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Haemodynamic and cardiorespiratory acute and chronic responses to different blood flow restrictions at rest and during exercise

Julien Desanlis

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Haemodynamic and cardiorespiratory acute and chronic responses to different blood flow restrictions at rest and during exercise

Réponses et adaptations hémodynamiques et cardio-respiratoires à différents niveaux de restriction du flux sanguin, au repos et à l'exercice

Thèse de doctorat de l'université Paris-Saclay

École doctorale n°566, Sciences du sport, de la motricité et du mouvement humain
(SSMMH)

Spécialité de doctorat : Sciences du sport et du mouvement humain
Graduate School : Sport, Mouvement et Facteurs Humains.
Réfèrent : Faculté des sciences du sport

Thèse préparée dans l'unité de recherche **CIAMS** (Université Paris-Saclay),
sous la direction de **François COTTIN**, Professeur des Universités,
la co-direction de **Dan GORDON**, Professor,
le co-encadrement de **Marie GERNIGON**, Maître de Conférences, HDR.

Thèse soutenue à Paris-Saclay, le 20 novembre 2024, par

Julien DESANLIS

Composition du Jury

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Rapporteur & examinateur

Rapporteur & examinateur

Examinatrice

Titre : Réponses et adaptations hémodynamiques et cardio-respiratoires à différents niveaux de restriction du flux sanguin, au repos et à l'exercice.

Mots clés : Restriction du flux sanguin ; ischémie ; occlusion ; oxygénation musculaire ; spectroscopie proche infrarouge ; consommation d'oxygène ; high-intensity interval training ; HIIT.

Résumé : L'ischémie induite par un brassard gonflé au repos (Ischaemic Preconditioning, IPC) ou à l'effort (Blood Flow Restriction, BFR) affecte les paramètres de la chaîne de transport de l'oxygène, en fonction de la pression appliquée. Cette hypoxie locale est utilisée pour optimiser les effets de l'entraînement. L'objectif de ce travail de thèse est donc d'étudier les réponses et adaptations hémodynamiques et cardio-respiratoires à différents niveaux de restriction du flux sanguin, au repos ou à l'effort. Afin d'étudier les réponses hémodynamiques au repos, la fiabilité des mesures d'oxygénation musculaire obtenues avec de la spectroscopie infrarouge (NIRS) est testée. Les études menées dans ce travail doctoral montrent une excellente fiabilité des mesures intra-session, mais un manque de fiabilité inter-sessions.

Au repos, l'oxygénation musculaire est affectée différemment entre une occlusion vasculaire partielle ou complète. Cependant, la réponse n'est pas différente entre une pression suprasystolique absolue et individualisée. A l'effort, quatre semaines d'entraînement aérobie à haute intensité couplé à différents niveaux de BFR améliorent la performance lors de l'épreuve maximale incrémentée et l'efficacité à intensité sous-maximale, sans distinction entre les pressions d'occlusion. Ces résultats ouvrent des perspectives de recherche sur les processus physiologiques impliqués dans les réponses à l'hypoxie locale et suggèrent des applications du BFR dans l'entraînement aérobie pour améliorer les paramètres cardiovasculaires et respiratoires.

Title: Haemodynamic and cardiorespiratory acute and chronic responses to varying blood flow restrictions at rest and during exercise.

Keywords: blood flow restriction; ischemia; occlusion; muscle oxygenation; near-infrared spectroscopy; oxygen uptake; high-intensity interval training; HIIT.

Abstract: Ischaemia induced by an inflated cuff at rest (Ischaemic Preconditioning, IPC) or during exercise (Blood Flow Restriction, BFR) affects the oxygen transport chain, depending on the cuff pressure. This local hypoxia is used to optimise training effects. The objective of this thesis is to study the haemodynamic and cardio-respiratory responses and adaptations to different levels of blood flow restriction, at rest or during exercise. The reliability of muscle oxygenation values obtained using near-infrared spectroscopy (NIRS) is studied to enable further investigations of haemodynamic responses. The results show excellent intra-session reliability but a lack of inter-session reliability.

At rest, muscle oxygenation is significantly affected by partial and complete occlusions differently. However, no significant differences are observed between absolute and individualised supra-systolic pressures. During exercise, four weeks of high-intensity aerobic training with various BFR levels improve cycling performance in a maximal incremental test and submaximal efficiency, regardless of the training pressure used. The results of the present PhD manuscript provide research perspectives on the physiological processes involved in responses to local hypoxia and suggest applications of BFR in aerobic training to improve cardiovascular and respiratory parameters.

"Ever tried. Ever failed. No matter. Try again. Fail again. Fail better."

Beckett (1983)

To my beloved parents,


Céline and Philippe.


TABLE OF CONTENTS


TABLE OF CONTENTS	6
CURRICULUM VITAE	8
ACKNOWLEDGEMENT	15
LIST OF FIGURES	18
LIST OF TABLES	19
LIST OF EQUATIONS	20
LIST OF ABBREVIATIONS	21
INTRODUCTION	25
PHYSIOLOGICAL BASIS OF BLOOD CIRCULATION	25
METHODOLOGICAL CONSIDERATIONS OF INDUCING ISCHAEMIA TO IMPROVE PERFORMANCE	28
<i>Description of ischaemic preconditioning</i>	28
<i>Background on blood flow restriction training</i>	30
CARDIOVASCULAR ADAPTATIONS TO BLOOD FLOW RESTRICTED EXERCISES	32
STRUCTURE OF THE PRESENT THESIS	34
CHAPTER 1 - INDUCING CARDIOVASCULAR AND HAEMATOLOGICAL ADAPTATIONS WITH BFR	37
1.1 FUNDAMENTALS OF BLOOD COMPOSITION AND EFFECTS OF BFR	40
1.2 BFR ENHANCES CARDIAC ADAPTATIONS	43
1.2.1 <i>Mechanisms for enhancing cardiovascular parameters</i>	43
1.2.2 <i>Enhancing cardiac output with training</i>	46
1.2.3 <i>Enhancing cardiac output with BFR</i>	47
1.3 BFR AFFECTS HAEMODYNAMICS	49
1.3.1 <i>Understanding haemodynamics</i>	50
1.3.2 <i>Effects of BFR on blood flow during exercise</i>	53
1.4 BFR ENHANCES VASCULAR REMODELLING	56
1.4.1 <i>Angiogenesis, arteriogenesis and vasculogenesis</i>	56
1.4.2 <i>Effects of BFR on vascular remodelling factors</i>	58
1.4.3 <i>Effects of BFR on capillary growth</i>	64
1.4.4 <i>Muscle oxygenation</i>	67
1.5 CONCLUSION	70
CHAPTER 2 - INCREASING AEROBIC CAPACITY AND POWER WITH BLOOD FLOW RESTRICTION	73
2.1 BFR AND MAXIMAL AEROBIC POWER	73
2.1.1 <i>MICT vs. HIIT</i>	74
2.1.2 <i>Effects of MICT and BFR on VO₂max</i>	76
2.1.3 <i>Effects of HIIT and BFR on VO₂max</i>	78
2.2 OXYGEN UPTAKE KINETICS FOLLOWING BFR TRAINING	80
2.2.1 <i>Principles of oxygen uptake kinetics and exercise intensity domains</i>	80
2.2.2 <i>Effects of BFR on oxygen uptake kinetics</i>	82
2.3 VENTILATORY AND LACTATE THRESHOLDS	86
2.3.1 <i>Biochemical responses to exercise</i>	86
2.3.2 <i>Principles of ventilatory and lactate thresholds</i>	88
2.3.3 <i>Effects of BFR on ventilatory and lactate thresholds</i>	93
2.4 CONCLUSION	96

CHAPTER 3 -	METHODOLOGIES AND RECOMMENDATIONS TO OCCLUDE BLOOD	
FLOW		97
3.1	TYPE OF CUFFS.....	97
3.1.1	<i>Cuff width</i>	98
3.1.2	<i>Cuff material</i>	99
3.1.3	<i>BFR devices</i>	100
3.2	OCCLUSION PRESSURE.....	103
3.2.1	<i>Arbitrary pressures</i>	103
3.2.2	<i>Individualised pressures</i>	108
3.3	CONCLUSION.....	115
CHAPTER 4 -	TECHNOLOGIES USED TO MONITOR THE EFFECTS OF OCCLUSION ON	
	CARDIOVASCULAR AND RESPIRATORY SYSTEMS	117
4.1	MONITORING THE EFFECTS OF RESTRICTED BLOOD FLOW ON MUSCLE PERFUSION.....	117
4.1.1	<i>Monitoring muscle perfusion</i>	118
4.1.2	<i>Near-infrared spectroscopy</i>	119
4.2	ASSESSING CARDIOVASCULAR PARAMETERS.....	131
4.3	ASSESSING CARDIO-RESPIRATORY PARAMETERS.....	134
4.4	CONCLUSION.....	136
AIM OF THE THESIS		137
SCIENTIFIC CONTRIBUTIONS		139
	<i>STUDY 1: Intra- and Inter-Day Reliability of the NIRS Portamon Device after Three Induced Muscle Ischaemias</i>	140
	<i>STUDY 2: Effects of occlusion pressure on haemodynamic responses recorded by Portamon near-infrared spectroscopy device across two visits</i>	166
	<i>STUDY 3: The effects of four weeks of high-intensity interval training coupled with blood flow restriction on maximal and submaximal physiological adaptations</i>	183
DISCUSSION AND PERSPECTIVES		209
	OVERVIEW OF THE MAIN RESULTS.....	210
	LIMITATIONS OF THE STUDIES.....	218
	PERSPECTIVES.....	220
GENERAL CONCLUSION AND RECOMMENDATIONS		227
SYNTHESE EN FRANÇAIS DU MANUSCRIT		229
REFERENCES		255

CURRICULUM VITAE

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Education

2020 – Present **Doctorant en Sciences du Sport, de la Motricité et du Mouvement Humain**

PhD candidate Sports and Human Movement Sciences

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Haemodynamic and cardiorespiratory acute and chronic responses to varying blood flow restrictions at rest or during exercise. Supervised by Pr. François COTTIN (University of Paris-Saclay), Dr Marie GERNIGON (University of Paris-Saclay), and Dr Dan GORDON (Anglia Ruskin University, Cambridge, UK).

2018 – 2020 **Master mention STAPS, parcours PCMPS**

MSc research programme in Sport Sciences

University of Paris-Saclay

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2017 – 2018 **Licence mention STAPS, parcours Entraînement Sportif**

BSc programme in Sport Sciences apply to coaching

University of Paris-Saclay

Ranked 1st – with honours

2015 - 2017 **Diplôme d'Etudes Universitaires Générales (DEUG)**

2nd year of BSc

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Ranked 1st – with honours

Scientific contributions

Peer reviewed publications in preparation

1. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon, D. The effects of four weeks of high-intensity interval training coupled with blood flow restriction on maximal and submaximal physiological adaptations. *In progress*.

Peer reviewed publications

1. **Desanlis J**, Gordon D, French C, Calveyrac C, Cottin F and Gernigon M (2024) Effects of occlusion pressure on hemodynamic responses recorded by near-infrared spectroscopy across two visits. *Front. Physiol.*
 - DOI: [10.3389/fphys.2024.1441239](https://doi.org/10.3389/fphys.2024.1441239)
 - IF WoS 2024: 3.2 | Quartile Scimago 2023: Q2
2. **Desanlis, J.**, Gordon, D., Calveyrac, C., Cottin, F., Gernigon, M. (2022). Intra- and inter-day reliability of the NIRS Portamon device after three induced muscle ischemias. *Sensors*, 22(14), 5165.
 - DOI: [10.3390/s22145165](https://doi.org/10.3390/s22145165)
 - IF WoS 2021: 3,8 | Quartile Scimago 2021: Q1

Peer reviewed abstracts

1. Gernigon, M., **Desanlis J.**, Colin A., Cottin, F. and Gordon D. (2023). Effects of low-volume high-intensity training with blood flow restriction on sub-maximal cycling efficiency. *Medicine and Science in Sports and Exercise*; 55(9S): 20.
2. **Desanlis J.**, Gernigon, M., Colin A., Cottin, F. and Gordon D. (2023). Effect Of Blood Flow Restriction Training On Cardio-respiratory Adaptations: *Medicine and Science in Sports and Exercise*; 55(9S): 20.
3. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon., D. Cardiorespiratory adaptations following four weeks of high-intensity interval training with blood flow restriction. *Graduate Journal of Sport Science, Coaching, Management, and Rehabilitation*. 2023; 1(1): 1.
4. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon., D. Enhanced sub-maximal oxygen uptake after 4 weeks of high-intensity training with blood flow restriction. *Graduate Journal of Sport Science, Coaching, Management, and Rehabilitation*. 2023; 1(1): 88.
5. Dunn, A., Pearson, J., Wojciechowski, G., Curtis, J., Gernigon, M., **Desanlis, J.**, Gordon, D. Strength and Neuromuscular Adaptations following a 4-week Isokinetic Strength Training program with Blood Flow Restricted Recovery in a Physically Active Population. *Graduate Journal of Sport Science, Coaching,*

Management, and Rehabilitation. 2023; 1(1): 78.

6. Pearson, J., Dunn, A., Wojciechowski, G., Curtis, J., Gernigon, M., **Desanlis, J.**, Gordon, D. Reactive strength index and neuromuscular adaptations after 4-weeks of isokinetic strength training with blood flow restricted recovery in a physically active population. *Graduate Journal of Sport Science, Coaching, Management, and Rehabilitation*. 2023; 1(1): 92.

Conferences

Oral communications

1. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon, D. Enhanced sub-maximal oxygen uptake after 4 weeks of high-intensity training with blood flow restriction. BASES 2023 Student Conference, Cambridge (UK), 24 – 25 April 2023.
2. **Desanlis J.**, Gernigon M., Calveyrac C., Cottin F., Gordon D. Reproducibility of the Portamon NIRS device after induced muscle ischemia at rest. Journée FéDeV 2022. 13 October 2022 (Palaiseau, France).
3. **Desanlis J.**, Gernigon M., Calveyrac C., Cottin F., Gordon D. Reproducibility of the Portamon NIRS device after induced muscle ischemia at rest. Student conference at the University of Szeged (Hongrie). 27 – 29 April 2022.

Poster communications

1. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon, D. Effects of high-intensity interval training with blood flow restriction on cycling efficiency and oxygen uptake. 28th Annual Congress of the European College of Sport Science (ECSS), Paris (France), 4 – 7 July 2023.
2. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon, D. Effect of blood flow restriction training on cardio-respiratory adaptations. 2023 ACSM Annual Meeting, World Congress on Exercise is Medicine®, and World Congress on the Basic Science of Physical Activity and Aging Biology, Denver (Colorado, USA), 30 May – 2 June 2023. DOI: 10.1249/01.mss.0000979912.42682.2d. [Link](#)
3. **Desanlis, J.**, Gernigon, M., Colin, A., Cottin, F., Gordon, D. Cardiorespiratory adaptations following four weeks of high-intensity interval training with blood flow restriction. BASES 2023 Student Conference, Cambridge (UK), 24 – 25 April 2023. [Link](#)
4. **Desanlis, J.**, Calveyrac, C., Cottin, F., Gordon, D., Gernigon, M. Reproducibility of the Portamon NIRS device after induced muscle ischemia at rest. 19^{ème} congrès international de l'ACAPS, Montpellier (France), 27-29 October 2021. sciencesconf.org/acaps2021:354911.

Other communications

1. Gernigon, M., **Desanlis, J.**, Colin, A., Cottin, F., Gordon, D. Sub-maximal oxygen consumption enhanced by 4 weeks of high-intensity training with blood flow restriction. 28th Annual Congress of the European College of Sport Science (ECSS), Paris (France), 4 – 7 July 2023.
2. Gernigon, M., **Desanlis, J.**, Colin, A., Cottin, F., Gordon, D. Effects of low-volume high-intensity training with blood flow restriction on sub-maximal cycling efficiency. 2023 ACSM Annual Meeting, World Congress on Exercise is Medicine®, and World Congress on the Basic Science of Physical Activity and Aging Biology, Denver (Colorado, USA), 30 May – 2 June 2023. DOI: 10.1249/01.mss.0000979908.94718.29. [Link](#)
3. Dunn, A., Pearson, J., Wojciechowski, G., Curtis, J., Gernigon, M., **Desanlis, J.**, Gordon, D. Strength and Neuromuscular Adaptations following a 4-week Isokinetic Strength Training program with Blood Flow Restricted Recovery in a Physically Active Population. Graduate Journal of Sport Science, Coaching, Management, and Rehabilitation. 2023; 1(1): 78. BASES 2023 Student Conference, Cambridge (UK), 24 – 25 April 2023. [Link](#)
4. Pearson, J., Dunn, A., Wojciechowski, G., Curtis, J., Gernigon, M., **Desanlis, J.**, Gordon, D. Reactive strength index and neuromuscular adaptations after 4-weeks of isokinetic strength training with blood flow restricted recovery in a physically active population. Graduate Journal of Sport Science, Coaching, Management, and Rehabilitation. 2023; 1(1): 92. BASES 2023 Student Conference, Cambridge (UK), 24 – 25 April 2023. [Link](#)
5. Gernigon, M., **Desanlis, J.**, Calveyrac, C., Gordon, D., Cottin, F. Effect of the level of vascular occlusion on muscle oxygenation at rest. 19^{ème} congrès international de l'ACAPS, Montpellier (France), 27-29 October 2021. sciencesconf.org:acaps2021:354913.

Popular science

1. Gordon, D., **Desanlis J.**, Gernigon, M. (2021). Blood flow restriction training: how Olympians use it to boost performance. The conversation UK. [Link](#)
2. Gordon, D., **Desanlis J.**, Gernigon, M. (2021). « Garrotter » ses muscles, la nouvelle technique pour améliorer ses performances sportives. The conversation Fr. [Link](#)

International mobility

- 2022 **Human Energetics and Performance Centre, Anglia Ruskin University, Cambridge (UK)**
February to August 2022 (6 months)
- *Supervisors:* Dr Dan GORDON & Dr Marie GERNIGON.
 - *Aim:* Undergo a scientific study about the effects of 4 weeks of high intensity endurance training with BFR on cardiovascular adaptations.
- 2020 **Human Energetics and Performance Centre, Anglia Ruskin University, Cambridge (UK)**
February to March 2020 (1 month – interrupted by COVID-19)
- *Supervisors:* Dr Marie GERNIGON & Dr Dan GORDON.
 - *Aim:* Undergo a pilot study about the effects of 4 weeks of sprint interval training with BFR on cardiovascular adaptations.

Grants & Awards

- 2022 **2nd best oral communication of the “Fédération Demenÿ-Vaucanson”**
Obtained on the 20/10/2022 at the “Journée FéDeV 2022”
- **Desanlis J., Gernigon M., Calveyrac C., Cottin F., Gordon D.** Reproducibility of the Portamon NIRS device after induced muscle ischemia at rest. Journée FéDeV 2022. 13 October 2022 (Palaiseau, France).
 - Award: 450€
- 2022 **International mobility Erasmus+**
- Aim: to spend six months at Anglia Ruskin University (Cambridge, UK) for research purposes.
 - Grant: 2,000€
- 2021 **PhD Scholarships from the doctoral school Sport, Movement and Human Motricity Sciences (SSMMH)**
- Rank: first
 - Grant: 3 years
- 2020 **International Excellence Internship Scholarships IDEX, Université Paris-Saclay**
- Aim: to spend three months at Anglia Ruskin University (Cambridge, UK) for the 2nd year of Master’s internship.
 - Grant: 1,860 €

Duties

2022 – 2024	Representative member at the research academic council of the University of Paris-Saclay.
2022 – 2024	Representative member at the « Solidarity and Development Fund for Student Initiatives (FSDIE Social) » commission.
2020 – 2024	Substitute representative member of the PhD students at the council of the Doctoral School Sciences du Sport, de la Motricité et du Mouvement Humain (SSMMH).
2020 – 2023	Representative member of the PhD students at the council of the Graduate School Sport, Mouvement, Facteurs Humains (GS SFMH).

Teachings

Since the beginning of the PhD, 368h of teachings have been completed in exercise physiology and research methodology.

Courses	Degree
Lecture	
<i>Exercise physiology</i>	<i>Undergraduate</i>
<i>Introduction to research</i>	<i>Undergraduate</i>
<i>Presentation of BFR training methodology</i>	<i>Master</i>
Tutorials	
<i>Exercise physiology</i>	<i>Undergraduate</i>
<i>Scientific determinants of performance</i>	<i>Undergraduate</i>
<i>Performance assessment for rehabilitation</i>	<i>Undergraduate</i>
<i>Introduction to research</i>	<i>Undergraduate</i>
Other teaching activity	
<i>Creation of video capsules on exercise physiology</i>	<i>Undergraduate</i>

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This PhD made me discover a new country and live abroad, in Cambridge for six months. I met fantastic people there. Thanks to Viv and Jorge, Ash, Mike and, of course, Nathan. In addition, I would like to thank Henry Chung, who let me stay with him in the lab when I was in my master's degree. Finally, a huge thank you to Monsieur Matthew Slatter, who took me under his wing for six months. Matt, you are a model of generosity and benevolence. You are, without a doubt, an inspiration.

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Alicia, you have been by my side for five years now, following and supporting me through all our adventures. You made sacrifices to come with me to Cambridge, where we shared unforgettable moments. Feeling your love every day has deeply strengthened me. I am incredibly fortunate to have you with me each day and so happy about the family we have formed with our furry daughter, Ollie. I love you so much la bib.

Finally, I dedicate this thesis to my parents, Céline and Philippe, and my brother Noé. Nono, growing up by your side was a true joy. I am genuinely happy that we share the same passions, from our early days of playing tennis to our current interests in Mixed Martial Arts and running. When we wrestle on the beach, and you manage to strangle me, I sometimes wonder if I am still your older brother or just your toy. You have become a confident man and a true athlete. It is really inspiring. I am proud of you.

My parents always cherished me, raising a happy and curious child with a lot of passions, including sport. I am now close to defend a thesis in sport sciences. Unbelievable. I am deeply grateful for all the love and support you have given me throughout the years. All that you have helped me discover, from the simplest trips in Bretagne to the most splendid beaches of La Digue, has engraved unforgettable memories and fuelled my thirst for curiosity. Merci beaucoup, je vous aime tellement.

I would like to pay tribute to my grandfather, Serge Lhomel, who passed away in 2017, at the beginning of my university journey. You are a true inspiration, with boundless knowledge and a remarkable sense of humour. You cherished us, and loved us so much. I wish you were here to see the end of this journey; I know you would be so proud of me.

I think about you every day and miss you deeply.

LIST OF FIGURES

Figure 0-1: Marie-Anne Lavoisier's drawing of an experiment on oxygen consumption at rest.....	26
Figure 1-1: Wagner's diagram.....	39
Figure 1-2: Effect of hypoxia on maximal oxygen uptake.....	40
Figure 1-3: Pressure changes across the different blood vessels of the vascular system.....	51
Figure 1-4: Illustration of angiogenesis and arteriogenesis.....	57
Figure 1-5: The impact of hypoxia on angiogenesis.....	64
Figure 2-1: Representative examples of endurance exercise protocols used to increase aerobic power.....	75
Figure 2-2: Oxygen uptake kinetics representation and parameters.....	81
Figure 2-3: Identification of ventilatory or lactate thresholds from a representative participant.....	90
Figure 2-4: Schematic representation of ventilatory and lactate responses to increasing work rate during a graded exercise test.....	93
Figure 3-1: Representation of the blood flow as the percentage of resting blood flow (%) in response to occlusion pressures ranging from 10 % to 100 % of AOP.....	112
Figure 4-1: Illustration of the three near-infrared imaging (NIRI) techniques that could be used for tissue oxygenation monitoring.....	122
Figure 4-2: Representative image of the lower leg obtained with magnetic resonance imaging.....	124
Figure 4-3: Evolution of DPF during ischaemia.....	128
Figure 4-4: PhysioFlow Q-Link device and electrodes placement for exercise.....	133
Figure 4-5: Illustration of the use of Douglas bags at rest or during exercise.....	134
Figure 5-1: Representation of $\dot{V}O_2$ kinetics during a moderate intensity exercise with BFR at 40 % of LOP, BFR at 50 % of LOP and without BFR.....	222
From Study 3	
Study 3 - Figure 1: Schematic representation of the study design.....	190
Study 3 - Figure 2: Design of the submaximal test.....	193

LIST OF TABLES

Table 2-1: Training protocols and associated VO₂max changes from Amani-Shalamzari et al. (2019)²⁸⁷79

From Study 3

Study 3 - Table 1: Participants characteristics.....196

Study 3 - Table 2: pre-and post-training anthropometric and blood assessments.....197

Study 3 - Table 3: Changes in gas exchanges, performance, and cardiovascular values between pre- and post-training maximal test199

Study 3 - Table 4: Haemodynamic changes between pre- and post-training maximal test199

Study 3 - Table 5: Changes in submaximal gas exchanges, heart rate and lactate production between pre-and post-training submaximal test.....201

LIST OF EQUATIONS

Equation 1: Fick principle.....	37
Equation 2: Fick's law of diffusion	38
Equation 3: Diffusive O ₂ transport equation.....	38
Equation 4 : Poiseuille's law defining the resistance of the blood vessels	51
Equation 5 : Poiseuille's law defining the flow rate (F) in the blood vessels.....	52
Equation 6: Fluid shear stress.....	58
Equation 7 : LOP equation. The LOP (mmHg) calculation is based on the cuff width (W) and the limb circumference (C).....	110
Equation 8: Optical Density in human tissue.....	120
Equation 9: Bouguer-Beer-Lambert equation for absorbance	121
Equation 10: Modified Beer-Lambert equation.....	121
Equation 11 : Calculation of DPF	121

From Study 3

Study 3 - Equation 1: bi-exponential model.....	194
Study 3 - Equation 2: tri-exponential model.....	194
Study 3 - Equation 3: Heavyside step function.....	194

ABBREVIATIONS

1-RM	One-repetition maximum
A	Absorbance
ac	Amplitude of the modulated light wave
ACE	Angiotensin-converting enzyme
ACSM	American College of Sport Medicine
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ANS	Autonomic Nervous System
AOP	Arterial Occlusion Pressure
Ap	Amplitude of the primary phase
Asc	Amplitude of the slow component
ATP	Adenosine triphosphate
ATT	Adipose tissue thickness
AUC	Area under the curve
BFR	Blood Flow Restriction
BFR	Breathing frequency
BRS	Baroreflex sensitivity
c	Concentration of absorbing molecules
Ca ²⁺	Calcium ion
C _a O ₂	Arterial concentration of O ₂
CI-95 %	95 % confidence interval
C-leg	Leg without any occlusion
CNS	Central nervous system
CO ₂	Carbon dioxide
COXIV	Cytochrome c oxidase complex IV
CP	Critical power
CS	Critical speed
CT	Continuous training
CV	Coefficient of variation
C _v O ₂	Arterial concentration of O ₂
CW-NIRS	Continuous wave near-infrared spectroscopy
d	Distance travelled by light
dc	Average value of the modulated light wave
DO ₂	Diffusive oxygen conductance
DPF	Differential path length
DTOF	Photon distribution of time-of-flight
ECG	Electrocardiography
EMG	Electromyography

eNOS	Endothelial nitric oxide synthase
EPO	Erythropoietin
EPOC	Post-exercise oxygen consumption
ETC	Electron Transport Chain
FDMD	Frequency-domain multi-distance
FD-NIRS	Frequency domain near-infrared spectroscopy
FMD	Flow-mediated vasodilation
FT	Fast Twitch
G	Losses produced by scattering
GET	Gas exchange threshold
H ⁺	Hydrogen ion
Hb	Haemoglobin
HCO ₃ ⁻	Bicarbonate ion
Hct	Haematocrit
HHb	Deoxyhaemoglobin
HIF-1 α	Hypoxia-inducible factor 1 α
HIIT	High-intensity interval training
HR	Heart Rate
HR _{max}	Maximal heart rate
HRR	Heart Rate Reserve
HRV	Heart rate variability
HS	Handheld pump and sphygmomanometer system
I	Emergent light
I ₀	Emitted light
ICC	Intraclass correlation coefficient
iEMG	Electromyographic activity
I-leg	leg under vascular occlusion
IPC	Ischaemic Preconditioning
K ⁺	Potassium ion
La ⁻	Lactate
LI	Low Intensity
LL	Low Load
LoA	Line of Agreement
LOP	Limb occlusion pressure
LT	Lactate threshold
LT1	Lactate threshold 1
LT2	Lactate threshold 2
MAO	Mesenteric arterial occlusion
MAP	Maximal Aerobic Power
MCT	Monocarboxylate transporter
MG	Medial gastrocnemius
MICT	Moderate-intensity continuous training
MLSS	Maximal lactate steady state

MMSS	Maximal metabolic steady state
MPS	Muscle Protein Synthesis
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mROS	Mitochondrial Reactive Oxygen Species
MRS	Magnetic resonance spectroscopy
mTOR	Mammalian target of rapamycin
MVC	Maximal Voluntary Contraction
$\dot{m}VO_2$	Muscular oxygen uptake
Na ⁺	Sodium ion
NHE-1	Sodium-hydrogen antiporter 1
NIR	Near-infrared
NIRI	Near-infrared imaging
NIRS	Near-infrared spectroscopy
NO	Nitric Oxide
O ₂	Dioxygen
O ₂ Hb	Oxyhaemoglobin
OBLA	Onset of blood lactate accumulation
PER	Periodized Training
PET	Positron emission tomography
PETCO ₂	End-tidal pressure of CO ₂
PETO ₂	End-tidal pressure of O ₂
PGC1- α	Peroxisome proliferator activated receptor Gamma coactivator 1- α
PGs	Prostaglandins
PHD	Prolyl Hydroxylase
Pi	Inorganic phosphate
PORH	Post-occlusive reactive hyperaemia
PPO	Peak Power Output
PT	Personalized tourniquet
\dot{Q}	Cardiac Output
\dot{Q}_{max}	Maximal Cardiac Output
RBC	Red Blood Cells
RCP	Respiratory compensation point
RER	Respiratory gas exchange ratio
RF	Radio-frequency
RI	Rapid inflation
ROS	Reactive Oxygen Species
RPE	Rate of perceived exhaustion
RST	Repeated-sprints training
SAT	Subcutaneous adipose tissue
SBP	Systolic Blood Pressure
SEM	Standard error of measurement

SIT	Sprint Interval Training
SPECT	Single-photon-emission computed tomography
SRS	Spatially-resolved spectrometers
SV	Stroke Volume
SV_{max}	Maximal Stroke Volume
TA	Tibialis anterior
tcpO ₂	Transcutaneous oxygen pressure
TD-NIRS	Time domain near-infrared spectroscopy
TD _p	Time delay of the primary phase
tEV	Total Erythrocytes Volume
tHb	Total Haemoglobin
ToF	Time-of-flight
TRS	Time resolved spectroscopy
TTE	Time to Exhaustion
$\dot{V}CO_2$	Rate of CO ₂ excretion
\dot{V}_E	Ventilation
VEGF	Vascular endothelial growth factor
VEGF-R2	Vascular endothelial growth factor receptor-2
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_2 SC$	Oxygen uptake slow component
$\dot{V}O_{2max}$	Maximal oxygen uptake; maximal aerobic power
$\dot{V}O_{2peak}$	Highest point reach by the oxygen uptake
VT1	Ventilatory threshold 1
VT2	Ventilatory threshold 2
$\mu's$	Scattering coefficient
μ_a	Absorption coefficient
φ	Phase of the modulated light wave
τ_P	Time constant of primary phase
τ_{sc}	Time constant of the slow component
ε	Molar absorptivity

INTRODUCTION



The human body can withstand weeks without food and days without water but only minutes without oxygen (or dioxygen; O₂)—the vital part of air ¹. This O₂, captured during respiration, flows through the arteries, thanks to the work of the heart, to reach all organs and tissue to produce energy ². O₂ is then indispensable for maintaining life and muscle contractions. Hence, the proposition to engage in training under reduced blood flow seems incredulous, if not outright insane. However, for over three decades, the practice of ischaemic preconditioning (IPC) at rest and blood flow restriction (BFR) has demonstrated notable improvements in performance despite its seemingly unconventional nature ^{3,4}. These methodologies, though distinct, converge in their capacity to elicit central and peripheral adaptations by reducing blood flow, thereby disrupting cardiac and vascular parameters and skeletal muscle oxygenation.

