. Besides, those median values at each timepoint were compared using the Steel–Dwass multiple comparison test. For all parameters, statistical significance was accepted at P value of <0.05 .

3 Results

We were able to obtain data from 22 patients (male/female; 14/8). Three patients were excluded from the study, because their muscle relaxation levels were mild or moderate before beginning the study.

The values of NIRS measurements and cardiopulmonary parameters at each of the timepoints are shown in the Table 1. The median values of oxyHb and deoxyHb at T2 and T3 were significantly lower and higher, respectively, when compared to those at T0 and T1, respectively ($P < 0.0001$). Both the median values of total Hb and mean values of TOI did not change significantly between the four timepoints. There were no significant changes in cardiopulmonary parameters.

The difference between changes in deoxyHb and oxyHb (deoxyHb–oxyHb) at T2 and T3 tended to be higher when compared to that at T0 and T1 in most of the patients, and the median values at T2 and T3 were significantly higher than that at T0 and T1, respectively (Fig. 1).

4 Discussion

The present results showed that the levels of oxyHb in the brachioradialis muscle significantly decreased and those of deoxyHb significantly increased with sugammadex-induced recovery of neuromuscular blockade, and the difference

between the change in deoxyHb and oxyHb values tended to increase with complete recovery of muscle contractility under general anaesthesia in most patients. These results suggest that reversal of NMB-induced muscle relaxation with sugammadex under general anaesthesia might increase oxygen metabolism in skeletal muscle.

Our results also showed that both the levels of total Hb, an indicator of tissue blood volume, and TOI, an indicator of tissue oxygenation, in the brachioradialis muscle did not change significantly throughout the study. As evaluated by NIRS, tissue oxygenation (TOI and rSO_2 : regional oxygen saturation) decreases when skeletal muscle activity is increased by exercise [4, 5]. On the other hand, tissue blood volume index (total Hb and nTHI: tissue oxygenation index) increases or decreases, since the changes in muscle blood flow are variable due to changes in vessel diameter depending on exercise intensity and intramuscular pressure [5, 6]. Furthermore, haemodynamic parameters, BP and HR, are increased via sympathetic tone in response to increase in muscle activity. Therefore, the level of increase in muscle oxygen metabolism with recovery of muscle contractility under general anaesthesia in this study quite slight, resulting in the lack of effect on oxygenation and blood volume in the muscle and in haemodynamics. However, van Beekvelt et al. [6] showed that oxyHb decreased by 2–3 μM and deoxyHb increased by 5–8 μM in the forearm during light exercise, even they did not change at all during the resting state, as a typical example. In this study, both the oxyHb and deoxyHb changed significantly during the resting state under general anaesthesia, although their variations were smaller than that in the above case. Therefore, it is thought that the level of increase in muscle oxygen metabolism with recovery of muscle contractility is anything but meaningless.

Previous clinical studies demonstrated that the administration of NMBs causes a reduction in total body oxygen consumption in mechanically ventilated patients, and suggested that the effect was caused by the reduction of oxygen metabolism in skeletal muscle [1–3]. However, only a few reports have evaluated the direct effects of

Table 1 Changes in NIRS measurements and cardiorespiratory parameters in brachioradialis muscle

	Timepoints			
	T0	T1	T2	T3
NIRS measurements				
Oxy-Hb (μmol/l)	0	−0.01 [0.14]	−1.15 [0.54] ^{*,†}	−1.52 [0.61] ^{*,†}
Deoxy-Hb (μmol/l)	0	0.11 [0.06]	0.86 [0.5] ^{*,†}	1.36 [0.61] ^{*,†}
Total-Hb (μmol/l)	0	0.06 [0.26]	−0.47 [1.01]	−0.34 [1.31]
TOI (%)	74.7 (7.18)	74.62 (7.11)	73.21 (8.29)	72.95 (8.26)
Cardiorespiratory parameters				
sBP (mmHg)	94.77 (7.23)	95.91 (7.62)	95.6 (7.6)	94.68 (7.38)
dBp (mmHg)	53.27 (5.42)	52.95 (5.49)	52.95 (5.57)	52.05 (4.89)
HR (counts/min)	61.41 (5.41)	61.32 (5.09)	60.36 (5.88)	60.09 (5.71)
SpO ₂ (%)	97.82 (0.96)	97.95 (0.84)	97.77 (0.87)	97.77 (0.75)
EtCO ₂ (mmHg)	37.45 (2.86)	37.45 (2.94)	37.27 (2.83)	37.41 (2.99)
PIP (cmH ₂ O)	15.09 (1.85)	15.05 (1.68)	15.55 (1.65)	15.77 (1.54)

Data are presented as mean (SD) or median (QD). * $P < 0.0001$ vs. T0, † $P < 0.0001$ vs. T1. T0 (baseline): 3 min before sugammadex administration; T1: immediately before sugammadex administration; T2: complete recovery of muscle contractility; T3: 3 min after T2. Hb: haemoglobin concentration; TOI tissue oxygenation index, sBP systolic blood pressure, dBp diastolic blood pressure, HR heart rate, SpO₂ percutaneous oxygen saturation, EtCO₂ end tidal carbon dioxide, PIP peak inspiratory pressure