PHYSIOLOGICAL BASIS OF BLOOD CIRCULATION

The Ancient Greek word referring to “blood” is “αἷμα” (*haema, hema*), meaning “hot”, or “incandescent” ⁵. Since Antiquity, blood is considered as an intricate fluid that plays an essential role in sustaining life within the human body ⁵. The discovery of intrinsic mechanisms of blood circulation has been attributed to William Harvey (1578 – 1657) with the publication of his book *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (An Anatomical Exercise on the Motion of the Heart and Blood in Animals) in 1628 ^{5,6}. Harvey elucidated the unidirectional flow of blood, originating from the heart and coursing through arteries before ultimately returning to the heart via the veins, making the concept of venous return evident.

The blood transports everything that must circulate in the body, such as nutrients, hormones, and O_2 . The essential role of O_2 in cellular respiration has been extensively investigated by Antoine Lavoisier (1743 – 1794) and Armand Seguin (1767 – 1835), French chemists, through a series of innovative studies ⁷. Their work and discoveries have been illustrated and documented by Marie-Anne de Lavoisier (1758 – 1836), which has contributed to help other researchers to replicate the studies of Lavoisier and Seguin ⁷ (Figure 0-1).



Figure 0-1: Marie-Anne Lavoisier's drawing of an experiment on oxygen consumption at rest.

The subject (A. Séguin, left) wears a face mask connected to a glass vessel. The expired air travels through the vessel where it meets potassium hydroxide and is collected on the right by water displacement. From Grimaux (1896) (8).

In the mitochondria, O₂ acts as a combusive agent driving the production of adenosine tri-phosphate (ATP) through oxidative phosphorylation, a cornerstone of aerobic metabolism⁹. This metabolic pathway unfolds within the inner mitochondrial membrane, specifically during the electron transport chain (ETC). Oxidative phosphorylation involves a series of redox reactions, where electrons are transferred along the ETC complexes, ultimately leading to the pumping of hydrogen ion (H⁺) across the inner mitochondrial membrane. This creates an electrochemical gradient that drives the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i) by the ATP synthase enzyme¹⁰. Although the aerobic metabolism of oxidative phosphorylation yields the production of ATP, ATP is also produced by non-aerobic (*i.e.* anaerobic) metabolism that does not require oxygen: phosphagen energy system and glycolysis¹⁰.

Notably, skeletal muscle relies extensively on the ATP generated through aerobic metabolism to fuel muscle contractions and subsequent force production¹⁰. Indeed, oxidative phosphorylation yields a significantly higher amount of ATP per molecule of glucose compared to anaerobic pathways^{9,11}. During exercise, the demand for ATP increases, inducing an upregulation of oxidative phosphorylation to meet the augmented energy requirements¹¹. Therefore, oxygen uptake ($\dot{V}O_2$) and muscular oxygen uptake ($m\dot{V}O_2$) increase to meet the elevated energy demands, leading to enhanced O₂ delivery to the working muscles through increased blood flow and perfusion^{12,13}. This increased O₂ availability supports the sustained ATP production required for prolonged physical activity. Consequently, the efficient utilisation of O₂ underscores its importance in meeting the energy demands of human physiology, particularly during prolonged physical exertion¹⁴.

When the delivery of O₂ to skeletal muscle is impaired whether by a decrease in available O₂ in the environment (*i.e.* hypoxia), or by a reduction in blood flow (*i.e.* ischaemia), the muscle perfusion in O₂ is decreased^{15,16}. Decreased O₂ availability accelerates the rate of fatigue and reduces the time to exhaustion during knee-

extensor and plantar-flexion exercises in hypoxia¹⁷⁻¹⁹. However, in a hyperoxia state (increased O₂ availability), the rate of fatigue is slowed and time to exhaustion is increased^{19,20}. Therefore, limiting the O₂ availability by either ischaemia or hypoxia alters the performance, shifting the ATP production towards anaerobic metabolism and increasing muscular fatigue²¹⁻²³.

During physical activity, there is a reduction in skeletal muscle O₂ levels, and the extent of this decrease is influenced by the intensity of the exercise²⁴⁻²⁶. But when exercise is performed under hypoxia or ischaemia, the tissue oxygenation shows a greater decrease than in normoxia¹⁶. The tissue oxygenation then reflects the balance between O₂ utilisation and O₂ supply, reflecting skeletal muscle oxidative capacity²⁷.

METHODOLOGICAL CONSIDERATIONS OF INDUCING ISCHAEMIA TO IMPROVE PERFORMANCE

While both BFR and IPC manifest their effects by reducing blood flow to skeletal muscles, these methods employ distinct approaches. BFR is applied during exercise to partially restrict, rather than completely occlude, blood flow. In contrast, IPC involves repeated arterial occlusions performed at rest, prior to exercise, with the participant in a supine position on a medical couch^{28,29}.

Description of ischaemic preconditioning

IPC consists of brief periods of blood flow cessation (ischaemia) and restoration (reperfusion), and can protect the heart from more prolonged episodes of ischaemia³⁰. IPC was first described by Murry *et al.* (1986) on a canine model after four cycles of 5 minutes occlusion, with a reduction of infarct size caused by a prolonged ischaemic period³¹. Almost forty years after the work of Murry *et al.*, (1986) an extensive body of research has been conducted in the scientific literature about the cardioprotective effects of IPC³². Despite the considerable

understandings in the complex processes involved in the cardioprotection, the signal transmission mechanisms remain unresolved³². Three hypotheses (neurogenic, humoral or systemic pathways), potentially acting together and not exclusively, have raised to explain the signal propagation from the preconditioned organ to the target organ^{29,30}.

In recent years, there has been growing interest in optimising traditional training methods and identifying ergogenic strategies to enhance training adaptations and performance in athletes³³⁻³⁸. However, the effectiveness of IPC in sports science, remains debated due to equivocal results in performance enhancement^{3,39,40}. Discrepancies between results found in the literature could arise from methodological issues such as the pressure inside the cuffs⁴¹, and the number and duration of ischaemia and reperfusion cycles that varies across studies^{41,42}.

In a recent meta-analysis, Salvador *et al.* (2016) have investigated the effects of IPC on exercise performance in healthy population⁴⁰. The authors have classified the performance into three categories: aerobic (effort > 90s), anaerobic (10-90s), and sprint or power performance (< 10 s) that could be criticised for their broad and arbitrary time ranges, which might not accurately capture the nuances of different physiological contributions to ATP production⁴⁰. However, the authors have found the largest effects sizes for aerobic and anaerobic exercises and an unclear effect for power and sprint exercise performances⁴⁰. IPC seem to induce a > 99 % chance of having a beneficial effect on aerobic exercise, but only shows a 57.9 % chance on anaerobic exercise⁴⁰. These findings are consistent with other reviews, that highlight the fact that the most robust performance enhancement with IPC are for aerobic exercises (> 90 s)^{39,42}. IPC has been shown to improve power output during short-duration cycling^{33,43}, recovery⁴⁴, muscle perfusion⁴⁵ and enhance aerobic adaptations to sprint-interval training without altering systemic hypoxic signalling and immune function³³. The fact that no difference in blood markers of systemic angiogenesis, vasodilation or inflammation has been found between IPC and non-

IPC groups, questioned the role of both humoral and systemic pathways in the signal propagation³³. However, methodological limitations such as the low number of blood sample or the timing of post-training blood sampling may have contribute to this absence of difference between groups³³.

The effectiveness of IPC may depend on factors such as the specific exercise type, IPC protocol, and individual characteristics of the athletes^{42,46}. Consequently, more research would be needed to understand the underlying mechanisms that contribute to IPC effects on exercise performance.

Background on blood flow restriction training

The first studies about blood flow restricted exercises in humans appeared in 1937 with the work of Alam & Smirk^{47,48} on the effects of muscle-ischaemia during and post-exercise on blood pressure. 40 years later, the same kind of studies were conducted to measure blood pressure, but also heart rate (HR), ventilation (\dot{V}_E) and blood flow⁴⁹. Other studies investigated the effects of acute reduction in muscle perfusion on physiological variables⁵⁰⁻⁵². In these studies, the ischaemia was induced using manual cuffs or air chambers. However, these studies did not use the muscle ischaemia in a training purpose. In 1993, based on the adaptations in aerobic capacity following training in patients suffering from intermittent claudication, Sundberg *et al.*⁵³ investigated the potential effects of muscle ischaemia as a training stimulus. The authors showed that ischaemic training with a 20 % blood-flow reduction was more effective than control training in improving endurance and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$), in the legs. Thus, using an air chamber to induce ischaemia, the authors have highlighted the effectiveness of training with a reduced blood flow^{53,54}.

Simultaneously, a new resistance training method known as KAATSU training emerged in Japan, developed by Dr. Yoshiaki Sato⁵⁵. This method involves decreasing blood flow to a muscle by applying an external constricting device, such

as a blood pressure cuff or tourniquet, to provide mechanical compression of the underlying vasculature⁵⁶. The purpose of applying mechanical compression is to restrict the venous blood flow returning to the heart, which limits the re-oxygenation process and leads to the accumulation of metabolites and early appearance of fatigue. The KAATSU training, which means “training with added pressure”²⁸ has gain interest worldwide and his now known as “Blood Flow Restriction” (BFR) training. BFR stands apart from other training techniques involving complete vascular occlusion^{57,58}. In BFR, blood flow is partially restricted while exercising rather than fully occluded, maintaining circulation because the pressure exerted by the cuffs is lower than that needed to induce arterial occlusion.

BFR training has been shown to have beneficial effects on muscular strength and hypertrophy in several populations, including older adults and injured athletes^{28,59–61}. The ability to improve strength and hypertrophy in these populations is crucial since they need to counteract the aging-induced muscle loss or get their pre-surgical intervention muscle mass back⁶². However, gains in strength after resistance training follow a dose-response relationship, which offers best results with high-loads^{62–64}. Indeed, to improve strength, the American College of Sport Medicine (ACSM) recommends a minimal load of 60 %, but with better results at 80 – 85 %⁶³. However, training with high-load may be impossible for specific population. The interest of BFR lies in the fact that coupled with low-load resistance training, BFR maximises the effects of training on muscle strength and mass. In addition, low-load resistance training with BFR is able to produce significant greater strength and muscle mass adaptations compared to low-load resistance training alone, and similar adaptations in muscle hypertrophy compared to high-load resistance training⁶⁵. Thus, an alternative exercise training programme combining low-load resistance training with BFR can result in acute and chronic benefits to skeletal muscles^{28,61,65,66}.

In the last 15 years, research on the effects of BFR has seen a growing interest, with hundreds of scientific publications. The most prolific countries publishing about BFR are the United States of America (USA) with 320 publications followed by Japan, Brazil, Australia and the United Kingdom (UK) ⁶⁷. In this field, the most prolific authors originate from three different universities: the University of Tokyo (Takashi Abe, Tomohiro Yasuda), the University of Oklahoma (Jeremy P Loenneke, Michael G Bemben, J Grant Mouser, Robert A Thiebaud), and the University of Mississippi (Scott J Dankel, Samuel L Buckner, Matthew B Jessee, Kevin T Mattocks) ⁶⁷. The BFR field of investigation finds itself at the crossroads of the sport sciences and clinical medicine areas, marked by a multitude of impactful publications ^{28,61,65}. As this discipline continues to evolve, it opens the door to innovative avenues of research and measurement techniques. Technologies such as electromyography (EMG) for muscle activity analysis, gas exchange analysis for respiratory assessments, near-infrared spectroscopy (NIRS) for real-time tissue oxygenation monitoring are becoming increasingly part of the methodology associated with BFR interventions ⁶⁸⁻⁷⁰. While these tools enhance the knowledge about BFR mechanisms, they offer new opportunities to optimise training protocols and improve clinical applications. Thus, the synergy between the rise of BFR and emerging technologies promises a bright future for this field of scientific research.

CARDIOVASCULAR ADAPTATIONS TO BLOOD FLOW RESTRICTED EXERCISES

Since 1968's Mexico City Olympics, altitude training has become popular to improve aerobic performance through the role of hypoxia ^{71,72}. It has been shown that chronic exposure to hypoxia leads to improved mitochondrial and capillary density and enhanced oxidative capacity ⁷³. However, altitude training requires complex logistic and expensive costs. Therefore, alternatives have been developed to induce a local hypoxia such as BFR and IPC through the control of blood flow.

The effectiveness of BFR training can be attributed to several key mechanisms of the O₂ transport chain. The O₂ delivery from the lungs to the mitochondria is affected by BFR training at the heart level, since cardiac adaptations have been found following endurance training with BFR⁷⁴. In addition, BFR has been shown to improve the blood flow, through the upregulation of angiogenesis-related factors such as the messenger ribonucleic acid (mRNA) expression of HIF-1 α , vascular endothelial growth factors (VEGF and VEGFR-2), and endothelial nitric oxide synthase (eNOS)⁷⁵⁻⁷⁷. Consequently, BFR also has an effect at a microvascular level, inducing capillary growth and muscle O₂ exchanges^{16,78,79}. These mechanisms collectively contribute to the effectiveness of BFR training in promoting muscle growth, endurance, and overall performance. Given that BFR training has an effect on cardiac, vascular and O₂ exchanges, the $\dot{V}O_2$ is then also impacted.

Low-intensity (LI) aerobic exercise performed with BFR has the potential to significantly improve the maximal O₂ uptake ($\dot{V}O_{2max}$) and time to exhaustion (TTE) in young healthy populations^{80,81}. In older adults, results on $\dot{V}O_{2max}$ are more contrasted, but aerobic exercise with BFR does show significant improvement in aerobic performance⁸². High-intensity aerobic exercise performed with BFR has also shown benefits on cardiovascular adaptations and performance⁸³, and could be an effective training method for high-level athletes⁴. However, the efficiency of endurance training with BFR compared endurance training alone, remains to be demonstrated, especially for high-intensity exercises or short-term training durations.

The effectiveness of BFR in increasing $\dot{V}O_{2max}$ could arise from methodological differences between studies. Indeed, several methodological aspects to induce an ischaemia could affect the level of blood flow reduction. Across the different studies found in the literature about BFR training, a wide range of cuff types are used, with different materials, shapes, sizes and technologies. Consequently, each different cuff will provide a different stimulus, even so the

pressure in the cuff is the same. In addition, selecting the pressure is also a source of bias, since arbitrary pressures and personalised pressures could be found. The role of pressure has been investigated on both upper and lower limbs⁸⁴, but a wide range of pressure has been recommended. Thus, the consequences of using either low or high percentage of personalised pressure on cardiovascular and respiratory adaptations remains to be investigated.

This suggests that blood flow restriction training coupled with aerobic exercise may offer distinct benefits for different age or level groups, highlighting its versatility and efficiency as a training strategy²⁸. BFR induces structural changes in skeletal, cardiac and microvascular tissue improving the aerobic performance, that may be triggered by greater hormonal and intracellular signalling responses⁸⁵. Thus, if BFR training has shown effectiveness in improving strength or hypertrophy, the main benefits of training with restricted blood flow may lay elsewhere. As for training in hypoxia, BFR coupled with aerobic training have shown promising results in improving cardiovascular and respiratory adaptations⁸². It is then not surprising that has been suggested as an effective countermeasure to the decrease in aerobic power during spaceflights⁷⁵. However, a substantial part of the literature remains to be explored about the effects of methodological variations on cardiovascular and respiratory adaptations.

STRUCTURE OF THE PRESENT THESIS

The primary objective of this present thesis is to explore the cardiac, vascular, and respiratory responses and adaptations to different degrees of blood flow restrictions. This investigation sheds light on the disturbances of the delivery and utilisation of O₂ in muscles, whether at rest with complete occlusions or during exercise after training with partial occlusions.

The present thesis will focus on the effects of BFR training on the components of the O₂ transport chain, through the relationship between cardiac structure and efficiency, blood flow and muscle oxygenation. Then the effects of BFR training on each component of the O₂ transport chain, focusing on improvements in $\dot{V}O_{2max}$ and submaximal $\dot{V}O_2$ efficiency will be discussed. Similarly, the technologies used to estimate cardiovascular and respiratory adaptations to aerobic training coupled with BFR will be investigated. Thus, available technologies such as NIRS, cardiac impedance or gas analysers to assess the muscle oxygenation, the cardiovascular responses and the gas exchanges will be described. Methodologies of inducing IPC and BFR are heterogeneous, therefore the methodological aspects of inducing a reduction of blood flow will be discussed. The effects of the type of material chosen and the pressure used to inflate the cuffs will be presented to better understand discrepancies in the literature.

This manuscript present original scientific contributions centred on the haemodynamic and cardio-respiratory acute and chronic responses to different blood flow restrictions at rest or during exercise. The first scientific contribution investigated the within- and between-session reliability of NIRS devices at rest, following vascular occlusion. In a second study, the effects of different degrees of arterial occlusion will be investigated, using both arbitrary and individualised pressures. Then, a third study will focus on the haemodynamic and cardio-respiratory adaptations following four weeks of aerobic training with two different individualised occlusion pressures, or without BFR. Finally, the results of the three scientific contributions will be discussed in a general discussion, providing a critical analysis of the findings in the context of existing literature and outlining perspectives for future research.

CHAPTER ONE

Inducing cardiovascular and haematological adaptations with BFR



Achieving a given $\dot{V}O_2$ is best represented by both perfusive and diffusive O_2 conductances ⁸⁶. In other words, the $\dot{V}O_2$ depends on both the delivery of O_2 into the muscle microcirculation (blood flow \times O_2 content) and the diffusion of the O_2 from the microcirculation to the mitochondria. To better understand the functioning of the O_2 transport system from lungs to mitochondria, in which each step contributes to limiting $\dot{V}O_{2max}$, it is most effective to use a graphical representation (Figure 1-1). This diagram should illustrate the relationship between $\dot{V}O_2$ and microvascular partial pressure in O_2 (P_{O_2}), based on the two primary transport equations: the Fick principle ⁸⁷ and the Fick's law of diffusion ⁸⁸⁻⁹⁰.

Equation 1: Fick principle

$$\dot{V}O_2 = \dot{Q} \times (C_{aO_2} - C_{vO_2})$$

$$\dot{V}O_2 = HR \times SV \times (C_{aO_2} - C_{vO_2})$$

Where $\dot{V}O_2$ is expressed in L/min, \dot{Q} represents the cardiac output (L/min), $C_{aO_2} - C_{vO_2}$ is the arteriovenous difference in O_2 concentration (mL/100 mL), HR is the heart rate (beats per minutes; bpm), and SV is the stroke volume (mL).

Equation 2: Fick's law of diffusion

$$\dot{V}O_2 = D_{MO_2} \times (P_{O_2\text{microvascular}} - P_{O_2\text{mitochondrial}})$$

Where D_{MO_2} represents the muscle O_2 diffusional conductance (cm^2/s), $P_{O_2\text{microvascular}}$ is the mean microvascular P_{O_2} within muscle capillaries (mmHg or Torr), and $P_{O_2\text{mitochondrial}}$ is the mitochondrial P_{O_2} (mmHg or Torr). This equation could be simplified given that: $P_{O_2\text{mitochondrial}}$ is negligible compared to $P_{O_2\text{microvascular}}$, and $P_{O_2\text{microvascular}}$ is proportional to muscle venous P_{O_2} (P_{vO_2}). Therefore, $P_{O_2\text{mitochondrial}}$ could be erased from the equation and $P_{O_2\text{microvascular}}$ replaced by P_{vO_2} times a constant, k . Thus, the diffusive O_2 transport equation could be written as follows:

Equation 3: Diffusive O_2 transport equation

$$\dot{V}O_2 = D_{MO_2} \times k \times P_{vO_2}$$

Both equations (Equation 1 and Equation 3) are based on the principle that the amount of O_2 remains constant as it is transported through the system (conservation of mass). Since both equations relate $\dot{V}O_2$ to P_{vO_2} , they could be plotted in the same diagram, known as the Wagner's diagram⁸⁸⁻⁹⁰ (Figure 1-1). In this diagram, the conservation of mass applies only at the intersection point of both curves, indicating the value of $\dot{V}O_{2\text{max}}$ for the given conditions of \dot{Q} , C_{aO_2} , D_{MO_2} . As a chain is only as strong as its weakest link, all steps of the O_2 transport chain significantly affect $\dot{V}O_{2\text{max}}$. Consequently, any changes induced by IPC or BFR training in the O_2 pathway should impact the subsequent $\dot{V}O_{2\text{max}}$.

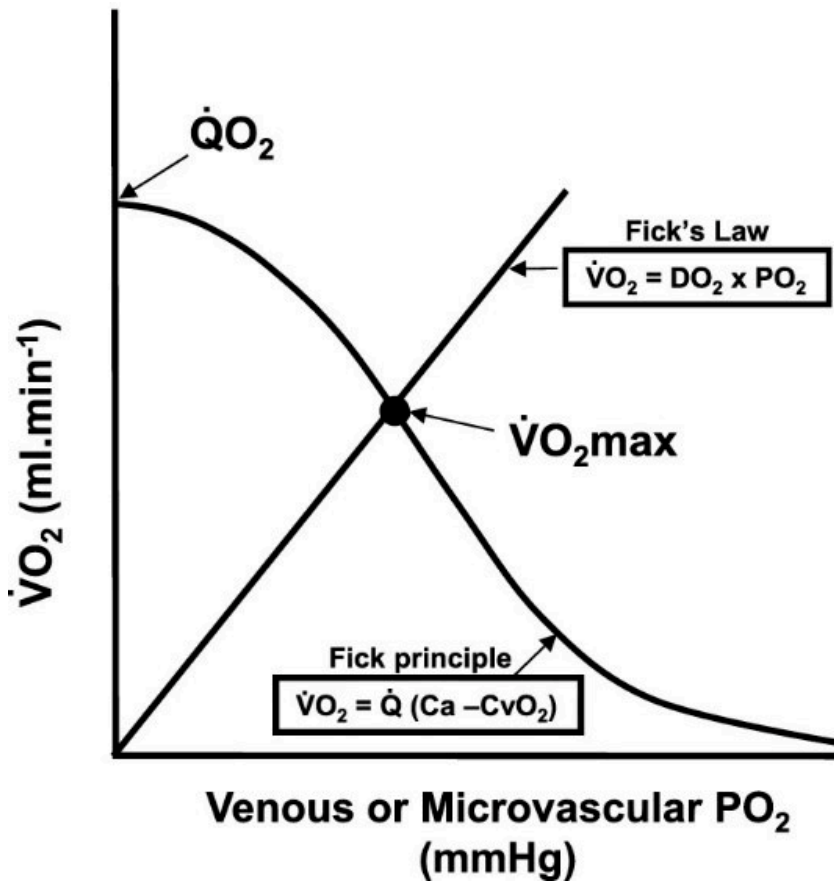


Figure 1-1: Wagner's diagram.

This diagram represents the perfusive (Fick principle, Equation 1) and diffusive (Equation 3) O_2 transport equations⁸⁸⁻⁹⁰. Given the conservation of mass principle, the intersection point between both curves is $\dot{V}O_{2max}$. Here, \dot{Q} represents the cardiac function, C_{aO_2} represents pulmonary gas exchange and blood $[O_2]$, and D_{MO_2} represents the muscle O_2 diffusional properties. From Poole et al. (2022)⁸⁶.

As endurance training enhances $\dot{V}O_{2max}$, both the ability to deliver O_2 through blood flow (perfusion) and its diffusion capacity must increase⁹¹. Any decrease in blood flow at rest (through IPC) or during exercise (through BFR) affects these O_2 transport parameters. Therefore, investigating the effects of IPC or BFR on subsequent steps of the O_2 transport process should offer insights into the mechanisms underlying cardiovascular and respiratory adaptations associated with IPC or BFR training^{3,81}.

1.1 FUNDAMENTALS OF BLOOD COMPOSITION AND EFFECTS OF BFR

The perfusive O_2 fluxes are characterised by the product of the blood flow and the arterial content in O_2 . Consequently, any decrease in CaO_2 (hypoxia) will decrease the O_2 delivered to the muscles and decrease the $\dot{V}O_{2max}$ (Figure 1-2).

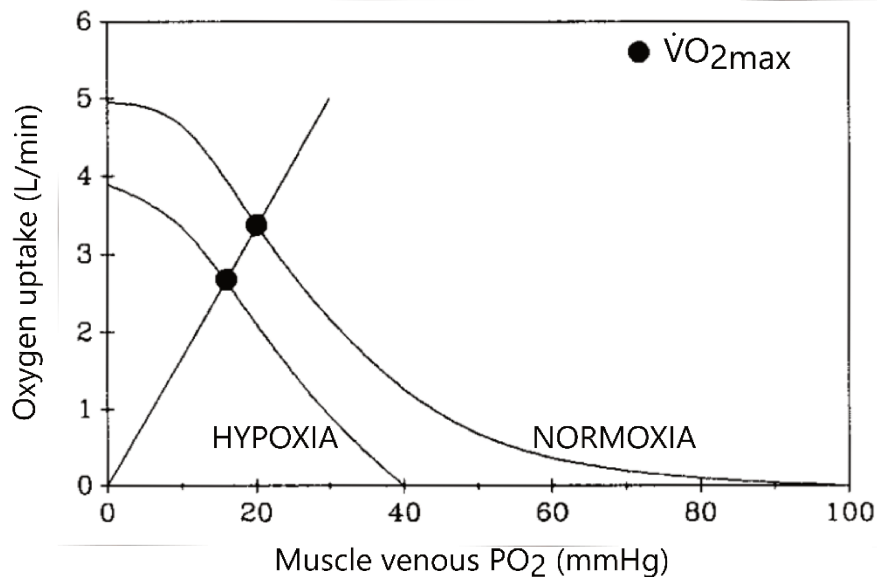


Figure 1-2: Effect of hypoxia on maximal oxygen uptake.

The decrease in CaO_2 caused by hypoxia decrease the perfusive capacity, inducing a reduction in VO_{2max} as shown by the Wagner's diagram. From Wagner (1996) ⁹².

The O_2 transport in the blood is facilitated by the presence of haemoglobin (Hb), an iron-rich protein, in the erythrocytes ⁹³. In the lungs, O_2 molecules bind to the iron atom within Hb, creating bright-red oxyhaemoglobin (O_2Hb). O_2Hb is then transported to the peripheral tissue capillaries, where it undergoes a reverse transformation into deoxyhaemoglobin (HHb) ⁹⁴. When the carbon dioxide (CO_2) produced by the cells reaches the red blood cell in the capillaries, a portion of the CO_2 binds to the Hb, with a preference for HHb ⁹⁴⁻⁹⁶. Returning to the lungs, the CO_2 is subsequently released from Hb and expelled from the body during exhalation.

Thus, erythrocytes have a crucial role in the transport of gases in the body, especially in the ability to meet the muscle demand in O₂ intake and CO₂ removal during exercise.

Haematocrit (Hct), representing the percentage of erythrocytes in blood volume, can be estimated through invasive or non-invasive methods. Non-invasive techniques do not require a blood sample but currently lack the accuracy and reliability to fully replace invasive methods⁹⁷. Invasive methods necessitate a blood sample ranging from approximately 10 µL (equivalent to 1-2 droplets) to around 100 µL⁹⁷. Two distinct invasive modes exist: laboratory-based and point-of-care analysis. Laboratory-based blood analysis methods are well-established and highly precise but require equipped laboratories for processing⁹⁷. Point-of-care methods, including visual inspection or the use of hemoglobinometer, are more accessible for users as they do not demand a fully equipped laboratory and allow for on-site analysis during blood tests. While visual analysis may be employed, it suffers from reliability issues⁹⁷. Therefore, hemoglobinometers are preferred as they offer reliability and accuracy and are convenient to use due to their portability and quick results display (~25 s)⁹⁷. The use of point-of-care hemoglobinometers can then be beneficial in sport sciences due to their ease of use and reliability, but cannot be used to screen patients in a medical field^{97,98}.

A blood sample's volume is constituted by plasma around 55 % and by white blood cells and platelets accounting for less than 1 %. Haematocrit then constitutes the remaining 45 % of the whole blood^{94,97}. However, a sex difference between males and females has been highlighted, with males demonstrating a higher Hct (41 – 50 %) than females (35 – 45 %)⁹⁹⁻¹⁰¹. The same difference is evident Hb, with males displaying higher Hb concentrations (13.5–17 g/dl) than females (12–16 g/dl)^{101,102}. These sex-based differences can be attributed to higher testosterone levels in men, facilitating the formation of erythrocytes, erythropoiesis. They may also arise from genetic differences in the erythropoietin gene and its receptor^{101,102}.

While the quantity of red blood cells (RBC) in the whole blood is important, the primary determinant of how effectively erythrocytes fulfil their O₂ transport function is the concentration of haemoglobin in the bloodstream. The greater the number of haemoglobin molecules within RBC, the greater their capacity to transport oxygen.

Endurance athletes typically exhibit lower Hct levels compared to sedentary individuals due to higher plasma volume, since training leads to a notable increase in total haemoglobin (tHb) mass. Kjellberg *et al.* (1949) observed higher tHb mass for athletes compared to untrained participants⁹⁹. These findings have been subsequently corroborated when comparing participants with different training status or when comparing individuals before and after a prolonged training period¹⁰³. The importance of tHb mass is underscored by the strong correlation ($r \sim 0.79$) existing between $\dot{V}O_2max$ and tHb, with a change of 1 g/kg in tHb inducing a 4.2 – 4.4 ml/min/kg change in $\dot{V}O_2max$ ¹⁰⁴. However, no such correlation exists between $\dot{V}O_2max$ and Hct or Hb, which both depend on blood volume. It is therefore crucial for an athlete to develop their ability to transport O₂ to the working muscles, by increasing the tHb mass or the total erythrocytes volume (tEV). However, significant training-induced tHb mass and tEV changes are slow and may require several years of training^{96,103,104}.

The control of erythropoiesis in following training in normoxia is not well understood⁹⁶, but the effects of training in hypoxia on erythropoiesis are well documented^{73,105,106}. Exposure to hypoxia increases the production of erythropoietin (EPO), an hormone that stimulates the production of RBC, increasing the O₂ transport capacity^{107,108}. Therefore, altitude/hypoxic training methods have emerged to induce beneficial haematological adaptations in athletes. However, the effects of passive (IPC) and active (BFR) training with induced local hypoxia on haematological changes present limited evidence. IPC may be effective in increasing RBC deformability for responsive participants^{101,109}, facilitating O₂ exchanges with

muscles in smallest capillaries. But, no studies have shown an increase in tEV following IPC protocol. For BFR, two studies have shown an increase in RBC quantity following endurance training (walking or cycling) ^{110,111}. Thus, BFR may serve as an efficient stimulus to activate EPO signalling and induce an increase in RBC quantity to potentiate the O₂ delivery to working muscles.

Thus, the blood composition of an individual influences its ability to respond to the elevated O₂ demand during exercise. Training in hypoxia has shown effectiveness in increasing the erythropoiesis and BFR training has shown promising results in inducing haematological changes. These adaptations are beneficial for enhancing performance but must be accompanied with haemodynamic changes to ensure an adequate O₂ delivery to working muscles.

1.2 BFR ENHANCES CARDIAC ADAPTATIONS

The first parameter of Fick principle (Equation 1) is \dot{Q} , or the product of HR and SV. As the heart pumps the blood throughout the vascular system, \dot{Q} represents the blood flow sent to the muscles by the heart. Endurance training leads to heart size enlargement, called cardiac hypertrophy^{112,113}. These structural changes caused by endurance training induce functional adaptations of the cardiac muscle, that increases the capacity of the heart to pump the blood.

1.2.1 Mechanisms for enhancing cardiovascular parameters

Long-term exercise training is accompanied by an healthy increase in the mass of the heart (especially left ventricular mass), that is called "athlete's heart"^{112,113}. Contrary to foetal cardiac growth that occurs through hyperplasia (increase in cell number), hypertrophy (increase in cell size) drives the growth of the heart after birth^{114,115}. The hypertrophic growth of cardiac myocytes (major constituent cells of the heart) is accompanied by an increase in protein accumulation caused by an accelerated rate of protein synthesis^{116,117}. Accelerating the rate of protein synthesis belongs to the properties of activated mammalian target of rapamycin (mTOR)

pathway, which has been shown to be implicated in exercise-induced cardiac hypertrophy¹¹⁸. During exercise, there is an afflux of blood in the heart due to an increased in venous return, increasing SV¹¹⁹. The greater volume of blood in the left ventricle stretches the myocardium and triggers the costamere that acts as a mechanoreceptor in the cardiac muscle cells¹¹⁸. The costamere is a structure that connects extracellular matrix to intermediates filaments within the muscle. This structure contains proteins that binds with integrin linked kinase (ILK), triggering a signalling pathway that leads to the activation of mTORC1 in the heart, and the subsequent cardiac hypertrophy^{118,120}. The mTOR pathway has then an essential role in the athlete's heart induced by training^{118,121-123}, inducing beneficial effects for health and performance^{112,113,115,118,124}.

The activation of mTOR pathway during BFR has been particularly investigated on skeletal muscles, since BFR has been shown to induce a cell swelling effect. Cell swelling is an increase in intracellular hydration¹²⁵ and is believed to be an essential regulator for cell function^{126,127}. Cell swelling during BFR training is a result of the pressure applied by the inflated cuffs, causing blood pooling and inducing a plasma fluid shift. This shift leads to changes in intracellular and extracellular water balance, increasing the influx of water into the muscle cells¹²⁸⁻¹³⁰. The detection of cell swelling occurs through an intrinsic volume sensor, that triggers the mTOR and mitogen-activated protein-kinase (MAPK) pathways, when activated¹²⁸.

Although the stimulation of the mTOR pathway in skeletal muscle by BFR exercise has been demonstrated¹²⁸⁻¹³⁰, no studies have shown greater mTOR signalling activation in the heart following BFR exercise compared to non-BFR exercise. BFR activates mTOR locally by detection of cell swelling¹²⁸. But, as the heart is not occluded during exercise, the blood pooling must happen distally to the heart. Consequently, the BFR mechanisms for mTOR activation in skeletal muscle does not align with a BFR-induced cell swelling inducing cardiac hypertrophy. However,

cardiac hypertrophy and mTOR pathway activation in the heart occur through myocytes stretching by a volume overload ¹¹⁸. But, when the blood flow is restricted by a cuff in a limb, the venous return to the heart is reduced ^{28,131}. Thus, BFR training should not be effective in inducing cardiac hypertrophy. However, even if the venous return is limited, during exercise with BFR, the volume of blood returning to the heart increases to fulfil muscular O₂ requirements ^{132,133}. Then, the stretching of the myocardium is still happening. Consequently, cardiac hypertrophy mechanisms described earlier could still take place in addition to concomitant muscular and vascular beneficial adaptations.

In addition, BFR exercise also stimulates VEGF production in skeletal muscle as well as in the cardiac muscle (in rodents) ¹³⁴, which therefore activates the VEGF angiogenic signalling cascade, that triggers the cardiac remodelling by forming new blood vessels in the myocardium ^{135,136}. Regarding signalling pathways, training with BFR in rodent model have resulted in significant increase in peroxisome proliferator activated receptor Gamma coactivator 1- α (PGC1- α) implicated in endurance exercise-induced adaptations by promoting mitochondrial biogenesis and angiogenesis ¹³⁷. BFR training also resulted in the highest expression of endothelial nitric oxide synthase (eNOS) in the myocardium, as well as the highest level of eNOS phosphorylation ¹³⁸. eNOS is known to synthesize NO, which stimulate the local expression of VEGF and promote the growth of blood vessels in the myocardium ^{135,136,139}. BFR training therefore improves cardiac function, potentially through the improvement of myocardial microvascular function caused by a greater activation of signalling pathways (VEGF-Pi3k-Akt-eNOS) ¹³⁸.

Consequently, while BFR exercise decreases venous return to the heart and reduces the volume overload that typically drives cardiac adaptations, it still results in an overall increase in blood volume returning to the heart due to the exercise itself. Furthermore, BFR has been demonstrated to stimulate cardiac angiogenesis in rodents. Therefore, despite the reduced venous return, BFR may remain an effective

stimulus for increasing \dot{Q} .

1.2.2 Enhancing cardiac output with training

During exercise, \dot{Q} increases to meet the O_2 requirement of the muscles. In elite athletes, the cardiac output increases to a greater extent compared to the general population during maximal exercise¹⁴⁰⁻¹⁴⁴. This difference between general population and elite athletes is caused by the effect of endurance training on the cardiac muscle, that increases \dot{Q} as shown by early studies on that topic^{140,141}. Given that \dot{Q}_{max} is the product of HR_{max} and SV_{max} , a significant increase in one or both parameter is needed to enhance \dot{Q}_{max} . While the main factor driving \dot{Q} increase during exercise is HR, HR_{max} remains relatively unaffected by training, and could even show a decrease. Therefore, the increase in \dot{Q}_{max} after training is due to a larger SV. The meta-analysis of Montero *et al.* (2015) has highlighted the fact that increase in \dot{Q}_{max} was significantly due to the sole increase in SV_{max} (medium effect size: standardized mean differences = 0.66^{145,146}), since HR_{max} was shown to decrease significantly after endurance training (mean difference = - 1.8 bpm). In an older population (mean age ≥ 40 years), the SV_{max} was also significantly increased whereas HR_{max} was unaltered, leading to an increased \dot{Q}_{max} after 8 to 52 weeks of endurance training¹⁴⁷.

This increase in the maximal quantity of blood ejected at each heartbeat following endurance training is likely to be influenced by either a greater filling of the left ventricle (*i.e.* left-ventricular diastolic filling) and/or a reduction in the volume of blood remaining after each ventricular contraction (*i.e.* end-systolic volume)¹⁴⁴. In other words, the SV is increased by either a greater filling or ejection effectiveness. Endurance training induced myocardium structural changes are associated with a greater capacity of the left ventricle to stretch and expand in response to filling with blood during diastole while maintaining adequate pressure (*i.e.* left-ventricular compliance). This stretching increases the sarcomere length within the cardiac muscle cells, leading to increased overlap between actin and myosin filaments,

which enhances the force of contraction during systole. Greater left-ventricular (LV) compliance in athletes has shown to induce larger SV during exercise through the Frank-Starling mechanism^{119,148–150}, that states that end-diastolic volume regulates the work of the heart¹⁵¹.

Endurance trained athletes have shown increased LV diastolic diameter and subsequent volume, LV mass, and LV wall thickness, enhancing the filling capacity and contractility of the heart^{143,144}. These findings are confirmed by longitudinal studies lasting 7 months (rowing) to 3 years (cycling), that show increases in LV cavity dimension from 51.5 to 53.3 mm and from 58.3 to 60.3 mm, respectively^{142,152}. Therefore, endurance training is characterised by a balanced increase in both LV diameter and mass, that is called “eccentric hypertrophy”^{144,153–155}. Indeed, one year of progressive endurance training (3 or 4 endurance training sessions per week during a 12-months period) of sedentary participants has led to cardiac morphological and functional adaptations¹⁵⁶. Twelve months after training beginning, participants exhibited significant increases in LV mass (mean difference = + 35 g or +21 %), in LV end-diastolic volume (mean difference = + 21 mL or + 18 %), and in SV_{max} (mean difference = + 15.5 mL or + 16 %) ¹⁵⁶. In addition, the results of pressure-volume curves suggest a reduced pericardial constraint and improved myocardial compliance after the training period¹⁵⁶. However, it is noteworthy that the training-induced cardiac remodelling in the study of Arbab-Zadeh *et al.* (2014), depends on training intensity. While the first three months of training were performed at lower-intensity, the LV mass increased through an increase in wall thickness (“concentric hypertrophy”), without significant increase in LV end-diastolic volume. But, when the training intensity and duration have been increased (after 6 to 9 months), the LV wall thickness decreased, and the LV end-diastolic volume increased significantly (“eccentric hypertrophy”) ¹⁵⁶.

1.2.3 Enhancing cardiac output with BFR

Tangchaisuriya *et al.* (2022) have investigated the effects of 12 weeks of

cycling training with either continuous training (CT) at 65-70 % of peak power output (PPO) or HIIT (4*4 min at 80 % of PPO) ¹⁵⁷. The results have shown that both training methods are able to elicit \dot{Q}_{max} adaptations following the training period, without differences between them. However, it is noteworthy that when BFR was coupled with HIIT (HIIT+BFR), the results also showed a significant \dot{Q}_{max} increase, while having the lowest weekly training volume (5934 KJ vs. 7723 KJ and 6116 KJ for CT and HIIT, respectively) and the lowest training mean cycling intensity (54 % of PPO). Therefore, although BFR decreases the venous return and the subsequent SV during exercise ^{28,131}, restricting the blood flow could still be an effective stimulus to induce cardiac function adaptations ¹⁵⁷.

The results from the study of Tangchaisuriya *et al.* (2022) have shown that increase in \dot{Q}_{max} in CT, HIIT and HIIT+BFR is driven by a significant increase in SV_{max} (+ 4 to 7 mL), while HR_{max} remains unchanged ¹⁵⁷. Unfortunately, studies on endurance exercise combined with BFR are lacking in the literature. Consequently, the impact of training with restricting the blood flow on cardiac morphological adaptations is not known yet. However, studies have investigated these adaptations in the rodent model. In a rodent model, it has been shown that the addition of BFR to mild endurance exercise for 10 weeks leads to a significant increase in cardiac hypertrophy index, whereas neither exercise alone or BFR alone has shown significant changes in cardiac hypertrophy index ¹⁵⁸. More specifically, the LV volume, and the volumes of myocytes and capillaries were significantly greater in the endurance-trained with BFR group than in the non-exercising control groups. The increase in LV volume in the endurance trained without BFR group was not significantly lower than training with BFR, and not significantly greater than control groups ¹⁵⁹. In addition, 8 weeks of training induced a significant increase in ejection fraction and fraction shortening, demonstrating a greater heart efficiency. However, there was no significant difference between either high-intensity resistance training (HIRT), low-intensity resistance training (LIRT), or BFR training ¹⁵⁸. Mild endurance training in rodents performed with BFR has also shown to significantly reduce the

LV end-diastolic pressure and time constant, and improve the heart contractility at rest compared to training without BFR ¹³⁷. These improvements in heart structure and function are associated with greater heart remodelling and performance ^{137,158,159}.

Although no studies on humans are available on cardiac morphological adaptations induced by endurance training with BFR, data is available on rodent model. Through mechanical (volume-overload) and chemical (signalling pathways) stimulations, BFR may be effective in inducing changes in the structure and function of the heart. Thus, endurance training with BFR could induce cardiac adaptations that will increase the \dot{Q}_{max} and, consequently, the $\dot{V}O_{2max}$. However, \dot{Q} and \dot{Q}_{max} are not the sole parameters that rules the blood flow that reaches the muscles. Indeed, the 100-fold increase in muscle blood flow during exercise is not induced by a 100-fold increase in \dot{Q} , but rather by the ability of the blood vessels to constrict and dilate ^{160,161}.

1.3 BFR AFFECTS HAEMODYNAMICS

The blood flow delivers nutrients, hormones, and O₂ to skeletal muscles. But, during exercise the demand of muscles increases, inducing a subsequent need for increasing blood flow. Matching the muscle requirements in O₂, especially depends on the available O₂ in the blood and the ability of the cardiovascular system to increase the rate of blood delivery ¹⁶². While these parameters could be enhanced through aerobic training ⁹⁹, exercising with restricted blood flow has been shown to improve vascular functions ¹⁶³.

1.3.1 Understanding haemodynamics

Blood vessels are divided in three main categories: the arteries, the capillaries and the veins. Their diameter range from 1.5 cm to 37.0 μm for arteries, from 20.0 μm to 5.0 mm for veins, and 5.0 to 10.0 μm for capillaries ⁹⁴.

The circulatory system consists of interconnected blood vessels that facilitate the circulation of blood driven by pressure differentials between the arterial and venous systems (Figure 1-3). Specifically, blood flows from high-pressure regions to low-pressure areas, traversing a sequence of large arteries, arterioles, and capillaries before returning to the heart via venules and veins. During the heart's systolic phase, the left ventricle maintains an average pressure of approximately 120 mmHg, while the right atrium, responsible for receiving venous blood, maintains a pressure close to 0 mmHg (Figure 1-3). This pressure contrast between the arterial and venous systems enables the continuous flow of blood from the heart to the peripheral tissues and back to the heart.

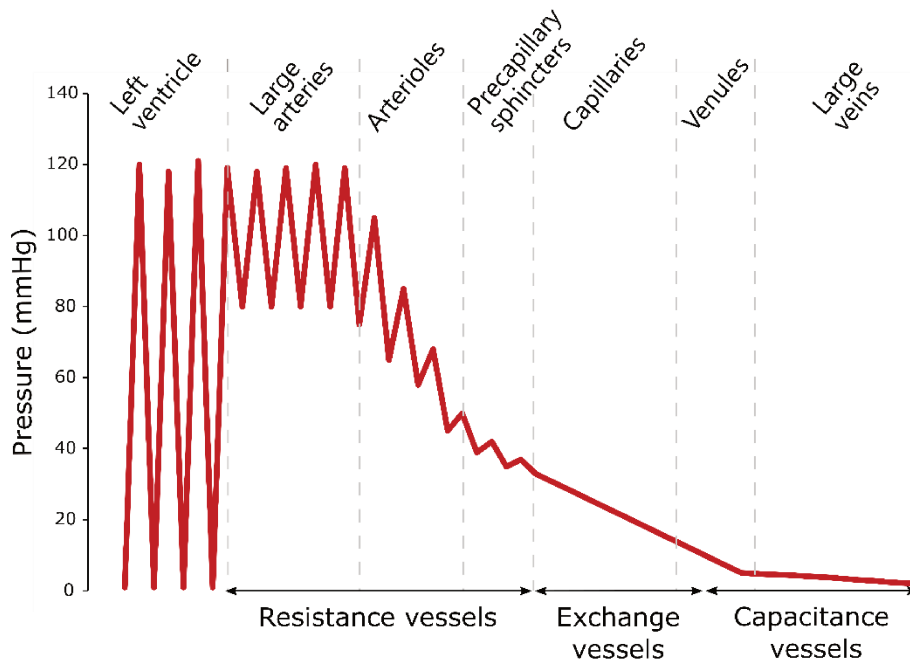


Figure 1-3: Pressure changes across the different blood vessels of the vascular system.

The blood pressure is higher in large arteries (≈ 120 mmHg) than in small capillaries ($\approx 20 - 30$ mmHg). Veins show the lowest blood pressure (until ≈ 4 mmHg). This gradient of pressure allows the blood to circulate across the vascular network in a closed loop. Adapted from Kenney, Wilmore & Costill (2017) ¹⁶⁴.

This gradient arises from the resistance of the vessels against the blood flow. This resistance is contingent upon several factors, including the length of the vessels, the diameter of their lumens, and the viscosity of the blood itself. To quantify this resistance, Poiseuille's Law is employed (Equation 4 and Equation 5) ¹⁶⁵, which can be expressed as follows:

Equation 4 : Poiseuille's law defining the resistance of the blood vessels

$$R = \frac{8\eta L}{\pi r^4}$$

Here, R represents the resistance pascal-seconds per cubic meter ($\text{Pa}\cdot\text{s}/\text{m}^3$), η signifies blood viscosity ($\text{Pa}\cdot\text{s}$), L corresponds to the vessel length (m), and r stands for the vessel's lumen radius (m).

Simultaneously, the blood flow equation can be expressed as follows:

Equation 5 : Poiseuille's law defining the flow rate (F) in the blood vessels

$$F = \frac{P2 - P1}{R}$$
$$F = \frac{(P2 - P1)\pi r^4}{8\eta L}$$

Where F is expressed in m³/s, and P2 – P1 stands for the gradient of pressure between two vessels (Pa).

Since the vessel diameter 'r' is raised to the power of 4, any decrease in diameter resulting from factors such as branching, plaque formation, or compression by an inflated cuff will result in a corresponding reduction in blood flow. For example, a 5 % reduction in the vessel's radius leads to a decrease in blood flow to approximately (0.95)⁴ = 0.81 of its original value. In other words, a 5 % reduction in radius causes a significant 19 % decline in blood flow. Any pathological or mechanical obstructions in these vessels will then impair the delivery of O₂ to the muscles. To counteract such limitations, the cardiovascular system must increase pressure to sustain the necessary blood flow, or increase their radius by relaxing smooth blood vessels muscles. This process called vasodilation is opposed to vasoconstriction, and requires an healthy and efficient endothelial function ^{166,167}.

As blood flow and pressure diminishes in the smaller arteries, arterioles and capillaries, these vessels have a crucial role in facilitating blood circulation and enabling essential exchanges with the body's tissues ¹⁶⁸. The capillaries gather to form a vascular bed, that arises from the terminal arteriole and end in the postcapillary venule. The microcirculation stands as an essential O₂ exchanges place ¹⁶⁹. Because of the high intrinsic O₂ affinity of Hb, several mechanisms are implicated to decrease this Hb-O₂ affinity and release the O₂ from the Hb, that could then diffuse to the cell tissues. The Bohr effect implies that Hb-O₂ affinity is reduced as

the blood pH decreases and CO₂ concentration ([CO₂]) increases, facilitating the diffusion of O₂ to the cells¹⁷⁰. This effect is illustrated by the fact that in situation of high ATP demand, the O₂ release from the Hb molecule is facilitated by the decrease in O₂-Hb affinity, to promote oxidative phosphorylation^{96,170}. Coupled with an increased blood flow due to the exercise-induced vasodilation of the muscle capillaries, the modulation of O₂-Hb affinity allows the exercising skeletal muscles to meet their O₂ requirements during exercise^{13,96,171–174}.

1.3.2 Effects of BFR on blood flow during exercise

Vasodilation and vasoconstriction correspond to the vasomotor function, reflecting the ability of the blood vessels to dilate or constrict, altering their diameter. Vasodilation occurs when more blood is needed to the tissues (*e.g.* to fulfil the O₂ requirements during exercise) and corresponds to a relaxation of the smooth muscle cells within the vessel walls, increasing the blood flow. Thus, during exercise, the muscle blood flow can increase almost 100-fold compared to resting state¹⁷⁴, thanks to the vasodilatory mechanisms. The relaxation of blood vessels may be exerted by the action of vasodilators secreted by the endothelium and the reduction of the vasoconstrictive effect of the sympathetic activity, called functional sympatholysis^{174,175}.

Even though the sympathetic nervous system influences vascular beds throughout the body, active skeletal muscle vessels can escape sympathetic vasoconstriction through functional sympatholysis^{171,175–177}. This response reduces noradrenaline's impact on alpha-adrenoceptors during exercise, in an intensity-dependent manner^{174,178}. Furthermore, the release of ATP, particularly from RBC in response to HHb formation, may contribute to exercise hyperaemia by promoting vasodilation and attenuating local sympathetic vasoconstriction¹⁷⁷. Overall, skeletal muscle blood flow balance is determined by the interplay between sympathetic activity, vasoconstrictors, and vasodilators, such as NO, prostaglandins (PGs), prostacyclin, ATP, adenosine, potassium (K⁺)^{160,179,180} and other compounds, such as

H⁺ and calcium (Ca²⁺) produced locally in the skeletal muscle tissue ¹⁸¹.

Under hypoxic conditions the decrease in arterial concentration of O₂ (CaO₂) serves as a potential signal for the increased compensatory vasodilation occurring in the vascular beds of skeletal muscle during hypoxic exercise ^{182–184}. It has been shown that, although sympathetic activity is elevated in hypoxia compared to normoxia, the sympathetic vasoconstriction in the vascular beds of working muscles is blunted to match the O₂ delivery ^{13,175,185}. However, this phenomenon may be attenuated by long-term exposure to hypoxia, which increases the CaO₂ through a greater amount of haemoglobin in the blood ⁹⁶. Overall, systemic hypoxia leads to compensatory vasodilation, promoting increased blood flow in skeletal muscle vascular beds during both rest and submaximal exercise to ensure adequate O₂ delivery ^{13,186–188}. Conversely, hyperoxia, characterised by an excess of O₂ in the tissues, reduces resting muscle blood flow and increases vascular resistance ^{13,189}. Thus, in both conditions that alters the O₂ availability, the blood flow is regulated to provide an adequate supply of O₂ to the tissue, inducing vasodilation or vasoconstriction mechanisms.

When the delivery of O₂ to the muscles is insufficient (i.e., ischaemia), the compensatory vasodilation acts less efficiently than in hypoxia. When the blood flow is occluded by an external device during exercise, the vasodilation is not powerful enough to restore the blood flow to pre-inflation levels ^{13,190,191}. Indeed, by gradually increasing the pressure in a pressure chamber, the blood flow of the exercising forearm is reduced by 20 – 25 % and the O₂ perfusion by 15 – 18 % ¹⁹¹. However, Daley *et al.* (2003) showed that under 50 mmHg of positive pressure, the blood velocity is restored at levels of ambient pressure ¹⁹². The authors suggest that under high levels of ischaemia, the restoration of blood flow is constrained by a systemic sympathetic vasoconstriction response activated by the autonomic nervous system. These findings are consistent with Renzi *et al.* (2010) showing that a decrease in flow-mediated vasodilation (FMD) of the popliteal artery when participants

underwent a walking task with BFR (up to 160 mmHg)¹³². This decrease in FMD of the popliteal artery compared to the control group (walking without BFR) demonstrates an impairment of the endothelial function and the ability of the arteries to constrict or dilate¹³². The reduction of the endothelial function may then explain the inability of the arteries to restore the blood flow to the muscle by vasodilation. The effects of occlusion were also investigated invasively by inflating a balloon in the brachial artery causing a reduction in forearm blood flow¹⁹⁰. At rest and during exercise with the inflated balloon, the blood flow was partially restored (75 %) without significant increase in blood pressure, indicating the essential role of vasodilator mechanisms in the restoration of blood flow. Vasodilator agents such as NO, adenosine and ATP release from the erythrocytes seem to have a combined effect on the vasodilatory compensation mechanism when the O₂ availability is reduced^{13,190}.

Beyond acute changes on blood flow and vasodilation, training with restricted blood flow could also induce chronic changes in endothelial function and blood flow regulation¹⁹³. Patterson & Ferguson (2010) compared the effects of resistance training with and without BFR in the leg on untrained females¹⁶³. For 4 weeks, one leg was trained with BFR whereas the other leg was trained without. The results showed that after 4 weeks, the post-occlusive blood flow was greater for the BFR-trained leg compared to the other leg, although they received the same resistance training protocol. These results suggest that BFR training may be a potent stimulus to enhance the vascular function of participants¹⁶³. Moreover, a recent meta-analysis highlighted the efficiency of BFR training in improving FMD ($p= 0.002$) compared to non-BFR training¹⁹³. Several mechanisms may explain the greater vascular function following BFR training: a greater secretion of vasoactive metabolites, an increased capillarity or a greater venous compliance¹⁶³.