NMBs on oxygen consumption and metabolism in regional skeletal muscle. Using a bilateral isolated forearm technique with tourniquets in humans, Rhee et al. [7], demonstrated that the time of decrease and maximum decrease in NIRS-evaluated muscular oxygenation (SmO₂) in the forearms did not differ between the non-paralyzed side and the side paralyzed with rocuronium. They, therefore, concluded that NMBs have no effect on skeletal muscle oxygenation. However, they might not have been able to detect the slight change in oxygen metabolism, because they only measured SmO₂. Conversely, Pape et al. [8], demonstrated that the administration of rocuronium significantly decreased skeletal muscle oxygen consumption (mVO₂) in the lower limb adductor muscles, which recovered after administration of sugammadex in pigs receiving constant ventilation under general anaesthesia. Their results support those of our study, suggesting that administration of NMBs decreases skeletal muscle oxygen metabolism even when the skeletal muscle is maintained at rest with general anaesthesia. The present results suggested that the effect of administering NMBs on the decrease in oxygen metabolism in regional

skeletal muscle might be slight. Considering that skeletal muscular mass comprises almost 30–40% of total body mass [9], the slight effect of NMB administration may contribute to decrease in whole body oxygen consumption in patients under general anaesthesia.

In this study, the muscle oxygen metabolism was only evaluated by NIRS, and all cardiorespiratory parameters were measured using non-invasive measurements. These factors were imitations to this study.

5 Conclusion

The present study showed that reversal of rocuronium-induced muscle relaxation by administration of sugammadex slightly increases oxygen metabolism in the brachioradialis muscle. Paradoxically, muscle relaxation induced by a non-depolarising muscle relaxant might decrease oxygen metabolism in skeletal muscle. This study might support the clinical finding that NMBs administration decreases whole body oxygen consumption in patients receiving mechanical ventilation under general anaesthesia.

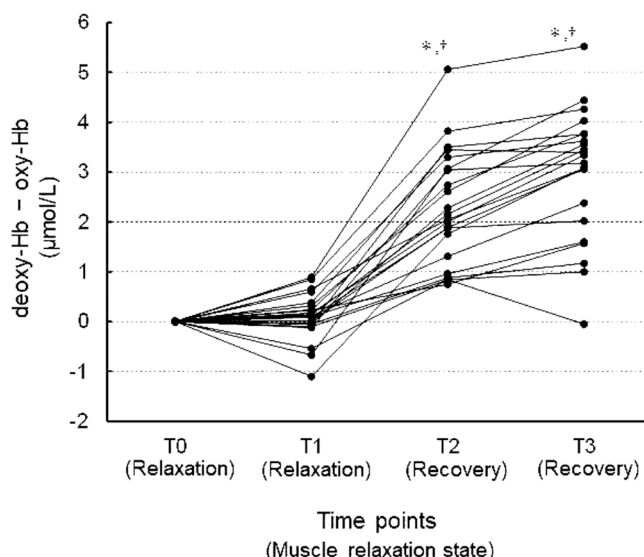


Fig. 1 Difference between changes in deoxyHb and oxyHb ($\text{deoxyHb} - \text{oxyHb}$) in each of the timepoints in individual patients. Hb (oxy- and deoxy-): haemoglobin concentration; T0 (baseline): 3 min before sugammadex administration; T1: immediately before sugammadex

administration; T2: complete recovery of muscle contractility; T3: 3 min after T2. There were significant differences in the median values between the timepoints. * $P < 0.0001$ vs. T0, † $P < 0.0001$ vs. T1

References

1. Marik PE, Kaufman D (1996) The effects of neuromuscular paralysis on systemic and splanchnic oxygen utilization in mechanically ventilated patients. *Chest* 109:1038–1042
2. Vernon DD, Witte MK (2000) Effect of neuromuscular blockade on oxygen consumption and energy expenditure in sedated, mechanically ventilated children. *Crit Care Med* 28:1569–1571
3. McCall M, Jeejeebhoy K, Pencharz P et al (2003) Effect of neuromuscular blockade on energy expenditure in patients with severe head injury. *J Parenter Enteral Nutr* 27:27–35
4. Nemoto EM (2021) Near-infrared spectroscopy (NIRS) of muscle HbO_2 and MbO_2 desaturation during exercise. *Adv Exp Med Biol* 1269:83–86
5. Messere A, Roatta S (2013) Influence of cutaneous and muscular circulation on spatially resolved versus standard Beer-Lambert near-infrared spectroscopy. *Physiol Rep*. <https://doi.org/10.1002/phy2.179>
6. van Beekvelt MCP, van Engelen BGM, Wevers RA et al (2002) In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. *Clin Physiol Funct Imaging* 22:210–217
7. Rhee KY, Kim TY, Oh IS et al (2015) Effect of muscle relaxation on the oxygenation of human skeletal muscle: a prospective in-vivo experiment using an isolated forearm technique. *Korean J Anesthesiol* 68:13–16
8. Pape A, Kertscho H, Stein P et al (2010) Neuromuscular blockade with rocuronium bromide increases the tolerance of acute normovolemic anemia in anesthetized pigs. *Eur Surg Res* 48:16–25
9. Kim J, Shen W, Gallagher et al (2006) Total body skeletal muscle mass: estimation by dual-energy X-ray absorptiometry in children and adolescents. *Am J Clin Nutr* 84:1014–1020

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