Apart from external application of a compression device, the blood flow could be reduced by the sustained contraction of the muscles inducing linear increase in intramuscular pressure¹⁹⁴. Therefore, the greater the exercise intensity, the greater the intramuscular pressure, implying prolonged periods of ischaemia during intense exercise. Training that involves sustained isometric contractions or repeated intense concentric contractions induces a mechanical ischaemia caused by the muscle tension during contraction. These changes induce endothelial and vasodilatory adaptations, since experts show greater blood flow during exercise and following arterial occlusion compared to sedentary controls¹⁹⁵. In addition, upon the release of the arterial occlusion the increase in blood flow, called post-occlusive reactive hyperaemia (PORH), is greater for experts compared to sedentary. Moreover, the greater PORH is not accompanied by any changes in blood pressure, meaning as already mentioned, that increase in blood flow were mainly due to vasodilatory mechanisms¹⁹⁵. These findings are not sport specific and have been found in climbers, tennis players, endurance-trained participants and manual workers¹⁹⁶⁻¹⁹⁸.

BFR has then been shown effective in increasing blood flow and vasodilatory mechanisms, that belongs to the perfusive elements of the Wagner's diagram (Figure 1-1). However, a greater blood flow in the same network length would decrease the O₂ diffusion capacities⁸⁸. Therefore, the increases in perfusive elements must be accompanied by a greater vascular network, increasing the ability to diffuse O₂ to the mitochondria.

1.4 BFR ENHANCES VASCULAR REMODELLING

1.4.1 Angiogenesis, arteriogenesis and vasculogenesis

Vasculogenesis, arteriogenesis, and angiogenesis are distinct processes essential for blood vessel formation and growth, each characterised by specific mechanisms and roles¹⁹⁹. Vasculogenesis refers to the *de novo* (from scratch) formation of primitive blood vessels from angioblasts during embryonic development, with a limited postnatal contribution²⁰⁰.

Arteriogenesis pertains to the remodelling and growth of pre-existing arteriolar connections into functional collateral arteries^{200,201} (Figure 1-4). It is driven by increased fluid shear stress that may occur during pathological (atheroma plaque) or mechanical (IPC/BFR) flow restriction^{4,202,203}. It involves the proliferation of endothelial and smooth muscle cells, with significant recruitment of monocytes providing necessary growth factors and cytokines^{200,201,204}.

Angiogenesis involves the sprouting of new capillaries from pre-existing vessels²⁰⁵ (Figure 1-4). This process is primarily induced by hypoxia and plays an essential role in responding to increased metabolic demands of tissues, such as during exercise or under conditions of low O₂ supply^{201,204}.

Angiogenesis



Arteriogenesis

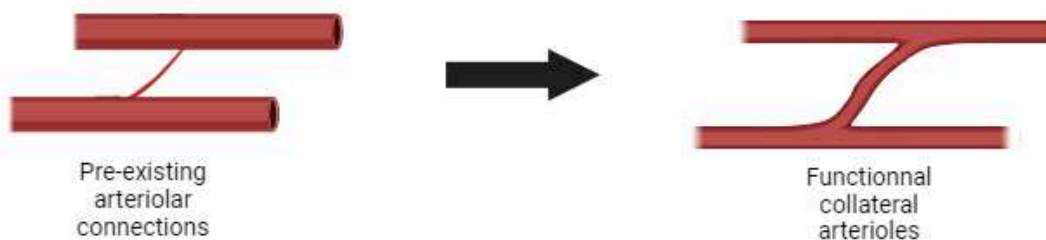


Figure 1-4: Illustration of angiogenesis and arteriogenesis.

Illustration of the mechanisms of angiogenesis (sprouting of new capillaries) and arteriogenesis (remodelling of arterial connections into functional collaterals). Original illustration adapted from multiple sources^{200,201,204}.

Thus, while vasculogenesis establishes the initial vascular framework during embryonic development, arteriogenesis expands pre-existing arteriolar connections, and angiogenesis enhances the capillary network, through distinct processes.

1.4.2 Effects of BFR on vascular remodelling factors

The vascular remodelling induced by arteriogenesis, and angiogenesis is driven by distinct stimuli, cellular activities, and growth factors. While angiogenesis is primarily triggered by chemical stimuli induced by hypoxia, arteriogenesis is triggered by the mechanical stimulus of shear stress²⁰¹.

1.4.2.1 Shear stress and arteriogenesis

Fluid shear stress in blood vessels refers to the frictional force exerted by the flowing blood on the endothelial lining of the vessel wall. This tangential drag force arises due to the velocity gradient between the faster-flowing blood in the centre of the vessel and the stationary endothelial layer. Shear stress is expressed as :

Equation 6: Fluid shear stress

$$\text{Shear stress} = \frac{4\mu F}{\pi r^3}$$

Where μ is the blood viscosity (Pa·s), F is the flow rate (Equation 5; m³/s) and r is the vessel radius (m)²⁰⁶. Consequently, any increase in blood viscosity due to haematological changes such as an increased tEV, or any increase in flow rate caused by exercise or cuff deflation, causes an increase in shear stress. This increase in forces against vessel wall activates the endothelial surface receptors and upregulates the adhesion molecules such as monocytes. Then follows the mitosis of endothelial and smooth muscles cells to transform the pre-existing connections into a functional collateral^{200,204}.

Therefore, BFR training may increase shear stress and potentially activate arteriogenesis mechanisms²⁰⁷. However, direct evidence linking BFR training with arteriogenesis, particularly when combined with endurance exercise, remains

limited. Thus, arteriogenesis could not yet be considered as a major vascular adaptative response of BFR training, but evidence is accumulating for the role of BFR in enhancing angiogenesis ^{193,207,208}.

1.4.2.2 Angiogenesis-related factors and vascular restriction

Angiogenesis involves complex pathways, and a key growth factor involved is VEGF ^{209,210}. VEGF is a protein secreted by various cells, including muscle cells, in response to hypoxic signals or mechanical stress. Once released, VEGF binds to its specific receptor, vascular endothelial growth factor receptor-2 (VEGF-R2), on the surface of vascular endothelial cells. This interaction triggers a cascade of intracellular signals that stimulate new endothelial blood vessels' migration, proliferation, and formation (Figure 1-5) ^{205,208,211,212}. HIF-1 α , a transcription factor, also plays an essential role in this process. Under hypoxic conditions, HIF-1 α accumulates and binds to specific regions of the VEGF gene, promoting its transcription and increasing VEGF production (Figure 1-5). Additionally, PGC-1 α positively regulates VEGF expression in muscle tissue. These coordinated mechanisms ensure an adequate angiogenic response based on tissue needs ^{205,208,211,212}.

During low-load resistance exercise with BFR, reduced muscle O₂ levels stabilise HIF-1 α , leading to targeted activation of VEGF transcription ²¹³. These events enhance the availability of VEGF at endothelial cell receptor sites, facilitating VEGF-induced angiogenesis ²¹⁴ and are mainly mediated by PGC-1 α in skeletal muscle ²¹⁵. Research indicates that BFR exercise can facilitate VEGF expression by enhancing HIF-1 α ^{211,216}, although conflicting views exist among other researchers ²¹⁷.

Larkin *et al.* (2012) investigated the effects of 120 unilateral knee extensions performed at 40 % of one-repetition maximum (1-RM) with and without BFR on serum and muscle VEGF ²¹⁶. The authors found that BFR significantly increased the

post-exercise muscle expression of VEGF, VEGF-R2, and HIF-1 α with a fourfold up-regulation of VEGF at 4h post-exercise. These findings are interesting since they highlighted that BFR is a potent stimulus of the VEGF pathway, from the HIF-1 α to VEGF-R2 expressions. Increased VEGF and HIF-1 α have been previously reported after knee extensions with or without BFR applied using a pressure chamber, with a significant difference between BFR and non-BFR in VEGF expression. However, no significant difference was found in HIF-1 α between both groups ²¹⁸. More recently, an higher expression of VEGF in the BFR group compared to the control group has been found following an acute session of knee extensions performed at 20 % of 1-RM ²¹¹.

Barjaste *et al.* (2021) reported significant increases in VEGF, HIF-1 α , and PGC-1 α protein expression at 3 hours after BFR-walking, while no increase was reported in the non-BFR group ²¹³. These findings confirmed an animal-based study, which showed increased PGC-1 α protein levels during low-intensity BFR aerobic training ²¹⁹. However, these findings contradict the results found in low-intensity cycling with BFR ^{220,221}. The authors reported no increase in VEGF, HIF-1 α or PGC-1 α above rest following a 15 min cycling bout at 40 % of $\dot{V}O_2peak$ (*i.e.* highest $\dot{V}O_2$ value reach during the test) with BFR, while 30 min cycling at 70 % of $\dot{V}O_2peak$ enhanced VEGF, HIF-1 α and PGC-1 α expression ²²¹. Differences in exercise modes in these studies reflect varied metabolic stress and mechanical tension, explaining the varying protein expression patterns. For example, low-intensity BFR walking better stimulated the expression of HIF-1 α , VEGF, and PGC-1 α proteins compared to low-intensity BFR cycling ²¹³.

However, using a 30-second sprint exercise with or without BFR, Taylor *et al.* (2016) elicited significant increases in PGC-1 α , VEGF, and VEGF-R2 for both groups and a significant increase in HIF-1 α , precursor of VEGF, only for BFR ²¹⁷. The increased HIF-1 α following BFR training may then induce enhanced capillary density ²¹⁷. However, this has not been demonstrated by the authors ²¹⁷. Thus, the intensity

of exercise protocols may also be essential in activating HIF-1 α during BFR exercise. Some studies found no significant differences in HIF-1 α protein levels between BFR and non-BFR conditions, suggesting that the degree of hypoxia might already be sufficient during non-BFR exercise at specific intensities ²¹⁸.

The stabilisation of HIF-1 α allowing the expression of VEGF occurs through the action of reactive oxygen species (ROS) ²¹². ROS are highly reactive compounds arising from diatomic O₂ characterised by unpaired electrons, making them particularly reactive. Oxidative stress arises when ROS production surpasses the antioxidant system's capacity to neutralise these molecules ²²². Acute oxidative stress can activate signalling pathways that promotes cellular and vascular adaptations ¹³⁰, while chronic oxidative stress might lead to detrimental outcomes like cell damage or death ²²³.

ROS generation necessitates periods of low-flow or intermittent ischaemia interspersed by periods of reoxygenation caused by blood flow restoration ²²⁴. At rest, protocols of IPC have shown effectiveness in increasing ROS production ^{225,226}, especially due to the reactive hyperaemia (*i.e.*, the increase in blood flow above baseline value) found after ischaemia ^{227,228}. The IPC-induced ROS generation is thought to confer the cardio-protective effects of this procedure ^{225,229,230}.

Studies indicate that acute resistance exercise can trigger ROS generation ^{28,231}, that might enhance angiogenesis, mitochondrial biogenesis, and muscle hypertrophy ²³². ROS also accompany skeletal muscle injury regeneration and are crucial for activating satellite cell-mediated skeletal muscle hypertrophy ^{233,234}. While exercise alone induces ROS production, lowering the tissue oxygenation by restricting the blood flow might affect mitochondrial-specific ROS emissions ²³⁵. This decrease in O₂ availability could significantly impact mitochondrial bioenergetics. Indeed, lowered O₂ levels create conditions conducive to the ETC being overly reduced, which can promote an optimal environment for increased ROS production (Figure 1-5) ^{236,237}.

A nuanced relationship emerges regarding oxidative stress and antioxidant responses to BFR exercise. The application of BFR during resistance exercise at low-load (LL; 20 % or 30 % of 1-RM) did not significantly raise ROS levels⁵⁷, but coupling the BFR to moderate intensity resistance exercise (70 % of 1-RM) did²³⁸. In the same way, one week of LL resistance training with BFR (20 – 30 % of 1-RM) did not increase oxidative stress²³⁹. However, Centner *et al.* (2018) found a systemic ROS increase after LL-BFR, but no increase in mitochondrial-ROS production was found²²³. Additionally, Petrick *et al.* (2019) found that after an acute bout of LL resistance exercise with BFR (11-cm wide cuff), mitochondrial ROS production rates were reduced by 30 % compared to pre-exercise, with a concomitant decrease in skeletal muscle oxygenation, which was not found in the non-BFR group²³⁷. Since the exercise intensity does not differ from these studies, the discrepancy between those findings could arise from the cuff size used in the studies. In fact, the methodology of inducing a BFR, including the cuff size, determines the magnitude of the restriction (*see Chapter 3 - Methodologies and recommendations to occlude blood flow, p. 97*). Thus, with cuff width of 3.3 cm⁵⁷, 11 cm²³⁷, and 20 cm²²³, the blood flow was not equally restricted⁸⁴. As LL-BFR seems to have a limited impact on ROS production²⁴⁰, it was suggested that oxidative stress may be load rather than BFR-dependent²⁸, but further research on this topic is needed.

Most of the literature has focused on the ROS production following resistance exercise with BFR, with a lack of evidence about the ROS generation following aerobic exercise with BFR. However, Christiansen *et al.* (2018) compared the effects of intermittent running bouts in hypoxia on PGC-1 α . Participants completed 3 exercise sessions: without (CON) or with blood flow restriction (BFR), or in systemic hypoxia (~3250 m). The authors showed that, although the muscle hypoxia was matched between BFR and systemic hypoxia groups, PGC-1 α only increased in BFR, indicating higher levels of oxidative stress caused by ROS production²²⁶. However, more research is needed to precisely evaluate the differences in ROS generation following aerobic session with BFR.

In summary, BFR exercise enhances angiogenesis through multiple mechanisms, such as ROS production and VEGF activation. Increased levels of ROS after IPC or BFR allow the stabilisation of the HIF-1 α protein and the transcription of the VEGF-A gene, thus contributing to the activation of the angiogenic pathway of endothelial cells (Figure 1-5) ²¹². BFR combined with exercise enhances the expression of critical regulators of VEGF expression (HIF-1 α , PGC-1 α , and eNOS), compared to exercise without BFR ^{193,208}, suggesting that BFR exercise is more effective for promoting angiogenesis. This angiogenic effect of BFR has the potential to develop the vascular network and to deliver the O₂ to the muscle better. A better O₂ delivery could be estimated by changes in the capillary density and muscle oxygenation ^{4,27,216}.

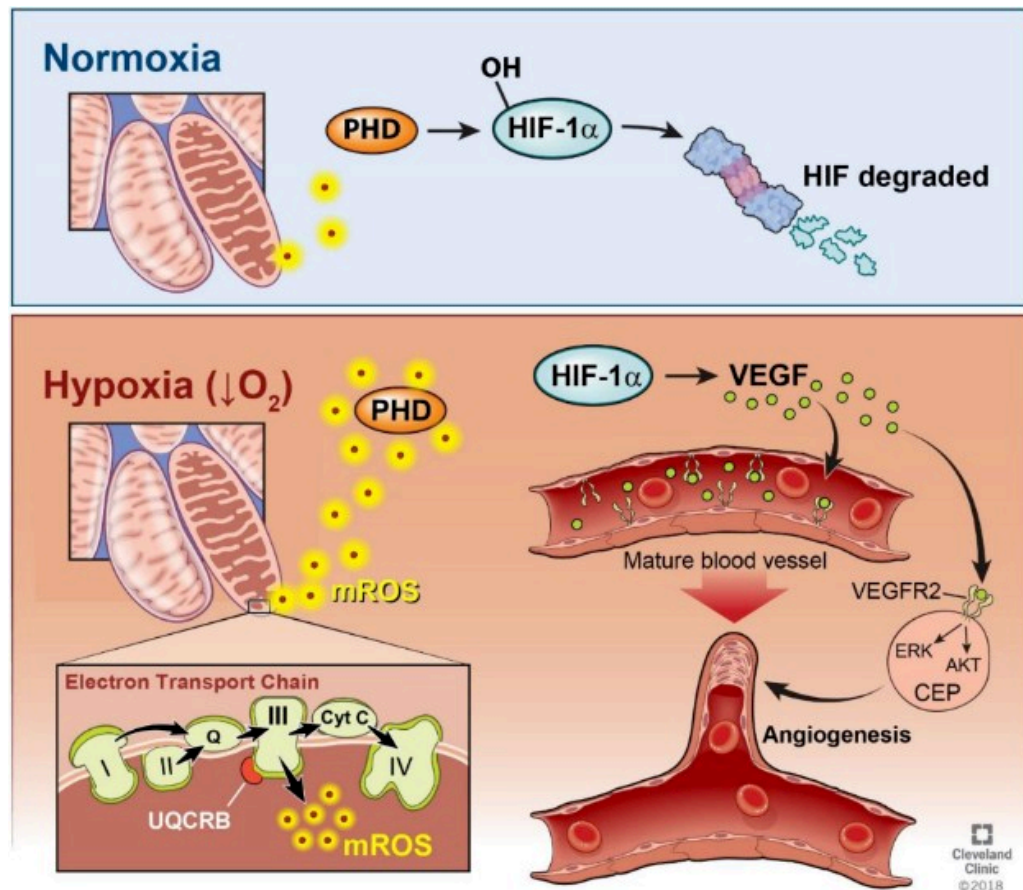


Figure 1-5: The impact of hypoxia on angiogenesis.

Hypoxia, whether induced by a systemic reduction in environmental O₂, a restriction of blood flow, or exercise, leads to a decreased level of O₂ availability in cells. The ETC subsequently generates mitochondrial Reactive Oxygen Species (mROS), which deactivate prolyl hydroxylase (PHD). Under normoxic conditions, PHD hydroxylates HIF-1α. However, in hypoxic conditions, the deactivation of PHD preserves HIF-1α. The stabilization of HIF-1α then initiates the production of VEGF, a crucial factor in angiogenesis. From Reichard and Asosingh²¹².

1.4.3 Effects of BFR on capillary growth

In healthy tissues, the growth and regression of capillaries is normal, and in harmony with the functional requirements²⁴¹. The term "capillary growth" or vascular density, that quantifies the number of capillaries within a designated area, is estimated using the capillary-to-fibre ratio, which corresponds to the number of capillaries surrounding muscle fibres²⁴¹. Capillary growth has a great importance in O₂ delivery to the working muscles in response to exercise and has been

hypothesised to play a role in performance enhancement following BFR training^{4,242}. Although studies have indicated that BFR training can enhance capillary-to-fibre ratio⁴, the connection between BFR training and capillary growth remains incomplete and requires further research²⁴³. As for markers of angiogenesis (VEGF-A, HIF-1 α , ROS), modes of BFR application may affect the subsequent adaptations in capillary growth.

One of the tools to investigate the effects of training in developing the capillary network, is to assess the available blood/muscle exchange area by quantifying the microvascular bed ability to filter fluids^{244,245}. This marker of capillary growth, known as microvascular filtration capacity, has been enhanced after an endurance-based training programme in elderly, eliciting a greater surface of fluids exchange²⁴⁶. Following resistance training with or without BFR, the microvascular filtration capacity has been significantly enhanced by 26 % in the BFR group, and has shown a tendency to increase in the non-BFR group²⁴⁶. In another study with lower intensity, six weeks of resistance training has shown a significant 14 % increase in capillary filtration following BFR training, but no changes following resistance training alone²⁴⁷. Thus, BFR training seems effective in enhancing the blood/muscle exchange surface, and consequently this better capillarisation improved the ability to deliver O₂ and nutrients to the working muscles.

Another way to investigate the effects of training on angiogenesis, is to quantify the capillarity in the skeletal muscle, through muscle biopsy samples. Three weeks of high-frequency (one or two daily sessions) low-load knee extensions training with BFR, has elicited microvascular remodelling in the *vastus lateralis*, but not in the non-BFR group²⁴⁸. This skeletal muscle microvascular remodelling is illustrated by a significant increase shown in capillaries per fibre up to 24 % and an increase of 34 % in the capillary density for the BFR group, although it has remained unchanged in the non-BFR group. It is noteworthy that increase in capillaries per fibre has occurred after only 8 days in the BFR-group, whereas similar improvement

usually require several months of training to occur^{249,250}. In addition, an increase in the ratio of total capillary area to myofiber area has been found in both BFR group (15-17 %), and non-BFR group (20 %), suggesting a better skeletal muscle perfusion capacity. This increase in capillary area to myofiber area, may then contribute to explain the enhancement in endurance capacity reported after BFR training²⁵¹. Hence, engaging in short-term high-frequency BFR training triggers angiogenesis, highlighted by an increased capillary count and an elevated capillary-to-muscle area ratio, which has the potential to enhance microvascular perfusion capacity²⁴⁸.

In an elite powerlifter population trained with or without BFR during 6.5 weeks by doing front squats, the number of capillaries around type I fibres has increased significantly in the BFR group, with a tendency for a greater increase compared to non-BFR group²⁵². In the BFR group, there has been a rise in the expression of the proangiogenic gene VEGF, coupled with a reduction in the levels of miR-16, a direct inhibitor of VEGF. With no changes reported in capillaries in contact to type II fibres, the BFR-induced angiogenesis seems specific to type I fibres²⁵². A similar finding was shown after six weeks of low-load RT with or without BFR²⁵³. Although no differences between BFR and non-BFR groups has been found, the BFR group has performed only two-third of the total workload performed by the non-BFR group, due to an earlier appearance of fatigue. Thus, the addition of BFR to resistance training has been an effective stimulus to induce angiogenesis, with a concomitant decrease in total exercise volume²⁵³. In addition, the BFR-induced angiogenesis around type I fibres may then be beneficial for improving aerobic capacity, since mitochondrial density and subsequent O₂ needs are the greatest in type I fibres²⁵⁴⁻²⁵⁷. This hypothesis is consistent with the findings of Christiansen & Bishop (2021), who have investigated the effects of high-intensity (105 % of participants' lactate turn-point; *for more details about lactate thresholds see 2.3 Ventilatory and lactate thresholds, p.86*) running bouts in hypoxic condition on angiogenesis²⁵⁸. The authors have found a significant 3.4-fold increase in VEGF-A following BFR running bouts, compared to control and hypoxia groups. This study highlights the

effectiveness of BFR aerobic exercise in inducing capillary growth. However, the authors have not reported any increase in HIF-1 α after the session. Thus, the capillary growth might be linked to other precursors than just HIF-1 α . This is consistent with findings of Mueller *et al.* (2014) who hypothesised that increased capillarisation following BFR training may arise from non-conventional signalling pathways, such as a VEGF expression induced by a PGC-1 α activation rather than HIF-1 α ⁷⁸.

Given these findings, angiogenesis would appear to contribute in the increase in aerobic capacity and endurance performance seen with BFR training. The local microvascular adaptations in the skeletal muscle such as the increase in muscle capillary density may contribute to a better oxidative capacity and the improvement of the $\dot{V}O_2max$ that may occur following BFR training ^{4,259}. Despite these findings, further research on the microvascular adaptation after BFR training are needed to better understand the role of BFR in capillary growth ²⁴³. Given that a greater capillary density facilitates O₂ microvascular exchanges ²⁶⁰, training with BFR should therefore induce changes in muscle oxygenation.

1.4.4 Muscle oxygenation

With the capillary growth following BFR training, changes in O₂ delivery should occur, leading to alterations in muscle oxygenation. The skeletal muscle oxygenation response can be estimated through the use NIRS, at rest or during exercise ²⁶¹. The muscle deoxygenation induced by exercise is reflected by the changes in deoxy[myoglobin+haemoglobin] (Δ [HHb]), that is the concentration of myoglobin ([Mb]) and haemoglobin ([Hb]) under the NIRS optodes which are not bound with O₂ ²⁶².

It has been suggested that Δ [HHb] reflects a metabolic stress caused by local hypoxia ^{263–265}, which is suggested to trigger the angiogenesis signalling cascade ²¹⁰. Thus, rather than execute invasive muscle biopsies to evaluate the effects of BFR on capillary growth, the use of non-invasive NIRS measurements may be used to

indirectly estimate angiogenesis after a training programme coupled with BFR ^{266,267}.

In fact, in response to resistance exercise coupled with BFR, muscle haemoglobin concentrations are significantly increased, mainly due to a large increase in [HHb] during exercise ^{216,264,268}. Similar findings were shown in both concentric and eccentric contractions that elicited greater deoxygenation when coupled with BFR ^{264,269}. As shown by the work of Willis *et al.* (2018, 2019) ^{16,69,270}, although aerobic exercise induces a muscle deoxygenation during repeated sprints ^{271,272}, adding BFR during the sprints potentiate this deoxygenation. In addition, the BFR stimulus induced greater Δ [HHb] responses compared to repeated sprints without BFR or in normobaric hypoxia ¹⁶. Finally, it is noteworthy that arms are more responsive to BFR-induced deoxygenation during exercise than legs ²⁷⁰. Moreover, a greater restriction of the blood flow, between 60 and 80 % of arterial occlusion pressure (AOP) ²⁷³, elicited a greater magnitude of deoxygenation compared to BFR at 40 % of AOP or without BFR ²⁶⁸.

While BFR is more effective at inducing significant deoxygenation compared to training without BFR, it is essential to investigate the muscular oxygenation adaptations following training with restricted blood flow to assess its potential role in promoting angiogenesis. In this way, Kacin & Strazar (2011) investigated the effects of low-load resistance training in ischaemia performed to failure on quadriceps muscle oxygenation ⁷⁹. The training consisted of 4 weeks of low-load knee-extension sessions, performed with one leg under vascular occlusion (ischaemic leg) and the other leg without any occlusion (control leg). Following the training period, the quantity of repetitions performed during the knee-extensions endurance test exhibited a significant increase of 63 % in the ischaemic leg and 36 % in the control leg, with a significant difference between legs. Despite greater numbers of repetitions, the O₂Hb at the end of exercise was increased in both ischaemic leg (56 %) and control leg (21 %), highlighting a lower exercise-induced hypoxia in the ischaemic leg. With no changes in HHb, these increases in O₂Hb led

to significant greater total haemoglobin (tHb) in both legs, with a significant higher tHb for ischaemic leg (+ 120 %) compared to control leg (+ 53 %). In addition, the deoxygenation tended to be slower after training in both legs. Notable improvements in muscle endurance capacity were found in the occluded leg, correlating with an increase in the delivery of O₂ to the muscles that may be triggered by skeletal muscle vessels angiogenesis⁷⁹. These results are consistent with other findings using high-load resistance training with or without BFR²⁷⁴. After 4 weeks of training, greater improvements in the skeletal muscle microvascular function assessed by tissue oxygenation, were found in the BFR group compared to the non-BFR group²⁷⁴.

The chronic muscle oxygenation adaptations have been also studied following high-intensity interval training (HIIT) coupled with BFR (HIIT+BFR) and compared to both HIIT and continuous training without BFR²⁷⁵. The results showed that only HIIT and HIIT+BFR groups significantly improved their peak power output in the graded exercise test by 5 % and 7 %, respectively, without differences between groups. At the end of the post-training test, the tissue saturation index (TSI) decrease was greater compared to the pre-training test only in the HIIT+BFR group. These greater deoxygenation and peak power during the graded test reflect the ability of the vascular network to bring more O₂ to the working muscles. Thus, it is plausible that the periods of ischaemia induced by BFR training triggered the angiogenesis pathways, which increased the skeletal muscle capillarisation and provides a better O₂ delivery to the muscles. Therefore, BFR training showed effectiveness in enhancing the oxidative capacity of the body, when coupled with high-intensity training²⁷⁵. These results are consistent with the findings of Keramidas *et al.* (2012), who have found a significant greater deoxygenation at the end of a maximal exercise test in the BFR groups, compared to non-BFR group²⁷⁶.

It is noteworthy that the study of Kacin & Strazar (2011) has resulted in a lower deoxygenation for BFR-trained leg at the end of the endurance test, whereas

the studies of Tangchaisuriya *et al.* (2022) and Keramidas *et al.* (2012) have shown a greater deoxygenation at the end of the maximal exercise test for the BFR group^{79,275,276}. The discrepancies between these studies arise from the intensity of exercise: a constant intensity for Kacin & Strazar (2011) (15 % of pre-training maximal voluntary contraction (MVC) force)⁷⁹, against an increasing intensity for the two other studies^{275,276}. Therefore, the O₂ consumption is kept relatively constant when the intensity of exercise is constant, whereas the O₂ consumption rises when exercise intensity increases. Consequently, these studies have shown the effectiveness of BFR increasing O₂ delivery and extraction capacity.

While limited research has explored the muscular oxygenation adaptations resulting from BFR training, these findings underscore the efficacy of BFR in enhancing O₂ supply to the muscles. This improvement of the microvascular function is then in favour of a greater level of angiogenesis associated with BFR training.

1.5 CONCLUSION

An individual's $\dot{V}O_{2max}$ is influenced by both O₂ perfusion and diffusion capacities. Therefore, increasing $\dot{V}O_{2max}$ necessitates increases in perfusive (blood flow and arterial content) and diffusive (DO₂) O₂ transport parameters.

During exercise, the blood flow to muscles can increase almost 100-fold compared to resting state¹⁷⁴, facilitated by the vasodilatory mechanisms and increases in \dot{Q} . BFR has been effective in enhancing \dot{Q}_{max} , through a combined action of mechanical (volume-overload) and chemical (signalling pathways) stimulations²⁷⁵. Training with BFR has demonstrated enhancements in vascular function, increasing post-occlusive blood flow and stimulating angiogenesis-related markers like HIF-1 α , VEGF, and PGC-1 α proteins^{163,193}. This angiogenesis, characterised by increased capillary growth, presents potential to improve microvascular perfusion capacity²⁴⁸. Utilising non-invasive NIRS measurements provides an indirect means to estimate angiogenesis following BFR training, obviating the need for invasive

muscle biopsies ^{266,267}. Chronic adaptations to BFR training shows greater enhancements in skeletal muscle microvascular function, highlighting BFR's efficacy in augmenting O₂ supply to muscles, though research on microvascular adaptations to BFR remains limited ^{74,274}.

Since BFR improves cardiac (HR, SV, \dot{Q}), vascular (angiogenesis) and diffusive (muscle perfusion) functions ^{69,193,248,277}, the O₂ delivery and diffusion to the mitochondria is enhanced. Thus, BFR should be effective in enhancing $\dot{V}O_{2max}$ and submaximal respiratory efficiency.

CHAPTER TWO

Increasing aerobic capacity and power with blood flow restriction



BFR training has been shown to impact every step of the O_2 transport chain. Consequently, several studies have investigated the effects of BFR on $\dot{V}O_{2max}$, that belongs to the main factors of performance⁹¹. However, $\dot{V}O_{2max}$ should not be the sole factor impacted by BFR training, but submaximal $\dot{V}O_2$ and associated biochemical responses during exercise should also be affected. Therefore, it is essential to monitor the effects of BFR on the aerobic capacity and aerobic power, as indicated by submaximal thresholds and by the highest rate of O_2 uptake, respectively²⁷⁸.

2.1 BFR AND MAXIMAL AEROBIC POWER

Both high-intensity, short-duration training and low-intensity, high-volume training are essential components of training programmes for athletes²⁷⁹. Both training methods have been employed to increase $\dot{V}O_{2max}$, with each method showing better improvements in central adaptations (high-intensity) or peripheral adaptations (low-intensity continuous training)²⁸⁰. Through its action on central and peripheral responses and adaptations, the impact of endurance training with BFR on $\dot{V}O_{2max}$ has been investigated in several exercise types and intensities^{81,281–283}.

2.1.1 MICT vs. HIIT

Moderate intensity continuous training (MICT) represents steady-state aerobic exercise performed at a moderate intensity for an extended duration (30 – 60 min) ²⁸⁴. While MICT has been found effective in enhancing $\dot{V}O_{2max}$ ²⁸⁴, it necessitates sustained adherence to the training in terms on session duration as much as training length. In response to this, interval training has gained prominence as a time-efficient alternative to induce significant increases in $\dot{V}O_{2max}$ ²⁸⁵. Several recent literature reviews and meta-analysis have indicated that HIIT demonstrates similar or superior improvement in $\dot{V}O_{2max}$ compared to lower intensity exercises, such as MICT ^{286–288}.

Contrary to MICT that involves a continuous exercise at lower intensities, HIIT is a training regimen that involves periods of exercise at higher intensities interspersed with active or passive rest (Figure 2-1). While the definition of HIIT is equivocal and varies between reviews, the concept is globally the same: the athlete should spend the longest time possible near $\dot{V}O_{2max}$ (> 90 % of $\dot{V}O_{2max}$). Indeed, it has been shown that training at a very high percentage of $\dot{V}O_{2max}$ induces a maximal stress on the O₂ transport systems and provides the most effective stimulus for enhancing $\dot{V}O_{2max}$ ²⁷⁹. In addition, repeated-sprints training (RST; 3 to 7s sprints, less than 60s of recovery) and sprint interval training (SIT; 30 s all-out sprints; 2-4min recovery) are highly intense forms of HIIT ^{279,289}. By increasing the exercise intensity compared to MICT, HIIT or SIT induce central and peripheral changes that affect both cardiac output and O₂ extraction, which consequently increases $\dot{V}O_{2max}$ ²⁸⁷.

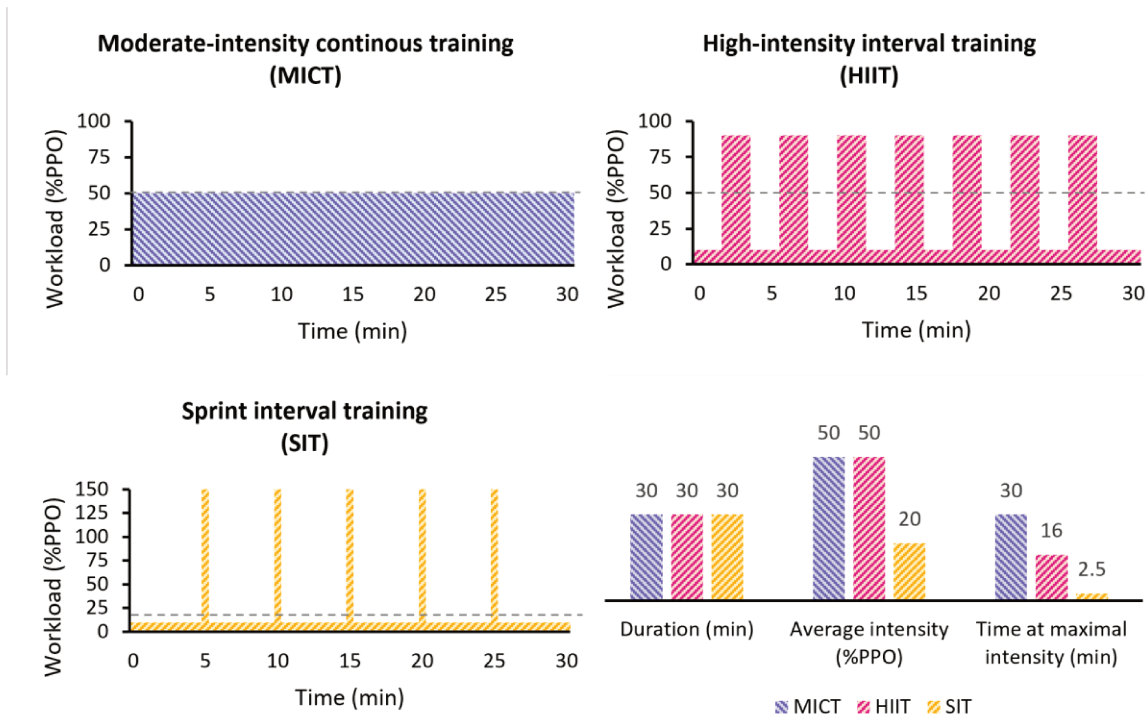


Figure 2-1: Representative examples of endurance exercise protocols used to increase aerobic power.

Protocols presented are moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), and low-volume sprint interval training (SIT). The intensity of exercise is presented as a percentage of peak power output (PPO) reached during a graded exercise test. MICT and HIIT are presented with the same training volume, while time spend at maximal intensity is lower for HIIT (16min) compared to MICT (30min). Based on MacInnis & Gibala (2017)²⁸⁷.

The increases in $\dot{V}O_{2max}$ following training are partly driven by peripheral adaptations such as increased skeletal muscle capillary density and mitochondrial content²⁹⁰⁻²⁹². Mitochondrial biogenesis is associated with signalling pathways (such as the expression of PGC-1 α) activated during both MICT or HIIT session similarly. The repeated activation of these pathways over time increases mitochondrial density, inducing a better reliance on aerobic metabolism (fat oxidation) during exercise^{290,293}. Studies have shown that mitochondrial content increase was greater following HIIT than MICT^{288,294}, but this result has not been

found in all studies²⁸⁷. Similarly, there is no consensus on a better effectiveness of HIIT compared to MICT in inducing greater skeletal muscle capillary density²⁸⁷.

Regarding central adaptations, Astorino *et al.* (2017) have demonstrated that HIIT induces significant improvement in \dot{Q}_{max} driven by a significant increase in SV_{max} , while HR_{max} remains unchanged^{295,296}. In Astorino *et al.* (2017), participants have followed HIIT (60s at 90 % to 110 % of peak power output, 75s of recovery) during 10 sessions (3 sessions per week) on a cycle ergometer²⁹⁵. The central adaptations to HIIT have induced a significant increase in $\dot{V}O_{2max}$ after only 10 sessions. In a complementary study, Astorino *et al.* (2017) have shown that SIT added to the previous 10 sessions of HIIT (HIIT + SIT) have resulted in a significant increase in SV , \dot{Q} and $\dot{V}O_{2max}$ compared to pre-training and post HIIT²⁹⁶. However, the addition of high volume HIIT (HIIT + HIIT) and periodized training (HIIT + PER; 3 sessions of high-volume HIIT, 3 sessions of SIT, and 4 sessions of low-volume HIIT) have not induced significant increase in SV or \dot{Q} compared to post HIIT values. Only HIIT+PER and HIIT+SIT have significantly increased $\dot{V}O_{2max}$ compared to post HIIT values²⁹⁶. These results are consistent with other studies that shows a significant increase in \dot{Q}_{max} with associated increase in $\dot{V}O_{2max}$ following HIIT or SIT regimens^{294,297}. In addition, the central adaptations following HIIT or SIT have been demonstrated in a recent meta-analysis, showing that 93 % of the studies analysed presented an increased SV and 80 % of the studies reported significant increases in SV_{max} or \dot{Q}_{max} ²⁹⁸. However, these findings are not shared by all studies that found a $\dot{V}O_{2max}$ increase following HIIT or SIT without a concomitant increase in \dot{Q}_{max} ^{299,300}.

2.1.2 Effects of MICT and BFR on $\dot{V}O_{2max}$

Early studies with restricted blood flow during exercise have shown an increase in $\dot{V}O_{2max}$ measured with Douglas bags following 4 weeks of one legged cycling training, with or without BFR⁵³. Using a pressure chamber to induce ischaemia (\approx 20 % reduction in leg blood flow), participants were endurance trained four times per week (45min per leg with and without BFR) at the highest tolerable

workload, maintaining a cadence of 60 rpm. After the training period, the BFR-trained leg showed a significant greater $\dot{V}O_{2peak}$ and significant greater $\dot{V}O_{2peak}$ improvements (+ 24 %) than the other leg (+10 %). The average workload during training was 68 watts, ranging from 46 watts session one to 81 watts in the last session. The intensity at the end of the training was estimated to be less than 50 % (between 29 % and 45 % of peak workload) of the average $\dot{V}O_{2peak}$ before training. An increased in $\dot{V}O_{2peak}$ after one-legged BFR training was also reported in another publication of the same group of researchers ⁵⁴.

Using inflatable cuffs to induce BFR, more recent studies have reported increase in $\dot{V}O_{2max}$ following low-intensity endurance training ^{301,302}. The participants followed a walk or cycling training ($\approx 40\%$ $\dot{V}O_{2max}$) protocol. After 8 weeks (24 sessions), Abe *et al.* (2010) have shown a significant increase in $\dot{V}O_{2max}$ (+ 6.4 %), whereas no $\dot{V}O_{2max}$ improvements have been found in the non-BFR group ³⁰¹. Each training session lasted 15 minutes for BFR group, compared to 45 minutes for non-BFR group. Therefore, despite a greater total workload, non-BFR training has not been shown efficient in improving $\dot{V}O_{2max}$, whereas a short duration training programme with BFR was ³⁰¹. Likewise, Park *et al.* (2010) have shown a significant increase (+ 11.6 %) in $\dot{V}O_{2max}$ after two weeks (24 sessions, 15 min/session) of BFR walk training, but no increase in the non-BFR group ³⁰². De Oliveira *et al.* (2016) have demonstrated that with only 12 sessions (26 min/session, 4 weeks) of low-intensity (30 % of peak workload) cycling with BFR, the $\dot{V}O_{2max}$ was significantly increased by 5.6 % while the non-BFR group has not shown any increase in $\dot{V}O_{2max}$ ³⁰³. Inconsistent with these findings, the study of Kim *et al.* (2016) has not shown significant increase in $\dot{V}O_{2max}$ following 6 weeks (18 sessions, 20 min/session) of low-intensity (30 % of heart rate reserve, HRR) with BFR ³⁰⁴. These results differ from other low-intensity studies with BFR reported increases in $\dot{V}O_{2max}$ using the same BFR protocol (as described in Abe *et al.* (2010)). However, de Oliveira *et al.* (2016) used larger cuffs (18-cm width vs. 5-cm) which may explain the benefits after

only 12 sessions, since the cuff width influences on the amount of restricted blood flow (see Chapter 3 - Methodologies and recommendations to occlude blood flow, p.97). In addition, the study of Kim *et al.* (2016) had 8 sessions less than the 24 sessions in Park *et al.* (2010) and Abe *et al.* (2010), with lower intensity (30 % of HRR vs. 40 % $\dot{V}O_{2max}$). Thus, the combination of lower intensity, less sessions and narrower BFR cuffs have not been a sufficient stimulus to induce significant $\dot{V}O_{2max}$ improvements.

The ACSM recommendations for $\dot{V}O_{2max}$ improvements indicate a beneficial effect of low-intensity exercise in sedentary, but moderate to vigorous intensity in trained athletes ²⁸⁴. In addition, at low-intensity, a length of 8 to 20 weeks are recommended to produce significant small (effect size < 0.6) increases in $\dot{V}O_{2max}$ (2-3 sessions per week, 20-30 min per session) ³⁰⁵. In the previous cited studies ^{301,302,304,306}, the session duration for BFR groups was 15 to 26 min per session, with 2 to 12 sessions per week during 2 to 8 weeks. Therefore, none of the BFR groups have met the recommendations for $\dot{V}O_{2max}$ improvements, but they still all showed $\dot{V}O_{2max}$ increases contrary to the matched intensity non-BFR groups ^{301,302,304,306}. It is then noteworthy that BFR stimulus, even at low-intensity, is efficient in enhancing $\dot{V}O_{2max}$.

2.1.3 Effects of HIIT and BFR on $\dot{V}O_{2max}$

The effects of BFR associated with either HIIT or SIT on $\dot{V}O_{2max}$ have been investigated in the literature ^{77,83,307-311}. Keramidas *et al.* (2012) showed no significant increases in both BFR and non-BFR groups after 6 weeks of training (3 sessions/week) ³⁰⁷. The sessions consisted of repeated bouts of 2-min work at 90 % of $\dot{V}O_{2max}$ and 2min of active recovery, with (90 mmHg) or without BFR. In the BFR group, the training has been performed at an intensity of 90 % of the $\dot{V}O_{2max}$ reached with the inflated cuffs. Therefore, both groups exercised at the same relative intensity (90 % of $\dot{V}O_{2max}$) but different absolute intensity (161 W for non-BFR, 125 W for BFR). While none of the experimental groups show a significant increase in

$\dot{V}O_{2max}$, they both have increased the peak power output reached at $\dot{V}O_{2max}$ (BFR \approx 20 %, non-BFR \approx 12 %). Thus, the greater performance seen in both groups is likely due to peripheral improvements as shown by the greater deoxygenation reached in the maximal exercise test ³⁰⁷.

In Amani-Shalamzari *et al.* (2019), participants were trained using different application of BFR and exercise intensities (for details see Table 2-1). The results have shown that all groups increased the $\dot{V}O_{2max}$ after 12 sessions (4 weeks), with a significant difference between CP_C-IE (+ 14.8 %) and IP-IE (+ 8.4 %). The addition of a greater blood flow restriction, inducing a greater venous occlusion, cell swelling and metabolite accumulation, with an increasing intensity may explain the greater improvements in $\dot{V}O_{2max}$ in the CP_C-IE group. Indeed, increasing the intensity throughout the training may induce a rise in skeletal muscle mitochondrial content, whereas a constant intensity seems to induce a plateau in mitochondrial content ²⁸⁷.

Table 2-1: Training protocols and associated VO_{2max} changes from Amani-Shalamzari et al. (2019) ²⁸⁷.

Groups	Parameters	Training protocol per week				% $\dot{V}O_{2max}$ changes
		1	2	3	4	
IP-CE	Pressure	160	190	210	240	+ 9.6 \pm 2.0 % *
	% $v\dot{V}O_{2max}$	60	60	60	60	
CP _P -IE	Pressure	160	160	160	160	+ 11.2 \pm 5.5 % *
	% $v\dot{V}O_{2max}$	60	70	80	85	
CP _C -IE	Pressure	240	240	240	240	+ 14.8 \pm 4.9 % *†
	% $v\dot{V}O_{2max}$	60	70	80	85	
IP-IE	Pressure	160	190	210	240	+ 8.4 \pm 2.4 % *
	% $v\dot{V}O_{2max}$	60	70	80	85	

Note: IP-CE, increasing BFR pressure with constant exercise intensity; CP_P-IE, constant partial BFR pressure with increasing exercise intensity; CP_C-IE, constant complete BFR pressure with increasing exercise intensity; IP-IE, increasing BFR pressure with increasing exercise intensity; Pressure: cuff pressure (mmHg); $v\dot{V}O_{2max}$: speed reached at $\dot{V}O_{2max}$.

* $p < 0.05$ vs pre-training values

† $p < 0.05$ vs IP-IE

Amani-Shalamzari *et al.* (2020) have found significant increases in $\dot{V}O_{2max}$ following specific HIIT (futsal training, similar physiological responses than HIIT³¹²) in both BFR and non-BFR groups³¹⁰. However, no significant differences were found between groups. Participants have received 10 sessions of specific futsal training with (110 % of systolic blood pressure, SBP) or without BFR. Therefore, BFR could also be applied during specific activities and integrated in the training of athletes³¹⁰.

Consequently, BFR appear to be effective in improving $\dot{V}O_{2max}$ when combined with HIIT or SIT. However, the beneficial effects of adding BFR to HIIT or SIT is not clear yet, since most of the studies reported concomitant increases in $\dot{V}O_{2max}$ in both BFR and non-BFR groups.

2.2 OXYGEN UPTAKE KINETICS FOLLOWING BFR TRAINING

MICT and HIIT are based on distinct domains of exercise intensity, which stimulate differently the physiological processes involved³¹³. The three-domains classification scheme is common in a performance context to describe exercise intensity and is best represented by the analysis of $\dot{V}O_2$ kinetics response to exercise intensity^{314,315}. Since BFR affect several steps of the O₂ transport chain, it also impacts the $\dot{V}O_2$ kinetics.

2.2.1 Principles of oxygen uptake kinetics and exercise intensity domains

Jones and Poole (2013, 13) have stated: "The study of $\dot{V}O_2$ kinetics, therefore, could be considered to involve the study of the physiological mechanisms responsible for the dynamic $\dot{V}O_2$ response to exercise and its subsequent recovery"³¹⁵. At the onset of a constant work exercise, the force applied by the muscles on the pedals increases immediately in a 'square-wave' fashion. However, the aerobic metabolism demonstrates considerable inertia inducing a $\dot{V}O_2$ response that follows an exponential rise. The curve could be modelled using various exponential and

linear models given the exercise-intensity domain and the presence or not of a $\dot{V}O_2$ slow component ($\dot{V}O_2$ SC). The $\dot{V}O_2$ SC is characterised by a slowly developing increase in $\dot{V}O_2$ during constant-work-rate exercise performed above the lactate threshold ³¹⁶. The modelling of the curve could be separated into three phases that provide usefulness information on the health status, training state and aerobic capacity of the participant ³¹⁵⁻³¹⁷ (Figure 2-2).

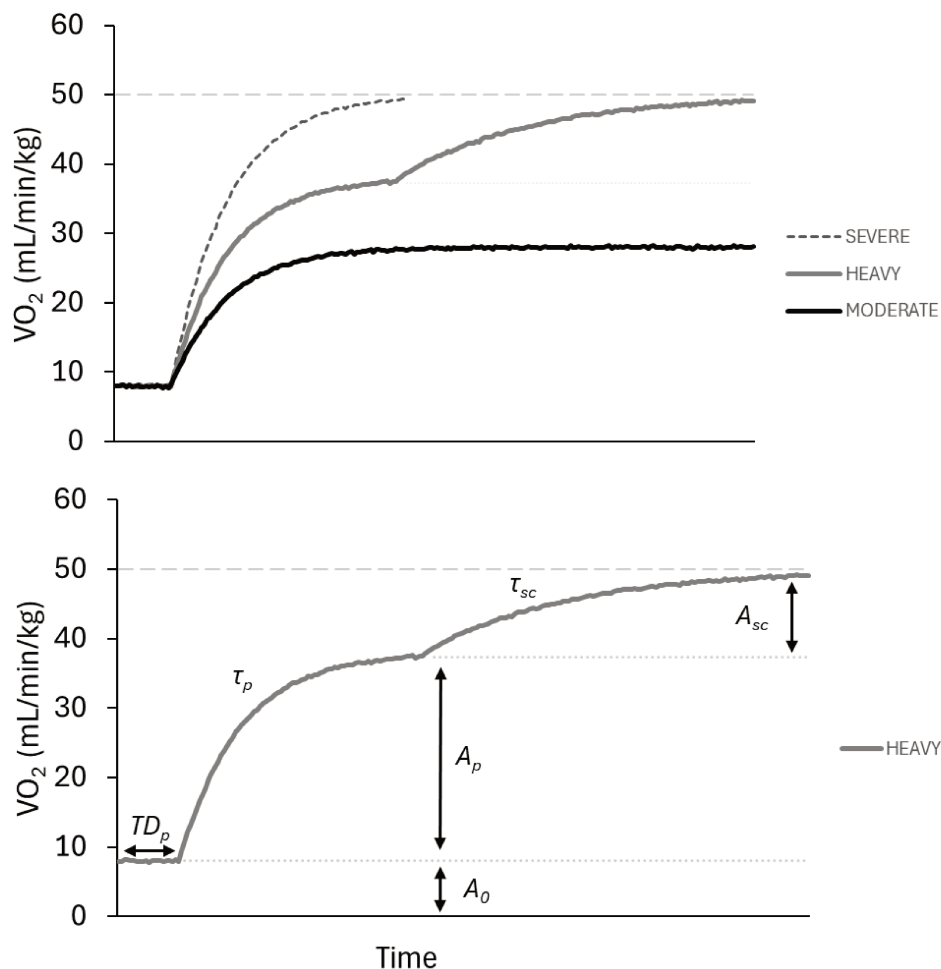


Figure 2-2: Oxygen uptake kinetics representation and parameters.

Schematic representation of $\dot{V}O_2$ kinetics in the moderate, heavy and severe domains (upper panel) and associated parameters for heavy domain (lower panel). A_0 is the $\dot{V}O_2$ at rest. TD_p is the time delay of the fast $\dot{V}O_2$ component. A_p and A_{sc} are respectively amplitudes of the fast and slow $\dot{V}O_2$ components. The τ_p and τ_{sc} are respectively the time constants of the fast and slow $\dot{V}O_2$ components. Further details about $\dot{V}O_2$ kinetics modelling are available in several reviews ^{315,317-319}.

Endurance trained athletes have shown a faster $\dot{V}O_2$ kinetics response compared to sedentary or trained participants with lower $\dot{V}O_{2max}$ ^{320–326}. The τ_p of regional-level competitive runners (12–17 s)^{327,328}, elite rowers (14 s)³²⁹ and well-trained cyclists (21 s)³³⁰ is much shorter compared to non-specifically-trained young healthy participants (~25–30 s)³¹⁷. In addition, endurance training has been shown to accelerate $\dot{V}O_2$ kinetics in longitudinal studies. Early $\dot{V}O_2$ kinetics study has shown faster $\dot{V}O_2$ kinetics after 10 weeks of endurance training (6 x 40 min of running or cycling)³³¹. In untrained participants, $\dot{V}O_2$ kinetics has been speeded in response to endurance training^{332–335}, with significant $\tau\dot{V}O_2$ decrease after as soon as 2 to 4 days of cycle training (2 h per day at 60 % of $\dot{V}O_{2max}$)^{332,333}. In trained athletes, modifying training intensity or volume has been efficient in accelerating $\dot{V}O_2$ kinetics. τ_p has been reduced by 5 and 7 s after respectively 4 and 8 weeks of increased volume training in trained cyclists³³⁶. Supplementing running training with severe-intensity bouts has been shown to reduce τ_p (pre: $26 \pm$ vs. post: 14 ± 1 s), but not $\dot{V}O_2$ SC amplitude³³⁷. In addition, SIT has shown effectiveness in accelerating $\dot{V}O_2$ kinetics with a ~25s speeding of τ_p and reduced $\dot{V}O_2$ SC after only six sessions of repeated 30 s sprints³³⁸.

The training-induced $\dot{V}O_2$ kinetics adaptations are mainly thought to be linked with the delivery of O_2 to the muscles (blood flow, capillary density)^{317,332,333} and the utilisation of O_2 by the muscle cells (oxidative enzyme activity)^{317,339,340}. Therefore, the increased capillary density and angiogenesis found after BFR training have been thought to induce $\dot{V}O_2$ kinetics adaptations when BFR is applied during endurance training.

2.2.2 Effects of BFR on oxygen uptake kinetics

Corvino *et al.* (2019) compared the effects of low-intensity (30 % of maximal aerobic power, MAP) interval training (LI), low-intensity (30 % MAP) interval training with BFR (LI+BFR), and high-intensity (~103 % MAP) interval training (HIIT) on the $\dot{V}O_2$ kinetics³⁴¹. Participants have been trained for 4 weeks (12 sessions) with pre-

training and post-training constant power tests that each consisted of three square-wave transitions at 30 % MAP (measured in pre-training graded exercise test). The results show no reduction in $\dot{V}O_2 \tau_P$ in LI, but a reduced $\dot{V}O_2 \tau_P$ ($P < 0.05$) in both LI+BFR (pre- versus post-training $\tau_P = 18.3 \pm 3.2$ versus 14.5 ± 3.4 s) and HIIT ($\tau_P = 20.3 \pm 4.0$ versus 13.1 ± 2.9 s), with not differences between LI+BFR and HIIT. While the intensity in LI is not high enough to induce $\dot{V}O_2$ kinetics adaptations, adding BFR induces a physiological stimulus that accelerated the τ_P . With 4 weeks of endurance training with BFR, intramuscular adaptations such as increased oxidative enzyme expression or speeding of activation of oxidative phosphorylation^{339,340}, should have occurred³⁴¹. Indeed, adding BFR to endurance training has resulted in increased muscle oxidative enzyme activity and increased capillary density^{242,246,248,342,343}. These findings are consistent with results from a recent study³⁴⁴ that has reported a significant reduction in τ_P after 4 weeks of training (~ 35 %) with BFR, but not without BFR. After 8 weeks of training, both groups have shown a pre- to post reduction in τ_P , with no differences between groups. Consequently, the reduction in τ_P has been accelerated in the BFR group (4 weeks), since the reduction in τ_P occurred later (8 weeks) in the non-BFR group³⁴⁴.

BFR application has been shown to induce a blood lactate accumulation associated with an increase in H^+ production compared to same intensity exercise without BFR. In addition, exercising with BFR is known to induce earlier fatigue apparition due to an earlier recruitment of fast-twitch fibres and a stimulation of group III and IV afferents. Since $\dot{V}O_2$ slow component (SC) is triggered by a loss of efficiency in muscle contractions and a greater recruitment of fast-twitch fibres, exercising with BFR has the potential to affect the $\dot{V}O_2$ SC. Indeed, Knight *et al.* (2004) applied BFR (75 % of thigh SBP) during a square-wave exercise at 60 % of individuals ventilatory threshold 1 (VT1), corresponding to moderate-intensity domain³⁴⁵. The authors have found an apparition of $\dot{V}O_2$ SC when the cuffs were inflated, but not when the cuff were deflated. Therefore, BFR has stimulate mechanisms involved in

$\dot{V}O_2$ SC, such as increased metabolic demand, decrease in tissue oxygenation and greater motor unit recruitment associated with a loss of muscular efficiency^{345,346}. This finding has also been reported in constant heavy-intensity domain, where $\dot{V}O_2$ SC has shown a greater amplitude in thigh BFR conditions. In addition, the authors have found that the amplitude of $\dot{V}O_2$ SC increases as a function of BFR pressure, since BFR at 40 % of AOP shows a lower SC amplitude compared to BFR at 50 % of AOP (6.7 ± 0.9 vs. 9.7 ± 1.0 ml/min/kg; $p < 0.05$)³⁴⁷. Consequently, while applying BFR already affects the $\dot{V}O_2$ kinetics, the changes in $\dot{V}O_2$ kinetics are function of the pressure applied. In addition to the greater amplitude of SC induced with greater pressure, a greater restriction of blood flow could also impaired O_2 delivery in different manners⁸⁴. A delay in O_2 delivery causes a slower $\dot{V}O_2$ kinetics response as shown in patients (e.g. peripheral arterial disease, type I and II diabetes, pulmonary diseases such as emphysema and chronic obstructive pulmonary disease) in whom the blood flow to the exercising muscles is reduced³¹⁷. In addition, exercising in supine position, which reduces the O_2 delivery, has shown to decelerate the $\dot{V}O_2$ kinetics^{348,349}. Salzman *et al.* (2021) have reported a significant slower τ_p (~ 40 % slower) when BFR was applied, with no differences between BFR at 40 % and 50 % of AOP³⁴⁷.

A fast $\dot{V}O_2$ kinetics is associated with a low O_2 deficit since a low amount on non-mitochondrial ATP is produced³¹⁷. However, when the $\dot{V}O_2$ kinetics are slowed, a greater amount of non-mitochondrial ATP is produced to meet the energy requirements, which induces a higher O_2 deficit³¹⁷. Given that a limited O_2 delivery capacity has shown to induce slower $\dot{V}O_2$ kinetics, the O_2 deficit during BFR should also be greater. In addition, the concept of O_2 deficit is strongly linked to the excess in post-exercise oxygen consumption (EPOC), representing the elevation in $\dot{V}O_2$ above resting levels after physical exercise, indicating sustained metabolic activity^{350,351}. Consequently, Mendonca *et al.* (2015) have investigated the effects of BFR on O_2 deficit and EPOC after 3-min walking bouts³⁵². The authors have found a significant greater O_2 deficit caused by BFR (1679 ± 250 vs. 1942 ± 396 mL)

compared to non-BFR exercise. In addition, the EPOC magnitude was significantly greater in BFR than in non-BFR condition, with a 27 % difference between both conditions (721 ± 279 vs. 917 ± 323 mL) ³⁵². The size of EPOC is linked to several exercise modality dependent factors, including exercise duration and intensity ^{351,353}. Here, the exercise duration was the same in both non-BFR and BFR groups ³⁵². In addition, both groups have walk at the same speed and have shown similar $\dot{V}O_2$, meaning that exercise intensity was equivalent between both groups ³⁵². Therefore, EPOC was not affected by exercise modality dependant variables, but rather by physiological responses to BFR. The elevated level of $\dot{V}O_2$ during recovery that characterised EPOC is mediated by replenishment of O₂ stores in blood and muscles, resynthesis of ATP and creatine phosphate, lactate removal from the blood, elevated ventilation and energy cost associated, and finally body temperature regulation ^{350,351,353–355}. Interestingly, BFR has been shown to directly affect some of these parameters, such as elevation of blood lactate ³⁵⁶, greater decrease in muscle tissue oxygenation ^{16,69,270}, greater creatine phosphate utilisation ^{357,358}, or increases in ventilation and heart rate ³⁵⁹. Therefore, BFR has the potential to induce higher O₂ deficit and higher EPOC compared to exercise without BFR.

By limiting the O₂ delivery to the working muscles, BFR slows the $\dot{V}O_2$ kinetics and consequently increase the O₂ deficit at the onset of exercise. In addition, the physiological responses to BFR exercise, such as metabolite accumulation ^{357,358,360}, greater motor unit recruitment ^{69,361,362}, stimulation of group III and IV afferents ^{363,364}, induce the apparition of a $\dot{V}O_2$ slow component at moderate-intensity exercise or a greater amplitude of $\dot{V}O_2$ SC than non-BFR exercise. Associated with the greater BFR-induced EPOC, restricting the blood flow during exercise notably change the $\dot{V}O_2$ response during exercise. Nonetheless, endurance training performed with BFR also induce physiological adaptations on $\dot{V}O_2$ kinetics, including a reduction in τ_p that reflects an increased oxidative enzyme expression or speeding of activation of oxidative phosphorylation ^{339,340}. The physiological responses and adaptations of $\dot{V}O_2$ kinetics to BFR training, such as the speeding and greater

reliance on oxidative phosphorylation, should therefore induce biochemical changes in the boundaries between exercise intensity. These boundaries are characterised by thresholds in the analysis of the ventilatory and lactate responses to exercise.

2.3 VENTILATORY AND LACTATE THRESHOLDS

Ventilatory and lactate thresholds belong to the key determinants of aerobic capacity identified by Whipp *et al.* (1982) ^{365,366}. Successful endurance training programmes are typically marked by a shift to the right towards higher power output or running speed within the lactate or ventilatory threshold ³⁶⁶. Consequently, it is essential to monitor these submaximal markers to evaluate the effectiveness of a training programme in inducing cardio-respiratory adaptations.

2.3.1 Biochemical responses to exercise

The intensity of an exercise determines the fuel used to provide ATP and the contribution of each metabolic pathway. The greater the intensity, the greater the reliance on anaerobic pathways to quickly meet the requirements in ATP of the exercising muscles. The increase in ATP hydrolysis and the greater reliance on anaerobic pathways induces an increase in lactate production and H⁺ accumulation, resulting from nonmitochondrial ATP hydrolysis and glycolysis ^{367,368}. Indeed, in a steady-state exercise, the H⁺ produced by glycolysis and ATP hydrolysis are consumed by the mitochondria as substrates for mitochondrial respiration ^{369–372}. However, when the exercise intensity and consequent H⁺ production from glycolysis and ATP hydrolysis increase, the rate of H⁺ acceptance by mitochondria is exceeded, leading to H⁺ accumulation in the cytosol of the muscular cell ³⁶⁸. While part of H⁺ is converted with pyruvate into lactate, the remaining accumulated H⁺ causes a decrease in cellular pH ³⁷³.

To delay the decrease in cellular pH, mechanisms of pH regulation are activated. One of the primary mechanisms is the transmembrane transport of H^+ out of the cell with the concomitant transport of sodium ion (Na^+) in the cell through the sodium-hydrogen antiporter 1 (NHE-1) ³⁷⁴. This lactate-independent H^+ transport accounts for the majority of H^+ efflux at low-intensity exercise ^{360,374}.

A second mechanism of pH regulation is the production of lactate from pyruvate and H^+ , which buffers an H^+ and evacuates H^+ outside of the cytosol ³⁶⁸. Indeed, through lactate/ H^+ co-transport systems (monocarboxylate transporters; MCTs), lactate and H^+ are diffused into the bloodstream with an obligatory 1:1 coupling ratio. This lactate-dependent H^+ regulation mechanism is the major contributor of H^+ release during exhaustive exercise (2/3 of muscle H^+ efflux) ^{367,368,375}. Consequently, when the intensity of exercise increases and mitochondria capacity is exceeded, the blood lactate concentration ($[La^-]$) is increased and could be monitored as an indirect marker of metabolic acidosis ^{368,376}.

In addition, intracellular pH is regulated using buffering systems (amino acids, proteins, bicarbonate (HCO_3^-), creatine phosphate hydrolysis, inorganic phosphate) ³⁶⁸. When H^+ are buffered by HCO_3^- , CO_2 and H_2O are produced ⁹. With the increasing buffering of H^+ , the CO_2 production then increases. Consequently, the partial pressure in CO_2 in the blood is increased, which is detected by peripheral chemoreceptors situated in the aorta or carotid arteries ³⁷⁷⁻³⁸⁰. The information of increased $PaCO_2$ will then be transmitted from the chemoreceptor to the central nervous system, that increases the ventilation to eliminate CO_2 through respiration ³⁸¹. Consequently, the increase in exercise intensity could be monitored using blood lactate and expired air assessments. These parameters have led to the determination of lactate or ventilatory thresholds, used to characterise exercise intensity ^{23,382-384}. However, the validity of thresholds' determination is nowadays questioned since more than 25 methods have been identified to calculate the lactate threshold, leading to heterogenous results and great disparity between methods ^{23,385}.

Skeletal muscle cells include different muscular fibres categories^{254–257}. Type I fibres, or slow-twitch muscle fibres contains a great quantity of mitochondria and have a greater blood supply than other fibre types. Their greater ability to produce ATP through mitochondrial respiration offers them a greater resistance to fatigue. Conversely, type IIa and IIb fibres, or fast-twitch fibres, have a lower quantity of mitochondria and a lower resistance to fatigue than slow-twitch fibres. However, they contain more glycogen and glycolytic enzymes than slow-twitch fibres, inducing a greater anaerobic capacity. In addition, fast-twitch fibres generate a greater force than slow-twitch fibres^{254–257}. Following the Henneman's principle, slow-twitch fibres are primarily recruited during exercise with an increasing fast-twitch fibres recruitment with increasing exercise intensity^{386–390}. As fast-twitch fibres are more reliant on glycolysis and cytosolic ATP turnover than slow-twitch fibres, H⁺ production and subsequent accumulation increases with increasing exercise intensity³⁶⁷.

2.3.2 Principles of ventilatory and lactate thresholds

Hill, Long and Lupton (1924) have stated that by using the respiratory quotient (also called respiratory exchange ratio; RER), it is possible to distinguish the acid-base changes occurring during exercise and recovery³⁹¹. RER is calculated as the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$. Later, Wasserman and McIlroy (1964) have demonstrated that the onset of anaerobic metabolism contribution during exercise could be detected using the RER, without performing invasive measurement of blood lactate³⁸². Therefore, both lactate and respiratory thresholds could be used to assess the contribution of anaerobic metabolism during exercise. However, nowadays, the concept of thresholds has evolved and led to a profusion of terminology and the criterion to detect those thresholds²³. While defining all the thresholds found in the literature would be redundant, defining the most widely used thresholds may help to better understand the relationship between exercise intensity and metabolic state.

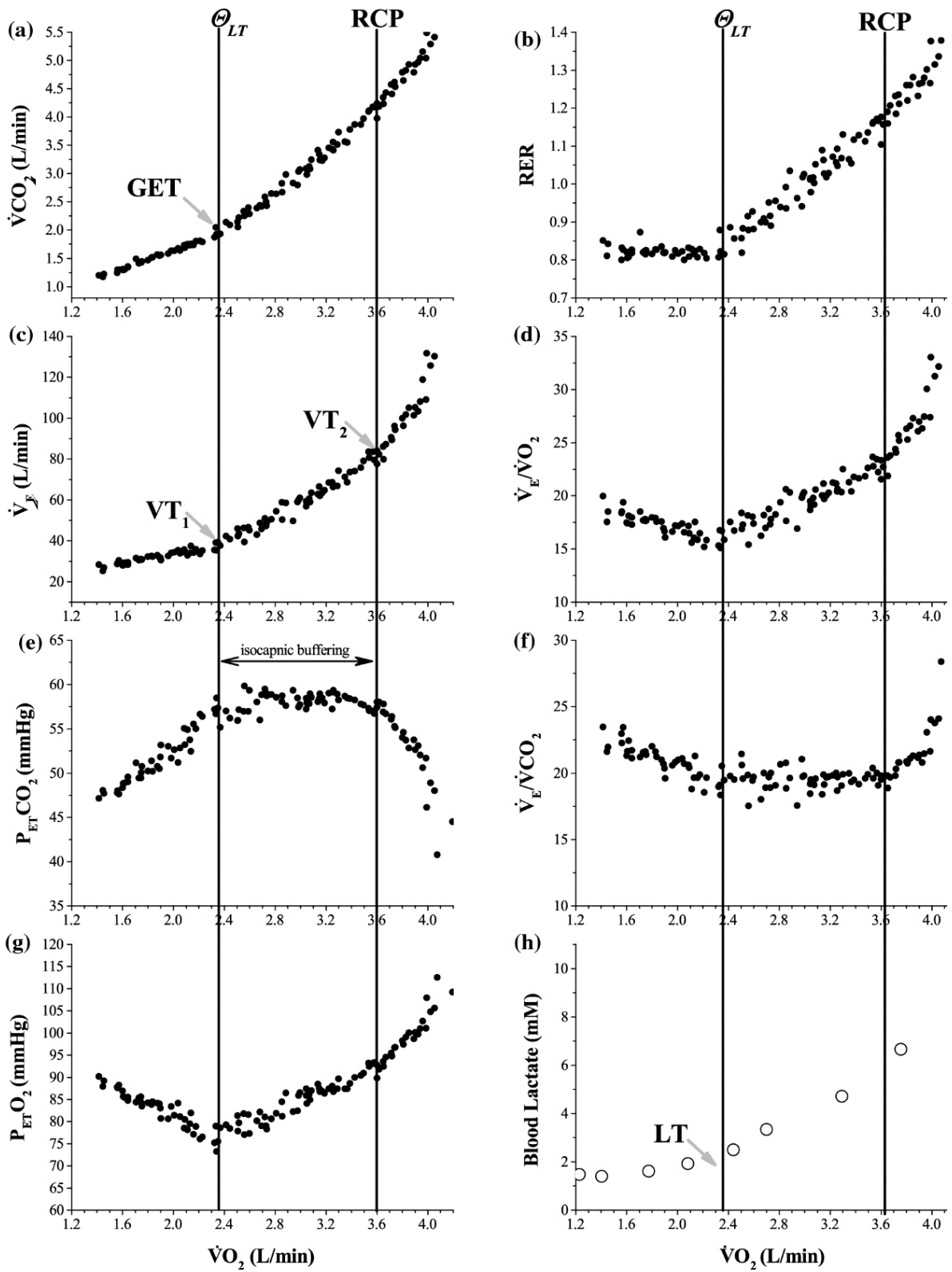
During an incremental exercise test, where the exercise intensity rises until volitional exhaustion, a first change in ventilatory and lactate curves could be found when a shift occurs in metabolism contributions to provide ATP³⁹². When a greater proportion of ATP is produced from anaerobic sources, the production of H⁺ and blood lactate increase, marking the onset of arterial lactate accumulation over resting values²³. Therefore, a first inflexion in the lactate curve over time could be found, named the lactate threshold (LT) (Figure 2-3). Above the LT, the increase in blood H⁺ associated with lactate combines with bicarbonate to produce an additional source of CO₂, leading to an increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$. The first inflexion in the $\dot{V}CO_2/\dot{V}O_2$ relationship (V-slope plot), and excess CO₂ plot is considered as the gas exchange threshold (GET)³⁸⁴. GET corresponds to the metabolic rate wherein the proportional evolution of excess CO₂ aligns with the rate of decrease in muscle and blood HCO₃⁻, independent of hyperventilation mechanisms³⁹³. The GET could be used as a non-invasive method to identify the LT, although there is a small difference due to buffering mechanisms between the LT and the following detection of GET^{23,394}. Since CO₂ delivery to the lungs drives ventilation ($\dot{V}E$), a disproportionate rise in CO₂ causes a corresponding increase in $\dot{V}E$. The ventilatory threshold (VT or VT1) is determined as a systematic increase in $\dot{V}E/\dot{V}O_2$ ^{382,394} (ventilatory equivalent in O₂) and end-tidal pressure of O₂ (PETO₂), and the point where end-tidal pressure of CO₂ (PETCO₂) begins to plateau³⁹⁵ (Figure 2-3). With the appearance of GET starts the region of isocapnic buffering, which is characterised by a relatively stable PETCO₂ while an increased CO₂ production that is due to a matched increase in $\dot{V}E$ ³⁸³. The isocapnic buffering region starts at GET/VT and ends at the respiratory compensation point (RCP) (Figure 2-3). The RCP marks the end of isocapnic buffering during a graded exercise test that maintains the arterial CO₂ level stable through an increase in $\dot{V}E$ ³⁹⁶. RCP reflects the metabolic rate at which the accumulation of H⁺ and associated increase in blood [La⁻] and CO₂ is no longer maintained stable³⁹⁴. In addition, the disproportionate increase in $\dot{V}E$ after RCP is mainly due to an increase in breathing frequency (BF) rather than an

increase in tidal volume, which has been shown to plateau after RCP³⁹⁷. The RCP, or also called ventilatory threshold 2 (VT2) is determined by the second inflexion point in the $\dot{V}E$ and $\dot{V}E/\dot{V}CO_2$ (ventilatory equivalent in CO₂) responses (Figure 2-3). RCP is also characterised by a fall in end-tidal pressure of CO₂ (PETCO₂), that was preceded by a period of stability (isocapnic buffering) (Figure 2-3). The RCP has been associated with the maximal lactate steady state (MLSS) and the critical power or speed (CP or CS), but their physiological significance has been fiercely debated in the literature^{23,398,399}. Indeed, the MLSS has been thought to underestimate CP or CS, while the RCP might overestimate the work rate or speed, as well as the $\dot{V}O_2$ ⁴⁰⁰, at which CP or CS occurs⁴⁰¹. This discrepancy may arise from methodological features in their determination, as highlighted by recent meta-analysis findings^{23,402}.

The MLSS and the CP/CS are not determined during an incremental exercise test; instead, they are calculated using specific protocols. The MLSS is the exercise intensity at which there is an equilibrium between the lactate transport to the blood and the lactate removal from the blood^{403,404}. The CP/CS is the power/speed asymptote of the hyperbolic relationship between power/speed output and time, determined using a series of constant power/speed output trials performed to the limit of tolerance⁴⁰¹.

Figure 2-3: Identification of ventilatory or lactate thresholds from a representative participant.

Gas exchange threshold (GET), respiratory compensation point (RCP), ventilatory threshold 1 and 2 (VT1, VT2) are assessed using a gas analyser. Lactate threshold (θ_{LT}) is assessed using $[La^-]$ in the blood. Rate of CO₂ excretion ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation ($\dot{V}E$), ventilatory equivalent of oxygen uptake (E/O_2), end-tidal pressure of CO₂ ($P_{ET}CO_2$), ventilatory equivalent of CO₂ ($\dot{V}E/\dot{V}CO_2$), end-tidal pressure of O₂ ($P_{ET}O_2$) and blood-lactate concentration are plotted in relation to rate of oxygen uptake ($\dot{V}O_2$). The vertical lines intersecting the abscissa of each figure indicate the O₂ associated with θ_{LT} and RCP. The methods of identification are presented in the text. From Keir et al. (2022)³⁹⁴.



In the moderate-intensity domain, $\dot{V}O_2$ rises until a steady state is reached within 2-3min, and lactate concentration stabilize at or below resting value after few minutes. In the moderate domain, the majority of ATP is produced aerobically with a contribution of anaerobic metabolism that does not result in any increase in blood lactate^{315,319,405,406}. The boundary between moderate- and heavy-intensity domains is therefore the GET (Figure 2-4). In the heavy-intensity domain, the $\dot{V}O_2$ slow component slowly drives the $\dot{V}O_2$ to a steady state below $\dot{V}O_{2max}$, but above the exercising $\dot{V}O_2$ value for the associate work-rate^{319,406}. The heavy-intensity domain lie between the GET and CP, with some criticisms to consider MLSS and RCP as boundaries between heavy- and severe-intensity domains^{407,408} (Figure 2-4). Another threshold has been used to delineate heavy- and severe-intensity domains, that is the onset of blood lactate accumulation (OBLA), measured during a graded exercise test⁴⁰¹. The OBLA corresponds to the exercise intensity at which the $[La^-]$ rise above the absolute value of 4 mmol/L during an incremental exercise test, which corresponds to a point where respiratory mechanisms (as indicated by \dot{V}_E) are sufficient to counterbalance the increasing H^+ and CO_2 production from anaerobic sources^{409,410}. In the severe domain, the exercise is performed at a greater intensity than CP, that is considered as the maximal metabolic steady state (MMSS)^{401,411,412}. Consequently, the increasing intensity causes an overwhelming rise in \dot{V}_E , $\dot{V}O_2$, blood and muscle lactate, and decrease in pH^{23,319,406}. The $\dot{V}O_2$ slow component causes $\dot{V}O_2$ to increase continuously without stabilising until $\dot{V}O_{2max}$ is reached or fatigue causes the termination of exercise^{319,406}. Therefore, the use of physiological thresholds to delineate the exercise intensity associated with either moderate, heavy or severe intensity domains. Since each domain is associated with different metabolic rate and physiological responses, the use of thresholds helps to discern the physiological adaptations expected with training³¹⁵. In addition, these thresholds are essential in monitoring the effects of training on aerobic capacity, since the delayed appearance of the GET and RCP is a sign of a successful training programme³⁶⁶.

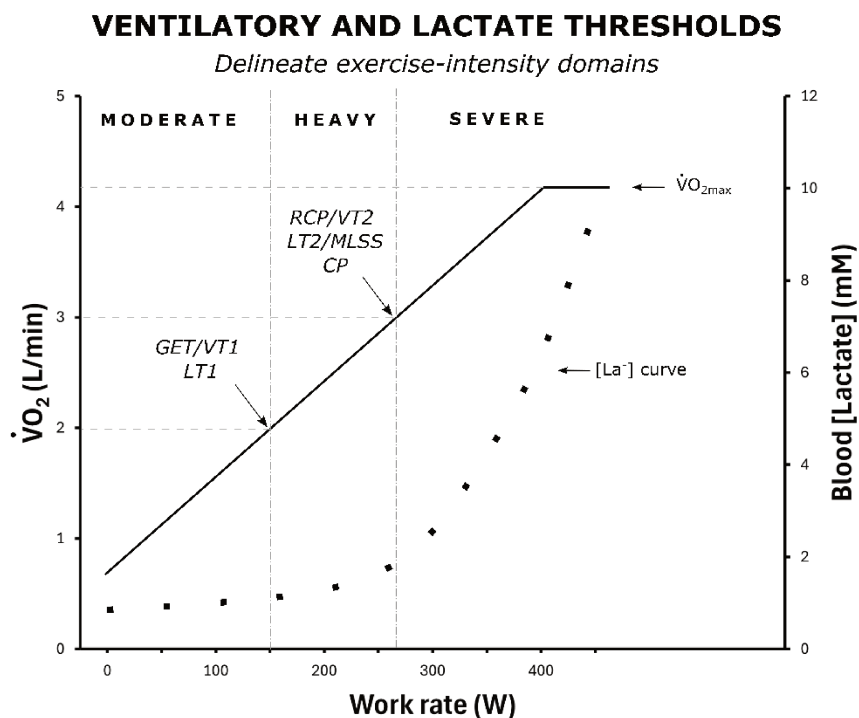


Figure 2-4: Schematic representation of ventilatory and lactate responses to increasing work rate during a graded exercise test.

The exercise-intensity domains are separated by ventilatory and lactate thresholds. Gas exchange threshold (GET), ventilatory threshold (VT1) and lactate threshold (LT1) delineate the moderate and heavy domains. Respiratory compensation point (RCP), ventilatory threshold 2 (VT2), lactate threshold 2 or lactate turn-point (LT2), maximal lactate steady state (MLSS) and critical power (CP) delineate the heavy and severe domains. This figure summarises thresholds but does not consider their physiological basis and discrepancies in their associated work rate. Although GET/VT and LT1 as well as RCP/MLSS and CP are displayed at the same arrows, they do not exactly appear at the same work rate^{392,393}. Modified from Poole et al. (2021)³⁹³.

2.3.3 Effects of BFR on ventilatory and lactate thresholds

Given the effects of BFR training on lactate production and pH regulation^{357,358}, the role of endurance training coupled with BFR on lactate and ventilatory thresholds have recently been investigated^{303,413-416}.

De Oliveira et al. (2016) have train participants for 4 weeks (3 sessions per week) with different regimens : low-intensity interval training with (LI-BFR, n = 10) or without (LI, n = 7) blood flow restriction (200 mmHg), high-intensity interval

training (HIT, $n = 10$), and combined HIT and BFR (BFR + HIT, $n = 10$, every session performed 50 % as BFR and 50 % as HIT) ³⁰³. Their results show an improved OBLA after all training regimens (LI-BFR, 16 %; HIT, 25 %; HIT + BFR, 22 %; LI, 6 %), with no difference between groups. Thus, adding BFR to LI training has produced a 16 % increase in power output reached at OBLA, compared to a 6 % increase in LI ³⁰³. These results are the first to demonstrate an increase in OBLA following endurance training coupled with BFR and are encouraging in the usefulness of BFR in delaying OBLA after endurance training. These findings are consistent with findings of Mitchell *et al.* (2019) that found a significant and meaningful increase in CP following SIT+ post-exercise BFR (+ 3.3 %) ³¹¹. However, the increases were in the same magnitude compared to increases found in the SIT group (+ 3.6 %). Consequently, these results have shown that short endurance training performed with BFR or postexercise BFR are effective in enhancing OBLA or CP, but do not show a greater increase compared to work-matched training ³¹¹.

Consistent results have been found by coupling BFR to resistance training (leg press, leg curl and leg extension), which shows a significant increase (+ 10.7 %) in $\dot{V}O_2$ reached at anaerobic threshold measured during an incremental ramp test to volitional exhaustion ⁴¹⁴. However, a recent study has found contradictory results ⁴¹⁵. Participants have followed a low-intensity (below lactate threshold) swimming training (15 sessions) with (75 % of AOP) or without BFR placed on upper limbs. $\dot{V}O_{2peak}$ LT1, and LT2 were measured during a swimming-specific incremental step test with $[La^-]$ concentration estimated between steps ⁴¹⁷. The results show notable $\dot{V}O_{2peak}$ improvements using the Bayesian approach, but no effects on either LT1 or LT2 ⁴¹⁵. Given previous results on the effects of BFR on lactate thresholds, these results were not expected. The discrepancies between these findings and previous results may arise from different factors. A first explanation may lay on the fact that BFR has been applied in upper limbs contrary to other studies ⁴¹⁵. Consequently, the occluded muscle mass and subsequent restricted blood flow is lower than a thigh

BFR. Given that relative contributions of arm strokes and leg kicks during swimming varies on an individual basis, physiological adaptations to either an upper or lower limbs occlusion may have been different. In addition, LT2 was calculated as $LT1 + 1.5 \text{ mmol/L}$ which does not represent a completely individualised calculation of lactate threshold. Calculating lactate thresholds using absolute values of blood lactate concentrations such as 2 and 4 mmol/L (for LT1 and LT2, respectively), has been criticised for lacking reliability between participants ⁴¹⁸. In addition, since swimming involves a different body position, more external pressure and less gravitational forces than terrestrial activities, the haemodynamic responses during exercise may be different and more work is needed to efficiently apply BFR on upper limbs during swimming ⁴¹⁹.

Although a relatively small amount of studies have investigated the effects of BFR on lactate or ventilatory thresholds, restricting the blood flow during or post endurance exercise seems effective in delaying ventilatory or lactate thresholds apparition ^{83,420}. However, none of the studies have reported a greater effectiveness or efficiency of BFR when compared to a non-BFR group. Given the various methodologies reported to calculate ventilatory and lactate thresholds, comparing those results is difficult. Therefore, additional work on submaximal physiological adaptations is needed to better understand metabolic and haemodynamics responses after adding BFR to endurance training.

2.4 CONCLUSION

The improvement in perfusive and diffusive O_2 conductances induced by cardiovascular adaptations to BFR have substantial impacts on maximal and submaximal $\dot{V}O_2$ ^{89,90}. These improvements of the cardiovascular system have been shown to delay the appearance of lactate and ventilatory thresholds, submaximal markers of aerobic capacity. These thresholds are essential to calibrate the training, as they delineate the exercise intensity domains ⁴⁰⁵. BFR has been shown to affect these domains, by slowing the $\dot{V}O_2$ kinetics and increasing its amplitude ^{352,421} during exercise. However, $\dot{V}O_2$ kinetics have been speeded after several weeks of BFR training contrary to the non-BFR group ³⁴¹. In addition, even with MICT, BFR has been shown effective in enhancing $\dot{V}O_{2max}$, while non-BFR groups did not ^{302,422}. But when BFR is associated with HIIT, BFR appears effective in increasing $\dot{V}O_{2max}$ but does not add a beneficial effect to HIIT ⁴²³. Given the small number of studies investigating chronic cardiovascular adaptations to HIIT+BFR or SIT+BFR, more research is needed to better understand if adding BFR to HIIT or SIT is beneficial. In addition, the discrepancies in the results could arise from methodological differences between studies, especially about the types of cuffs used and the selected pressure.

CHAPTER THREE

Methodologies and recommendations to occlude blood flow



The disruption of blood flow is influenced by various parameters such as cuff pressure, cuff material and width, as well as the individual's blood pressure and limb characteristics ^{28,130,424,425}. A broad range of occlusion pressures, materials and techniques are found in the literature, whether for BFR or IPC ^{41,426–428}. But, the heterogeneity in the ways of inducing a blood flow restriction also leads to heterogeneity in the reported outcomes ^{41,426–428}. Consequently, it is important to consider methodological factors when implementing a restriction of the blood flow for training purposes to ensure safety and optimise the effectiveness of the technique ^{28,429}.

3.1 TYPE OF CUFFS

Either in IPC or BFR, blood flow is restricted using an inflatable cuff. While IPC typically requires wide cuffs, those used in BFR studies vary in size, ranging from 3.0 to 20.5 cm ⁴³⁰. Rolnick *et al.* (2023) recently highlighted the extensive range of cuff types employed in BFR studies ⁴³¹, indicating a diversity in cuff width and material within the literature. However, this heterogeneity significantly impacts blood flow disruption and the strain exerted on blood vessels ^{424,430,431}. Furthermore, the past 25 years have seen the emergence of various BFR devices incorporating different technologies, which also influence the pressure stimulus ^{427,431,432}. Consequently, this

equipment diversity introduces haemodynamic discrepancies that necessitate further investigation.

3.1.1 Cuff width

Crenshaw *et al.* (1988) have highlighted the fact the cuff width has an impact on the transmitted pressure to the deeper tissues⁴³³. The authors compared the effect of four cuff widths (4.5, 8, 12, and 18 cm) on blood flow in human legs and found that the amount of pressure required for elimination of blood flow decreased as cuff width increased⁴³³. Wide cuffs transmit a greater percentage of the tourniquet pressure compared to narrower cuffs. Thus, a lower cuff pressure is required to completely occlude the blood flow, minimising the risks of soft-tissue damage⁴³³.

However, the use of wide cuffs is associated with a greater perceived pain during exercise endured by the participants. This greater perceived pain, which belongs to the barriers to BFR training⁴³⁴, may have participated in the use of narrower cuffs. Nowadays, a lot of different cuff widths are used in the BFR literature, but they do not produce the same vascular restriction. On the arms, occluding the arterial inflow necessitated different average pressures: 145 – 160 mmHg with a 5-cm wide cuff, 123 – 125 mmHg with a 10-cm wide cuff, and 120 – 122 mmHg with a 12-cm wide cuff^{84,435}. On the legs, this amount of pressure was raised to 148 mmHg with a 12-cm wide cuff⁴³⁶ and 144 mmHg with a 13.5-cm wide cuff⁴²⁴. In addition, the use of narrow cuffs (5-cm) has led to heterogeneous results: an average of 235 mmHg⁴²⁴, 344 mmHg⁴³⁶, and more than 500 mmHg⁴³⁷. In these three comparative studies, the authors mentioned that capacity of the cuff inflator was exceeded for some participants when using the 5-cm wide cuff (> 300 mmHg or > 500 mmHg)^{424,436,437}. Consequently, the occlusion of arterial blood flow is achieved at lower absolute pressure for wide cuffs compared to narrow cuffs^{424,436,437}.

The above-mentioned results are presenting discrepancies regarding the pressure required to occlude the blood flow. For instance, the same cuff width (5-cm) required 235 mmHg in a study ⁴²⁴, and 344 mmHg in another study ⁴³⁶. The differences could have arisen because the two cuffs used were from different manufacturers (Hokanson vs. KAATSU) and made of different materials (nylon vs. elastic).

3.1.2 Cuff material

Loenneke *et al.* (2012) have demonstrated that wider nylon cuffs (13.5 cm) required a lower pressure to occlude the blood flow compared to narrower elastic cuffs (5 cm) ⁴²⁴. But, the differences on the transmitted pressure between both cuffs has been hypothesised to be linked to cuff material. Consequently, a study from the same team investigated the effects of two cuffs with different materials (nylon vs. elastic) but same width (5 cm) on lower limbs arterial occlusion pressure ⁴³⁸. They found no significant differences between both cuff types (median for elastic: 235 mmHg and nylon: 225 mmHg). However, in this study 16 participants were recruited but half the sample has been excluded due to inability to induce arterial occlusion pressure (overreaching device limits). Thus, the influence of cuff type on applied pressure seems limited, but data presented have methodological issues. In order to address these issues, comparing the cuff material on upper limb will reduce the pressure required for arterial occlusion and avoid excluding participants. The effects on upper body stimulus of two widely used cuffs were then compared: Hokanson (5-cm wide, nylon) and Kaatsu (3-cm wide, elastic) ⁴³⁹. The results showed that resting AOP was reached at a significant higher pressure using elastic cuffs (246 ± 71 mmHg) than nylon cuffs (139 ± 14 mmHg) ⁴³⁹. This could be explained by the fact that the nylon cuff cannot be stretched as the elastic cuff, which compress more soft tissue and occlude the limb at much lower pressure ⁴⁴⁰. However, in the study of Buckner *et al.* (2017), both the cuff materials and widths differ (3 cm and 5 cm) ⁴³⁹. This parameter should not be excluded to explain the pressure required to occlude

the brachial blood flow, since larger cuffs require less pressure than narrower cuffs. The authors addressed this argument by stating that Jessee *et al.* (2016) only found 25 mmHg difference between their two nylon cuffs (5-cm and 12-cm) on upper limbs⁴³⁵. In addition, Jessee *et al.* (2016) only found a 3-mmHg difference between two cuffs with 2-cm difference in width (10-cm and 12-cm)⁴³⁵. Same results were reported by Mouser *et al.* (2017)⁸⁴. Thus, the 2-cm difference in width between the 3-cm cuff (KAATSU) and the 5-cm width cuff (Hokanson), should not show a significant difference on the pressure to occlude the blood flow. However, the 2-cm difference between 3 and 5 cm represents a difference of 40 %, whereas the 2-cm difference between 10 and 12-cm represent a difference of only 17 %. Interestingly, between two 4.5-cm and 8-cm width cuffs (difference = 44 %), Crenshaw *et al.* (1988) reported a difference of \approx 90 mmHg to occlude the arterial blood flow⁴³³. Consequently, the 2-cm width difference in the study of Buckner *et al.* (2017) might has a larger role than suggested⁴³⁹.

If the resting AOP value was altered by elastic cuffs, similar responses between the two cuffs were showed for the number of repetitions, the torque, the muscle activation or the rate of perceived exhaustion (RPE) during exercise at 40 % of the AOP⁴³⁹. But interestingly, the elastic cuffs elicited greater discomfort during exercise than nylon cuffs⁴³⁹. This discomfort could be attributed to the higher absolute pressure in the elastic cuffs compared to the nylon cuffs.

3.1.3 BFR devices

First studies on the effects of blood-flow restricted exercise in male used a large pressure chamber (21 m³) with both legs and cycle ergometer inside the chamber^{51-53,441}. The chamber pressure was increased by 50 mmHg above atmospheric pressure, which has been demonstrated to result in approximately a 20 % reduction in leg blood flow during exercise⁴⁴². Later, Sato developed a BFR device (model M.P.S.-700) that consisted of belt containing a small pneumatic cuff connected to an electronic pressure gauge for monitoring the pressure⁴⁴³. This kind

of electronically controlled units, such as KAATSU Master (Sato Sports Plaza, Tokyo, Japan), the Delfi Personalized Tourniquet System (Delfi Medical Innovations System, Vancouver, BC, CAN), and the Hokanson rapid cuff inflation system (Hokanson, Inc., Bellevue, WA, United States) have been developed for BFR training and used in the laboratory as well as in clinical routine ⁴³⁷. The advantage of such devices is their ability to inflate the cuffs to a very precise amount of pressure and maintain this pressure throughout the whole exercise duration.

These BFR devices are able to better regulate the applied pressure while inducing lower haemodynamic and perceptual responses than non-autoregulated BFR devices ⁴³². Using autoregulated devices allows a significant higher number of repetitions until failure compared to non-autoregulated devices ⁴⁴⁴. This is accompanied with less discomfort and exertion, which reduces the excessive perceptual demands of BFR training and may help to strengthen long-term compliance with this kind of training ^{434,444}. Yet, it is important to choose carefully the BFR device since they could show differences in cuff and then, in applied pressure. Weatherholt *et al.* (2019) conducted a study comparing the Delfi Personalized Tourniquet System and KAATSU devices to examine the impact of cuff width on limb occlusion pressure (LOP) ⁴³⁷. The study reported significant differences in LOP between the two units, with Delfi measuring 239.4 mmHg and KAATSU measuring 500 mmHg ⁴³⁷. These results are explained by the difference in cuff width of both devices (11.5 cm and 5 cm, respectively).

Furthermore, BFR systems use diverse technologies for cuff pressure regulation, alongside employing cuffs of varied shapes, materials, and sizes. These factors introduce potential variations in cuff-to-limb interface pressure, which may lead to heterogeneous perceptual and cardiovascular responses. Therefore, Hughes *et al.* (2018) examined whether different BFR systems, both autoregulated and non-autoregulated, exert equivalent pressure on the limbs and physiological responses ⁴³². Then, the authors compared three BFR systems among the most used: an

automatic rapid- inflation (RI) system (E20 rapid cuff inflator, Hokanson, Bellevue, WA, USA) with a straight nylon cuff (13 cm × 124 cm, 0.5 mm thick); an automatic personalized tourniquet (PT) system (Delfi Medical, Vancouver, BC, Canada) with a variable contour nylon cuff (11.5 cm × 86 cm, 2.5 mm thick); a manual handheld (HS) pump and sphygmomanometer system with straight nylon cuff (8 cm × 100 cm, 1.5 mm thick) (The Occlusion Cuff, Sussex, London, UK). The authors have chosen three systems with different methods of pressure regulation: automatic pressure adjustments (RI); automatic pressure adjustments around the targeted pressure (PT); and no automatic pressure regulation (HS). Using pressure sensors placed between the cuff and the limb (dominant leg), the interface pressure for each system was estimated during a complete arterial occlusion at rest, and at 40 % and/or 80 % of the AOP at rest and during exercise. The results of this study emphasise the discrepancies in interface pressure between BFR devices⁴³². The pressure needed for blood flow cessation (as indicated by AOP or LOP) was significantly greater for PT and HS compared to RI, which is mainly explained by a greater width for RI cuff. At rest, the interface pressure at 40 % and 80 % of LOP was significantly lower than the set pressure in all three systems. However, the interface pressure during the exercise trial (80 % of LOP) was significantly greater than the set pressure for RI and HS systems, exceeding the clinical limit of $\pm 15\text{mmHg}$ ^{432,445} for HS only. These results are noteworthy since applying too much pressure may damage the tissues or induce vascular or nerve injuries^{273,446}. In addition, both RI and HS system induce greater pain and cardiovascular responses than the PT system. After exercise, there was a statistically significant increase in mean arterial pressure observed in both the RI and HS systems, but not in the PT system. This increase in cardiovascular response could be particularly concerning for specific populations (elderly or patients with cardiovascular diseases)⁴⁴⁷. In addition, a greater cardiovascular work caused by some BFR systems could therefore induce discrepancies in cardiovascular adaptations in long-term studies. Finally, having a greater interface pressure than set pressure, exceeding the clinical limit, may induce an unintentional arterial

occlusion. This is noteworthy since it is known that during high-intensity exercise, the muscle contractions reduce the blood flow due to an increase in intramuscular pressure^{448,449}. Thus, combining high-intensity exercises with manually regulated BFR, could lead to an “higher-than anticipated” pressure in the limb compared to rest⁴⁴⁴. Therefore, utilising an autoregulated device that releases air during muscle contractions appears to be a safer approach and may result in more consistent responses.

However, the cost of such devices may be prohibitive for large scale training studies, but also for practitioners, athletes, or gyms. A Delfi PTS system, as presented in Hughes *et al.* (2018), typically has a price of approximately 6,000 €, while a pneumatic tourniquet with regulated pressure costs around 4,500 €. On the other hand, manually inflated cuffs come at a lower cost, ranging from 100 to 200 €. Consequently, since manual cuffs will still be used in research or in recreational use, it may be relevant to reduce the pressure recommendations to avoid adverse effects linked to an elevated pressure, when combined with high-intensity exercises.

3.2 OCCLUSION PRESSURE

Compared to ischaemic training, which implies the performing of the exercise with arterial occlusion⁵³, BFR aims to induce a venous but not an arterial occlusion⁵⁵. Thus, the amount of pressure in the cuff needs to be chosen carefully to reduce arterial inflow to the active muscles, but also occlude the venous return in order to induce a blood pooling near the working muscles⁴⁵⁰. On the other hand, IPC requires sufficient pressure to fully occlude blood flow in the limb without causing vascular or nerve damage⁴⁴⁶.

3.2.1 Arbitrary pressures

The use of absolute pressure to induce a restriction of the blood flow consists of inflating the pressure cuff of all the participants in a group at the same amount of pressure. For example, early studies on BFR used fixed pressures of 50, 70, and

100 mmHg to restrict the arm blood flow⁴⁴³. Similarly, incremental arbitrary values ranging from 160 to 240 mmHg were used for the legs⁴⁵¹, and are still found in recent literature⁴⁵². Such fixed values for the legs (i.e., increments in 10 mmHg each day or week in cuff pressure from 160 mmHg to 240 mmHg at the end of the training period) have been selected to induce a blood pooling in vessels distal to the cuff by restricting the venous blood flow⁴⁴³. These values have emerged from the use of a specially designed device (model M.P.S.-700) that will later evolved into the KAATSU devices^{55,443}, that is still used nowadays^{213,453,454}.

According to a recent systematic review⁴²⁶, 56.6 % of BFR studies made the choice to use arbitrary fixed pressures, whether for the arms or the legs. This approach has shown to induce physiological benefits in different populations using resistance or aerobic training. Takarada *et al.* (2000) showed acute and long-term changes in muscular function following resistance exercise performed with BFR at the arm⁴⁴³. After one session of dumbbell curl exercises with BFR using a varying pressure of 0 to 100 mmHg, the authors found an elevation in electromyographic activity (iEMG), postexercise hyperaemia and plasma lactate concentration with the increase in occlusion pressure⁴⁴³. In addition, older women (58.2 ± 6.6 yr) followed 16 weeks of dumbbell curl training with (110.0 ± 7.1 mmHg) or without arm BFR. The results showed greater improvements in cross-sectional area and isokinetic strength of the elbow flexor (30, 60 and 90 °/s) for the LI (50 % of 1-RM) group with BFR compared to the LI group without BFR. Moreover, the isokinetic strength improvements in the LI group with BFR ($+ 18.4 \pm 1.5$ %) were not different than those in the high-intensity (HI; 80 % of 1-RM; $+22.6 \pm 2.0$ %) without BFR. Thus, using arbitrary pressures to restrict the blood flow was efficient to induce acute and long-term changes in muscular function⁴⁴³. In a study focused on the lower body, participants were trained for 2 weeks (24 sessions), performing resistance exercises (squat and leg curl) with or without BFR⁴⁵¹. The occlusion pressure was set at 160 mmHg for all participants on the first session and increased by 10 mmHg each day until the final pressure of 240 mmHg. The results showed greater ($p < 0.05$) increases

in squat (16.8 %) and leg curl (22.6 %) 1-RM strength in the BFR group compared to the group without BFR (8.9 % and 1.3 %, respectively). The use of BFR with fixed or increasing arbitrary values then enhance the effects of resistance training.

The same occlusion pressure protocol was applied to aerobic training during exercise⁴⁵⁵ or during recovery between sets²¹⁷. In the studies of Abe *et al.* (2006, 2010), participants were trained using walk (50 m/min) exercises performed with or without BFR^{251,455}. The use of 160 – 240 mmHg occlusion pressure during exercise induced greater strength and hypertrophy than without BFR⁴⁵⁵. In addition, the walk exercise was more challenging for the BFR group, as $\dot{V}O_2$ and HR during exercise were significantly higher in the BFR group⁴⁵⁵. However, 6 weeks of walk training (67 m/min; 45 % of HRR) were not enough to induce long-term adaptations in $\dot{V}O_2$ for either group²⁵¹. More recent study also failed to induce long-term $\dot{V}O_{2max}$ changes after low-intensity aerobic training with arbitrary occlusion pressures³⁰⁴. An explanation could lie in the fact that, while BFR walk increases HR compared to control group, the stimulus is not high enough to reach the targeted training intensity. Indeed, BFR group only reach 45 % of HRR during training, despite the author's target of at least 50 % to improve aerobic capacity in older adults (≥ 60 years)²⁵¹. A recent literature review have stated that with a training intensity of 50 % of HRR, only small $\dot{V}O_{2max}$ gains could be expected in sedentary older adults (67.45 ± 5.25 years)³⁰⁵. But expected $\dot{V}O_{2max}$ gains with LI training (≤ 50 % of HRR) imply longer training duration (8 – 20 weeks) than the current protocol (6 weeks). It is noteworthy that with a 8 weeks long low-intensity (40 % $\dot{V}O_{2max}$) cycle training with or without BFR (160 – 210 mmHg incremental arbitrary pressures), significant improvements in $\dot{V}O_{2max}$ were only found in the BFR group³⁰¹. In this study the HRR reached by BFR group (50 – 65 %) was higher than control group (37 – 46 %), which could explain this difference in post-training $\dot{V}O_{2max}$ ³⁰¹. But, the exercise intensity is not the sole factor inducing $\dot{V}O_{2max}$ adaptations since interval training performed at 90 % of $\dot{V}O_{2max}$ (80 – 90 % HR_{max}) with (90 mmHg) or without BFR for 6 weeks failed to increase post-training $\dot{V}O_{2max}$ ³⁰⁷. While the intensity was high in this study,

the BFR stimulus was not powerful enough or the training not long enough to induce $\dot{V}O_{2max}$ adaptations as in other studies^{297,308,456}. However, the authors found higher peak power output and muscular deoxygenation in both groups, with greater changes in BFR group³⁰⁷. These results suggest that peripheral rather than central adaptations may have occurred with BFR³⁰⁷ such as increased capillarisation⁴⁵⁷ or increased mitochondrial activity²⁹⁴.

While a majority (56.6 %) of BFR studies use arbitrary pressure⁴²⁶, this result seems to be emphasized in IPC studies, targeting performance improvements. Indeed, in their review O'Brien & Jacobs (2021) reported more than 75 % (61 on 81 studies) of the IPC studies using arbitrary pressures⁴¹. Most of the studies used tourniquet pressure in the range 200 – 250 mmHg that has classically been found in clinical IPC procedure performed before cardiac surgeries^{41,458}. Moreover, these pressures have been associated with favourable changes in the literature^{45,459,460}. But, the beneficial effects of IPC in sport sciences are equivocal and questioned in a number of recent reviews^{41,461,462}. While significant improvements due to IPC have been found (increased $\dot{V}O_{2max}$, strength, O₂ perfusion, peak power output, performance)^{33,45,459,461,463–466}, the field suffers from heterogeneous findings that may be linked to huge methodological variations between studies^{41,461}.

One of the differing criteria between protocols is the occlusion pressure applied during IPC⁴¹. As stated earlier, the commonly used pressure range of 200-250 mmHg in IPC studies implies a 50 mmHg (25 %) difference between pressures. In BFR studies, cuffs are inflated in a wide range of arbitrary pressures from 10 to 270 mmHg, with most studies using pressure greater than 100 mmHg⁴²⁶. However, using the same pressure for all participants overlooks physiological disparities between participants. Indeed, physiological characteristics such as blood pressure at rest exhibit a great inter-individuals' variability^{467–469}. For instance, Landahl *et al.* (1986) reported a SBP of 123 ± 14.4 mmHg in 369 normotensive females aged 38 years⁴⁶⁷. Thus, the SBP lies between 94 and 152 mmHg (± 2 SD) for approximately

95 % of participants in this study. Therefore, applying the same arbitrary pressure to individuals with blood pressures of 94 mmHg and 152 mmHg will result in different percentages of AOP and subsequent blood flow reductions for each participant. In addition, the blood pressure varies between young and old ^{467,470}, between gender ^{471,472}, between arms ⁴⁷³, between sitting and lying down ⁴⁷⁴, between home and clinic measurements ⁴⁷¹, and between ethnic groups ⁴⁷⁵⁻⁴⁷⁷. Then, applying the same absolute amount of pressure to restrict blood flow may lead to a light occlusion in some participants and a full occlusion in others. Consequently, with wide ranges of pressure used between studies and different occlusion stimuli relative to AOP between participants, heterogeneous responses to arbitrary occlusion pressures are expected ⁴¹.

Beyond the concern that arbitrary pressures could produce inadequate results, the fact that arbitrary pressure may induce complete occlusion in some participants is particularly concerning, as exercising with BFR increases cardiovascular demand. ⁴⁷⁸. Consequently, exercising with a fully occluded limb will increase the additional cardiovascular work and the risk of cardiovascular adverse events such as arterial damages ^{41,273,426}. Moreover, Noordin *et al.* (2009) highlighted the fact that a greater cuff pressure may cause nerve injuries ⁴⁴⁶. Although the complications linked to tourniquets use has been demonstrated ⁴⁷⁹⁻⁴⁸¹, this argument needs to be put in perspective. Complications and nerve damages occurring after tourniquets use in clinic have been commonly reported (adverse events reported in 66 % to 77 % of patients) ^{446,482,483}. These damages occurred in the 1980's with old tourniquets devices, before the introduction of microcomputer-based tourniquet system that include pressure regulator ^{445,446,484}. In a questionnaire survey in Norway dating from 2006, the number of 26 complications (e.g. skin necrosis, thrombosis, nerves damages) were reported out of 63 484 operations (0.041 %) with the use of a tourniquet ⁴⁸⁵. In addition, the study of Ochoa *et al.* (1972) is often cited to show the anatomical changes in nerves structure consecutive to a tourniquet occlusion, such as nodal lesions and nerve demyelination. However, this

study was performed on apes with a cuff pressure inflated up to 1000 mmHg, which is four times higher than the pressure used in the studies about IPC (\approx 250 mmHg)⁴⁸⁶. Thus, high pressure may lead to nerve injuries, but it seems relatively uncommon, especially using recommended pressure and application time. Finally, another issue with arbitrary pressures is the fact that higher pressures may induce higher discomfort^{361,487,488}. Indeed, to ensure complete occlusion for each participant, arbitrary pressures often exceed the AOP for most participants. Consequently, IPC values range from 200 to 250 mmHg, even though the pressure required for arterial occlusion should be closer to the average arterial pressure values (\approx 120 to 130 mmHg in a normotensive population)^{467,469}. Using numerical rating scale (0 to 10), discomfort link to IPC intervention has been rated 4/10 (arm) and 7/10 (leg)^{489,490}. Discomfort produced by BFR during exercise has been rated between 4.5 and 8.7 out of 10^{491,492}. Consequently, if the pressure added is higher for some participants, it could reduce the adherence to the study^{426,478}. It then becomes an issue for the investigator, who needs to recruit more participants. But it also shows an ethical issue regarding the publication of the results : the results published will only be based on the most resilient or resistant participants, which is not representative of a population. It would then be hard to generalise the findings to the whole population.

In order to improve the safety, the applicability and the efficacy of IPC or BFR, the same relative restriction should be applied to all participants. Several studies in the last few years have encouraged the researchers to replace the arbitrary pressure by individualised pressures^{28,273,425,426}. The transition to using relative pressures is underway, as the number of publications with arbitrary pressures decreases each year, while the number of publications with individualised pressures increases⁴²⁶.

3.2.2 Individualised pressures

Participants present individual differences in limb characteristics, body composition and haemodynamics, that leads to heterogenous amount of restricted blood flow in the limb when using arbitrary pressures⁴⁹³. To overcome this lack in

consistency in the magnitude of the haemodynamic response, individualised pressures have been recommended, in order to induce the same relative BFR for all participants using the same equipment ^{273,435,494–496}.

To ensure consistent restriction of blood flow among all participants, establishing a common reference point relative to blood flow becomes necessary. Some have used the brachial SBP as an objective value to base the cuff pressure ^{357,358,497–500}. However, the use of brachial SBP to set a pressure for lower limbs has been discouraged since SBP values differs between upper and lower limbs ^{501,502}. In addition, the limb circumference and the cuff width should be taken into account when using the SBP to select a cuff pressure ⁴²⁵.

A simple approach for standardising the measurement of blood flow across participants involves estimating its absence. This is achieved by gauging the pressure at which the inflated cuff fully occludes blood flow in the arteries, leading to the determination of AOP for all participants ^{503,504}. Once the AOP is known, it becomes possible to express the blood flow restriction as a percentage of AOP. Consequently, even when participants have varying absolute AOP values, expressing the BFR as a relative percentage of AOP ensures uniformity in the applied restriction across all individuals.

The AOP, which is the lowest amount of pressure needed to occlude the arterial inflow in the limb, could be assessed reliably using a portable Doppler ultrasound ⁵⁰⁵. The method consists of placing the Doppler probe on the skin above a superficial artery to detect the pulse, and inflating the cuff until no pulse is detected. The cuff is then slowly deflated until the reappearance of the pulse ⁵⁰¹. The cuff pressure value is then the AOP. Another method for determining AOP is using an incremental step protocol. Starting with deflated cuffs (= 0 mmHg), cuff pressure is increased in 30 mmHg (arms) or 40 mmHg (legs) increments (30 s inflation followed by a 10 s deflation) until the absence of a pulse. Upon the absence of arterial inflow during inflation, the cuff pressure is systematically reduced in 10

mmHg increments until the pulse reappeared. The AOP is then determined as the nearest 10 mmHg, representing the lowest cuff pressure at which the pulse was not detected⁴²⁵.

In the literature, the terms AOP and LOP are often used interchangeably. However, a difference between both terms may be highlighted. The LOP is defined as the minimum pressure required with a specific surgical-grade tourniquet to occlude all arterial blood flow into the limb distal to the cuff⁴²⁷, although the AOP estimates the occlusion of a single superficial artery. Consequently, even if the superficial artery is occluded, deeper arteries may not be occluded. The reliability of AOP values as then be questioned, and the use of LOP has been preferred^{427,506}.

Once LOP is known for each participant, the restriction could be applied given a percentage of LOP. However, the assessment of LOP is not always possible, especially for lower limbs. Indeed, depending on the cuff specifications, the pressure needed to occlude the limb may exceed the technical limitations of the device (> 300 mmHg)⁴²⁵. Thus, it becomes impossible to precisely determine the LOP and apply the same percentage of LOP. Consequently, LOP predicting equations have been developed throughout the years taking into account some of LOP determinants, for specific cuff widths (*i.e.*, 5 to 18 cm)^{435,507} or based on cuff width and limb circumference^{273,446,508} (Equation 7).

Equation 7 : LOP equation.

The LOP (mmHg) calculation is based on the cuff width (W) and the limb circumference (C). From McEwen et al. (2019)²⁷³.

$$LOP = 67 + \frac{C}{0.06 \times W}$$

The selection of the LOP percentage for BFR training has exhibited considerable variability across studies, with the majority of investigations employing a wide range of 30 % to 80 % of LOP ⁴⁹³. Recent guidelines on BFR methodology propose utilizing values between 40 % to 80 % of LOP during aerobic exercise, as this range has been found effective in promoting physiological adaptations without compromising safety ²⁸.

It is noteworthy that the percentages of LOP used do not reflect a percentage reduction in blood flow, but rather a percentage relative to a pressure value ⁸⁴. Therefore, when literature mentions BFR training at 40 % or 80 % of LOP, it does not correspond to a reduction of 40 % or 80 % in blood flow ⁸⁴. Interestingly, the haemodynamic response following increasing levels of occlusion has yielded contradictory results in the literature. Some studies demonstrate a near-linear relationship between blood flow reduction and occlusion pressure in both upper and lower limbs ^{131,509,510}. Other studies ^{84,511} focusing on the upper limbs showed that the relationship between occlusion pressure and blood flow reduction was not truly linear (Figure 3-1). In addition, no significant difference in blood flow reduction between occlusions at 40 % and 80 % of LOP were found. The findings from Mouser *et al.* (2017) also suggest a similar reduction of blood flow between 50 % and 80 % of LOP, with the same response for three different sizes of cuffs ⁸⁴. The differences between these studies are likely explained by the nature of the pressure applied. Contrary to the studies that found a near-linear relationship between occlusion pressure and blood flow using absolute pressures, Mouser *et al.* (2017) applied pressures relative to the LOP of each participant ^{84,511}. Consequently, a line of agreement (LoA) could be drawn to indicate a true linear relationship between blood flow and occlusion pressure (Figure 3-1). It is then clear that the reduction of the blood flow does not follow this LoA, indicating that the relationship between pressure and blood flow reduction is not truly linear.

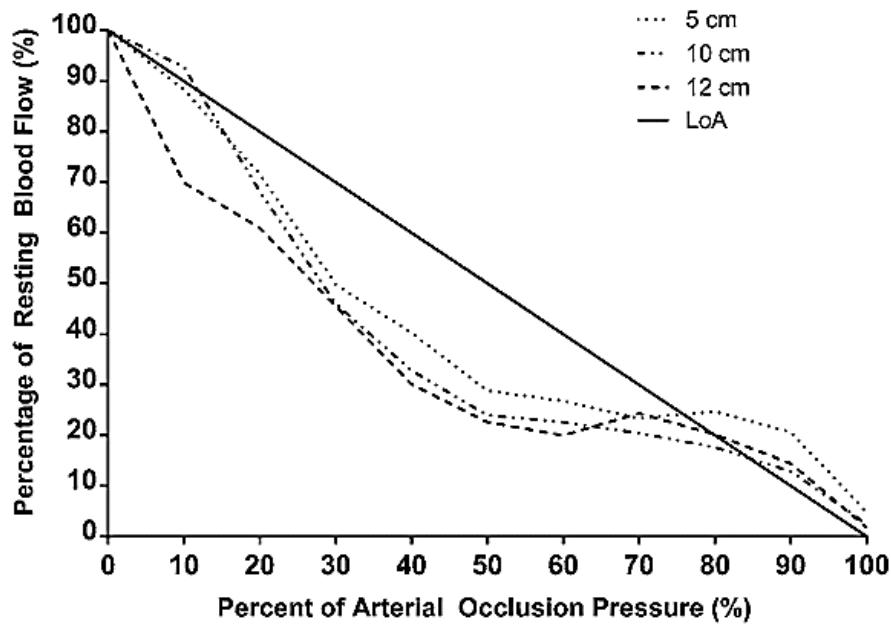


Figure 3-1: Representation of the blood flow as the percentage of resting blood flow (%) in response to occlusion pressures ranging from 10 % to 100 % of AOP.

The straight black line represents the line of agreement (LoA), and the dashed lines represent the different cuffs width used in the study from Mouser et al. (2017) ⁸⁴. The decrease in resting blood flow percentage with increasing pressure does not differ significantly between 40 % and 80 % of AOP and does not follow the LoA. Consequently, the relationship between pressure and blood flow reduction is not truly linear. From Mouser et al. (2017) ⁸⁴.

The aim of individualised pressure is to overcome individuals' characteristics, to standardise the stimulus for all participants. But the range of applied BFR pressure is wide, with pressure ranging from 30 % to 100 % of AOP ⁴⁹³. In their recent review, Patterson et al. (2019) have recommended using between 40 – 80 % of AOP for resistance or aerobic BFR training ²⁸. But there is no clear explanation of these range based on physiological responses to BFR. The findings of Mouser et al. (2017), that showed no significant differences in blood flow reduction between 40 % and 80 % of AOP ⁸⁴ may have been used to justify this range but are not mentioned as a

justification in the review ²⁸. In addition, it has been shown recently that BFR literature lack of arguments when selecting a cuff pressure ⁴⁹³. In a recent systematic review, none of the studies comparing two BFR pressures provided a justification of the pressure used ⁴⁹³. And only 7 studies among the 36 studies that used a single pressure provided a justification. In total, 13.5 % of the included studies of this systematic review, provided a justification for the pressure used. The provided justifications were that the pressure used has been shown to be effective in another study, the use of lower pressure to reduce the risks or the discomfort ⁴⁹³.

Cuff pressures between 40 % and 80 % of AOP are reported to be efficient stimulus in both resistance and aerobic training with BFR ²⁸. Thus, it remains to be determined whether 40 % of AOP is sufficient to achieve the same physiological adaptations to BFR training as 80 % of AOP. Similar adaptations in hypertrophy, strength, and muscle endurance following 8 weeks of low-load (30 % of 1-RM) unilateral elbow flexion training with either 40 % or 90 % of AOP ⁶⁸. However, the greater discomfort has almost always been associated with greater pressure ⁶⁸. This results on muscle adaptations are consistent with the findings of Loenneke *et al.* (2015) suggesting that muscle activation was not increased with higher pressure than 50 % of AOP ⁵¹². However, a difference between low and high occlusion pressure may be seen at lower exercise intensity. Indeed, after 12 weeks of lower limb resistance training at 20 % or 40 % of 1-RM with either BFR at 40 % or 80 % of AOP, increasing occlusion pressure was more effective in enhancing muscle mass ⁵¹³. This finding was only shown at very low intensity (20 % of 1-RM), but not with higher load (40 % of 1-RM), highlighting the importance of occlusion pressure when the load is low. This is particularly useful for participants that are unable to perform task with higher loads, such as elderly or patients in rehabilitation. The findings of another study indicate that vascular adaptations may be facilitated by higher pressures of occlusion ⁵¹⁴. In this study, the participants completed 8 weeks of resistance training (15 % of 1-RM) with BFR at 40 % or 80 % of AOP, or without BFR at 15 % or 70 % of 1-RM. The results showed a significant greater vascular

conductance after training in the high-pressure (80 % of AOP) and the high-load groups, only. These results were found in both upper (forearm) and lower (calf) body vascular conductance⁵¹⁴. The pressure applied by the cuff has been hypothesised to increase the circumferential strain on the microvasculature⁵¹⁵, leading to a blood pooling and a release of angiogenic factors. With the results of Mouser *et al.* (2019), it appears that neither training at 15 % of 1-RM with or without BFR at 40 % of AOP did result in any sufficient strain on capillaries wall⁵¹⁶. However, a higher occlusion pressure increases the circumferential strain on microvasculature and induce vascular adaptations⁵¹⁴.

The greater vascular adaptations caused by higher cuff pressure could arise from the impact of a greater pressure on haemodynamics. In their study, Singer *et al.* (2020) asked their participants to perform one set of 30 repetitions of unilateral isotonic (30 % of peak torque) knee extension at four different occlusion pressures (0 %, 60 %, 80 % and 100 % of AOP)⁴²⁸. As for resting condition, the increase in blood flow during exercise was significantly blunted as cuff pressure increased (100 % < 80 % \approx 60 % < 0 %, all $p \leq 0.011$). Consequently, the muscle oxygenation measured through NIRS decreased significantly in a gradual manner, with the exception of 80 % and 100 % of AOP showing no significant differences, but a tendency ($p = 0.098$). However, the concentration of HHb was the greatest at 100 % of AOP highlighting a greater venous occlusion and metabolites byproducts accumulation with higher pressure, as supported by Yasuda *et al.* (2010)⁵¹⁷. While not significant, the SV during exercise seems to be decreased gradually with increasing pressure, caused by a greater blood pooling with high pressures⁴²⁸. Consequently, to preserve \dot{Q} and meet the O₂ requirements, the HR is higher in the BFR conditions compared to no BFR, with the greatest pressure (100 % AOP) showing the highest HR⁴²⁸.

While it is now acknowledged that cuff pressure should be individualised in BFR, the efficiency of different levels of occlusion pressure during exercise have

given heterogeneous results in the literature. The pressure in the cuffs needs to be selected rationally, aiming to be adapted to a given population or a targeted physiological adaptation. Future research is needed to better understand the effects of BFR in the recommended range of 40 % to 80 % of AOP. However, these questions about the level and the individualisation of cuff pressure need to be investigated also in the IPC literature. As for BFR, the effects of lower or higher occlusion pressure on haemodynamics needed to be studied to help choosing between pressures and bring more homogeneity and safety to the research.

3.3 CONCLUSION

Both IPC and BFR have led to heterogeneous responses or adaptations, that could arise from methodological differences. The disruption of blood flow is influenced by various parameters such as cuff pressure, cuff material and width, as well as the individual's blood pressure and limb characteristics^{28,130,424,425}. Regarding cuff characteristics, a wide range of bladder widths (3.0 to 20.5 cm) have been reported in the literature when associated with BFR. These width differences impact the transmitted pressure to the deeper tissues, with wider cuffs requiring less pressure than narrower cuffs to induce the same blood flow restriction⁴³³. In addition, when the width of the cuff is too small (< 5 cm), the capacity of the cuff inflator may be exceeded, resulting in an inability to restrict consistently the blood flow^{424,436,437}. This finding may be exacerbated by the material in which the cuff is made. Indeed, cuffs may be built of nylon or elastic matter, comprising different physical properties relative to pressure transmission to the tissues. Elastic cuffs have been shown to require higher pressure to occlude blood flow compared to nylon cuffs^{439,440}. In addition, a study has reported a greater discomfort with elastic cuffs, which may be due to the greater pressure required⁴³⁹. Finally, the type of device used to induce BFR plays an essential role in the pressure applied to the limbs with autoregulated devices providing safer and more accurate stimulus⁴³². Regarding

cuff pressure, it could be set arbitrarily (*i.e.* same absolute value for all participants) or individualised (*i.e.* relative to individual's characteristics such as AOP). While using arbitrary pressure is easier, it should be avoided since it provides different haemodynamic responses between participants. Using absolute pressures may also provide an insufficient stimulus or, conversely, induce an arterial occlusion, which should be avoided during BFR to avoid adverse effects ^{41,273,426}. Thus, individualised pressure based on AOP or LOP equations should be preferred to provide more consistent and safer responses ²⁷³. Since a comparable reduction in blood flow found between 40 % and 80 % of AOP has been found ⁸⁴, it is recommended to use between 40 % and 80 % of AOP when training with BFR ²⁸. However, the long-term effects of training with either 40 % or 80 % of AOP have not been yet investigated. Additionally, there is a lack of evidence on the impact of BFR methodologies on cardiovascular adaptations, which may be monitored using various technologies throughout the O₂ transport pathway

CHAPTER FOUR

Technologies used to monitor the effects of occlusion on cardiovascular and respiratory systems



Although the impact of BFR on cardiovascular and respiratory adaptations has been detailed in previous chapters, this chapter focuses on the methodological aspects of monitoring the physiological responses to BFR and IPC. The elements of the O₂ transport chain will be investigated, from muscle oxygenation to cardiovascular and respiratory responses.

4.1 MONITORING THE EFFECTS OF RESTRICTED BLOOD FLOW ON MUSCLE PERFUSION

Training with BFR has been shown to enhance endothelial function and angiogenesis, increasing muscle blood flow and oxygenation^{163,193}. Monitoring the effects of training on muscle oxygenation, could therefore provide pertinent information on the growth of new capillaries and enhancing O₂ delivery to the muscle tissue^{27,261}. Monitoring muscle perfusion not only sheds light on immediate effects of a restriction of the blood flow, but also may help to develop strategies that enhance vascularisation, tissue repair, and performance outcomes⁵¹⁸.

4.1.1 Monitoring muscle perfusion

Monitoring the muscle perfusion could be achieved by investigating the vascular network morphology using invasive muscle biopsies or image-based modelling techniques ⁵¹⁹. Muscle biopsies could be applied to investigate the capillary density and the capillary-per-fibre ratio providing insight into the muscle vascularisation. However, muscle biopsies involve risks, such as pain, infection and damage to muscle tissue ⁵²⁰. Muscle biopsies are also complex as they necessitate dedicated staff, are costly and time consuming. Thus, due to their invasive nature and the availability of non-invasive imaging techniques (described in the following paragraph), biopsies are not directly used to measure muscle oxygenation ⁵²⁰.

Image-based modelling techniques may have two- or three-dimensional (2D, or 3D) resolution and penetration depth that ranged from 1 m with X-rays or magnetic resonance imaging, to only several μm with laser speckle contrast imaging or light microscopy ⁵¹⁹. Among the different imaging techniques, two main categories emerged: tracer- and nontracer-based imaging ⁵²¹. Tracer-based imaging techniques rely on the concepts of radiolabelling and particle detection, such as the positron emission tomography (PET) or the single-photon-emission computed tomography (SPECT). However, these techniques require qualified staff and invasive radiolabelling, which requires intravenously injecting the tracers or incorporating them into the tissues of interest during exercise ⁵²¹. Moreover, the main issue is the high-cost of both devices (SPECT: \$400,000 - \$600,000; PET: around \$2 million) that prevents them from being used. Non-tracer-based imaging techniques rely on the specific properties of a substrate, such as magnetic properties or light absorption, to identify and quantify the metabolites concentrations ⁵²¹. One of these techniques is the magnetic resonance spectroscopy (MRS), that is complimentary to the magnetic resonance imaging (MRI) (> \$225,000) ⁵²¹, but has limitations due to the cost and the type of exercises achievable in the machine. Beyond the cost of the presented imaging techniques, they all present severe limitations for use in exercise

physiology field, such as small penetration depth or sampling rate, X-ray radiation, invasive injection, but mainly the restricted types of exercises achievable due to spatial resolution or available space ⁵¹⁹.

Blood perfusion in tissues could also be non-invasively investigated using laser Doppler velocimetry and related techniques, such as laser speckle contrast imaging, laser Doppler flowmetry or laser Doppler imaging ^{522,523}. These imaging techniques are used to assess the vascular function in the tissue ⁵²⁴, allowing the reliable measurement of PORH ⁵²⁵ and effective in assessing microvascular function ^{524,526}. However, laser Doppler measurement techniques have limited penetration depth (300 μm – 1mm) ^{527,528} and are mainly used for clinical purposes to investigate skin micro-perfusion ^{527–529}. The same limitation in penetration depth occurs for transcutaneous oxygen pressure (tcpO₂) that provides indirect measurement of skin tissue perfusion but cannot be used for skeletal muscle perfusion investigation ⁵³⁰.

Another non-invasive technique is the Doppler ultrasound, that consists of using high-frequency sound waves that bounce off red blood cells moving through the blood vessels to create images of blood flow in the body ⁵³¹. The assessment of the blood flow in the tissue provides useful information about muscle perfusion, by reflecting the rate of O₂ delivery to the muscles. Doppler ultrasound is often use in combination with NIRS, a technology that uses light absorption properties of the red blood cells to provide information on the muscle oxygenation ⁵³¹.

4.1.2 Near-infrared spectroscopy

NIRS application to monitor local muscle oxygenation has been used since the 1980's, and applied during exercise in the following years ^{261,532,533}. NIRS could be used either at rest or during exercise, with the advantage of being non-invasive, allowing its use in all types of populations ^{534–536}. Across the different types of NIRS technologies, some devices are portable and easy to use ²⁷, which facilitates its utilisation in exercise physiology.

4.1.2.1 Absorbance & transmittance of the light

When light penetrates tissue, a series of intricate interactions unfold, that involve reflection, absorption, scattering and transmittance. A portion of the incident light is reflected off the tissue surface (reflection), some light is absorbed by the tissue (absorption), part of the light is scattered in multiple directions as it traverses the tissue (scattering), and a fraction of the incident light manages to pass through the tissue (transmittance) ⁵³⁷⁻⁵³⁹.

By employing transmitters and receivers, both incoming and outgoing light can be estimated. In a fully transparent medium, where light passes without hindrance, all the light that is generated by the transmitter is collected by the receiver, resulting in 100 % transmittance. Conversely, in a fully opaque medium, the emitted light can reach the receiver, resulting in an absence of transmittance.

The presence of chromophores, substances that absorb specific wavelengths of light, into constituent molecules of the medium cause a certain degree of light absorption ⁵⁴⁰. Thus, the light emitted at precise wavelengths is absorbed by molecules' chromophores. Thus, the quantity of absorbed light could be estimated ⁵⁴⁰. The concept of optical density, also referred as absorbance, is a measure of how much light a substance absorbs. It is quantified using the logarithm of the ratio between incoming and outgoing light (Equation 8) ²⁶²:

Equation 8: Optical Density in human tissue.

$$\text{Optical Density (Absorbance)} = \text{Log} \frac{I_0}{I}$$

Where optical density is dimensionless, I_0 is the emitted light, and I the emergent light.

Initially observed by Bouguer in 1729 ⁵⁴¹, it was later postulated by Lambert ⁵⁴² that absorbance depends on intensity and path length, that is the distance the light must travel to reach the receiver. In 1852, Beer made the statement that

absorbance is also dependent upon molecular concentration ⁵⁴³. Consequently, an elevated molecular concentration results in enhanced light absorption by chromophores, leading to a correspondingly higher level of absorbance. After, several major discoveries in spectroscopy, photochemistry and electromagnetic theory ⁵⁴⁴, the modern expression of the (Bouguer-)Beer-Lambert absorbance (A) equation, in a non-scattering medium, is given by Equation 9 ⁵⁴⁴:

Equation 9: Bouguer-Beer-Lambert equation for absorbance

$$\log_{10} \frac{I_0}{I} = A = \varepsilon \cdot c \cdot d$$

Where A is the absorbance, ε is the molar absorptivity (L/mol/cm), c is the concentration of absorbing molecules (mol/L), and d is the distance travelled by the light, that is, the transmitter-receptor distance (cm). However, this formula cannot be applied to biological tissue since they are highly scattering media, and therefore increase the path length of light and may modify absorption ²⁶². Thus, modification has been brought to the Beer-Lambert equation to counterbalance light attenuation. Modified Beer-Lambert law is given by Equation 10 ⁵⁴⁵:

Equation 10: Modified Beer-Lambert equation

$$A = \log_{10} \frac{I_0}{I} = \varepsilon \cdot c \cdot d \cdot DPF \cdot G$$

Where, G is the losses produced by scattering, and DPF is the differential path length factor that accounts for the additional path length caused by scattering. DPF is described as the relationship between both absorption (μ_a) and scattering coefficient (μ'_s), as follows (Equation 11) ⁵⁴⁶:

Equation 11 : Calculation of DPF

$$DPF = \frac{\sqrt{3\mu'_s}}{2\sqrt{\mu_a}}$$

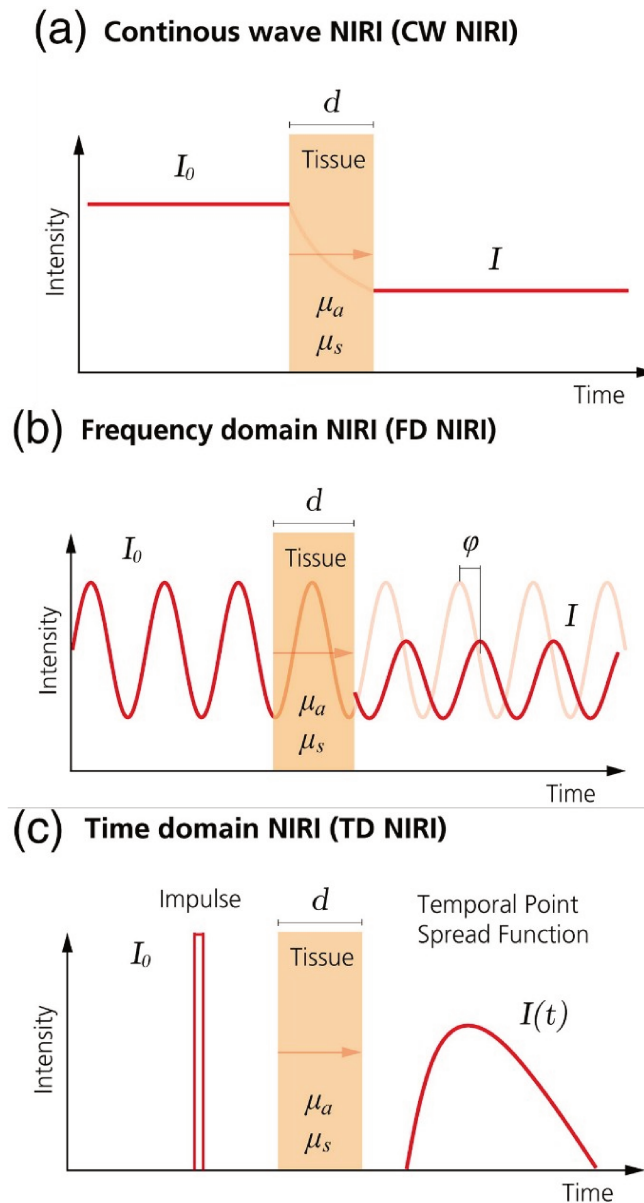


Figure 4-1: Illustration of the three near-infrared imaging (NIRI) techniques that could be used for tissue oxygenation monitoring.

Continuous wave (a) emits light at constant intensity, and only focus on intensity changes through tissue. The DPF is assumed constant. Frequency domain (b) modulates emitted light intensity, measuring detected light intensity and phase shift (time of flight). Time domain (c) emits rapid light pulses, capturing photon arrival times. I_0 : incident light signal, I : transmitted light signal, d : thickness of the medium, μ_a : absorption coefficient, μ_s : scattering coefficient, φ : phase delay, and $I(t)$: temporal point spread function of the transmitted light signal. From Scholkmann et al. 2014⁵⁴⁷.

4.1.2.1 NIRS technology

NIRS technology includes three types of oximeters (Figure 4-1) that differs in the signal sent to the detector: continuous wave NIRS (CW-NIRS), Time Domain NIRS (TD-NIRS), and Frequency Domain NIRS (FD-NIRS) ²⁶².

4.1.2.1.1 Continuous Wave NIRS

CW-NIRS oximeters are the oldest and still the most used type of commercial NIRS devices, mainly due to their low cost ^{262,548}. CW-NIRS instruments emit a light at a constant intensity (usually between 700 and 900 nm) in the infrared spectrum, that traverse the tissue and is detected by a receptor (Figure 4-1). Thus, the absorbed light is calculated from the detected light, providing changes in light attenuation ²⁶². The CW-NIRS assumes a constant DPF and G (and consequently a constant light scattering), implying that light attenuation arises only from concentration changes of absorbing molecules (*see Equation 10*). In the near-infrared (NIR) region, the main chromophores are Hb and Mb. Thus, CW-NIRS imply that light attenuation arises only from O₂Hb, HHb and Mb concentration changes. Using multiple source-detector distances (Figure 4-2), spatially-resolved spectrometers (SRS) provide a quantitative measure of tissue oxygenation percentage (%TSI or StO₂), and an estimation of oxy- and deoxy[Hb+Mb] and total[Hb+Mb] changes in the tissue. However, the CW-NIRS do not allow the estimation of absolute concentration of oxy- or deoxy[Hb+Mb], since DPF is unknown ²⁶². The reliability of CW-NIRS have been investigated and found reliable in intra-session measurements, but not between sessions ⁵⁴⁹ (*see STUDY 1, p. 140*).

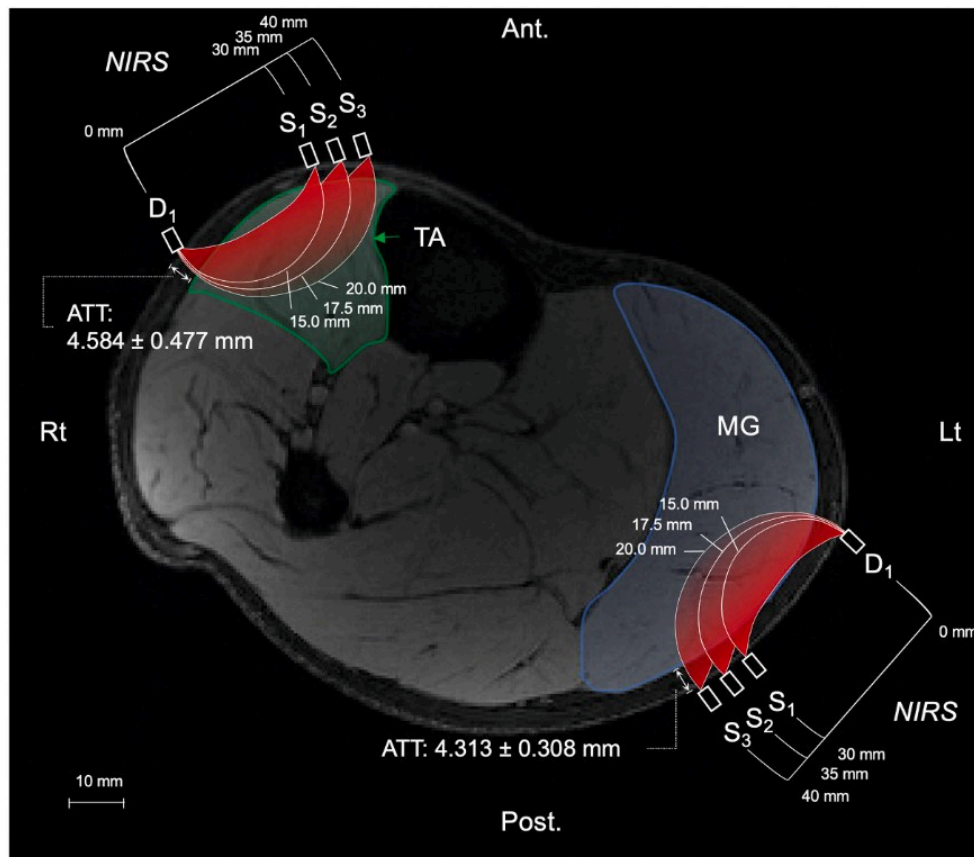


Figure 4-2: Representative image of the lower leg obtained with MRI.

The medial gastrocnemius (MG) and the tibialis anterior (TA) muscles are indicated in red and blue, respectively. CW-NIRS devices are represented for MG and TA oxygenation monitoring. The devices have source-detector distances of 30, 35 and 40 mm (S_1 , S_2 and S_3 , respectively). The banana shaped light curve in the tissue is represented in red between the source (S_1 , S_2 , S_3) and the detector (D_1), with associated penetration depth. The adipose tissue thickness (ATT) is provided for each muscle to better understand the necessity of having low ATT for muscle oxygenation monitoring. From Luck et al. (2023)⁵³¹.

4.1.2.1.2 Time Domain NIRS

Time domain spectroscopy, also called time resolved spectroscopy (TRS), rely on the calculation of photon time of flight and light attenuation. A pulsed light source, typically a laser, emits a short light pulse (≈ 100 picoseconds) into the tissue, that is detected with a sub-nanosecond scale temporal resolution (Figure 4-1). Thus, the photon distribution of time-of-flight (DTOF) is measured at a fixed distance from the light source (in the range of 10 to 40 mm). Due to the scattering in the tissue, the signal is delayed, broadened and attenuated. Then, the detection-time curve is fitted depending on the source-receptor distances to calculate the absorption coefficient, the reduced scattering coefficient and the mean path length, using the diffusion theory. Thus, the calculation of absolute deoxy[Hb+Mb], oxy[Hb+Mb], and total[Hb+Mb] is possible contrary to CW-NIRS that calculates the concentration changes to a fixed baseline^{262,550,551}. However, the reproducibility or reliability of TD-NIRS devices has not been extensively assessed, except for two studies^{552,553}.

4.1.2.1.3 Frequency Domain NIRS

FD-NIRS (Figure 4-1) relies on the principle of amplitude-modulation a technique in which the amplitude (or intensity) of the carrier signal wave is varied in proportion to a separate additional electromagnetic signal^{262,554}. The intensity of the source signal is modified by addition of electromagnetic wave. In the case of FD-NIRS, the carrier signal is a continuous wave of around 690 or 830 nm, which is amplitude-modulated by a radio-frequency (RF) signal at 100 or 110 MHz. When using a frequency-domain multi-distance (FDMD) device, which has multiple source-detectors distances that range from 1.0 to 5.0 cm, it is possible to calculate the average value (dc), amplitude (ac), and the phase (φ) of the modulated light wave at the different inter-optodes distances^{262,555}. Since the initial phase is known, the phase shift (*i.e.* displacement of the wave in time) provides information on the time taken by the signal to go through the tissue. In addition, because the detected signal intensity and phase shift are known, it is then possible to estimate the absorption

and scattering coefficients of the tissue, for each wavelength. Thus, the DPF could be calculated and the absolute concentration of oxy- and deoxy[Hb+Mb] in micromolar units could be estimated, using the modified Beer-Lambert equation ²⁶². Contrary to CW-NIRS measurements, the FD-NIRS *in-vivo* measurements are less sensitive to adipose tissue thickness, and consequently may be used in a broader population ⁵⁵⁶. However, studies about the reproducibility and reliability of FD-NIRS devices are lacking ⁵⁵⁷.

4.1.2.2 Differential Pathlength Factor (DPF)

The DPF in NIRS is a parameter that accounts for the increase in optical path length due to scattering in the tissue. It is used to estimate the actual distance travelled by near-infrared light in the tissue, which helps for quantifying changes in chromophore concentration ²⁶².

When the emitted light passes through the tissue, the scattering of the media causes the near-infrared light to travel a greater distance to reach the receiver. In addition, since both the near-infrared light transmitter and receiver are placed on the skin, and muscle tissue is a few millimetres deep, it forms a triangle between the emitter, the receiver and the tissue. Consequently, the light needs to have a banana-shaped ^{558,559} path to monitor the skeletal muscle oxygenation status (Figure 4-2). The actual path travelled by the emitted light, is then much longer than the source-detector distance on the surface of the skin ^{545,560}. Thus, DPF has been added to the traditional Beer-Lambert equation, to compensate for the extra distance travelled by the photons in the media until the receiver. The DPF is a constant, multiplicative correction factor, that could be estimated by the calculation of the time-of-flight (ToF) measurements divided by transmitter – receiver distance ^{262,545}. The DPF measurement could also be estimated using FD-NIRS ⁵⁶¹, that compared the emitted and received light intensity as well as the phase shift in the signal that corresponds to the ToF ⁵⁵⁸. However, this factor was found to be age, sex, tissue and wavelength dependent ^{558,561–564}. In addition, since the DPF calculation is complex and subjective,

a large proportion of CW-NIRS devices recommend a constant DPF of 4 for the estimation of skeletal muscle oxygenation status⁵⁶⁵. This value reflects an average of DPF distributions observed in individuals at rest^{565,566}.

However, the use of a constant DPF value for all the participants is disputed, since the DPF is highly variable between participants and muscle tissues²⁶². The DPF is determined as the mean of the dispersion of values in photon flight times through scattering mediums like biological tissues. But the distance travelled in the media by the first and last 5 % of detected photons ranged from 2.6 to 9.2 times the source-detector distance⁵⁴⁵. Since scattering decreases with increasing wavelength, the DPF values for 690 nm and 832 nm wavelengths are variable, such as witnessed for a measurement site in the calf (5.8 for 690 nm and 5.3 for 832 nm), or in the forearm (4.4 for 690 nm and 3.9 for 832 nm)⁵⁶¹. Due to anatomical differences, women show higher DPF values compared to age-matched men, that are on average from 23 % to 24 % greater than men (calculated from Duncan *et al.* (1995)⁵⁶¹ in forearm and calf, respectively). Moreover, coefficients of variation between participants ranged from 16 to 20 %, highlighting potential calculation errors that could occur when using constant DPF for all participants. Similar findings were reported more recently in the gastrocnemius, using a FD-NIRS system, with DPF values of 5.2 and 4.5 and CV values of 16.8 % and 18.5 %, at 690 and 830 nm, respectively⁵⁴⁶.

NIRS finds its predominant application in the observation of physiological responses to exercise, venous or arterial occlusion or both with BFR exercise. Interestingly, both exercise and ischaemia (Figure 4-3) induce changes in DPF values⁵⁶⁶. Thus, oxygenation status provided by the device may be biased if DPF is kept constant. Using a constant DPF fixed at 4.0 during vascular occlusion, does induce exaggerated values of [O₂Hb] and [HHb], leading to significant underestimations of TSI at rest (-11 %), at maximal deoxygenation (-50 %) and at maximal reperfusion (-10.5 %)⁵⁶⁵. Similarly, during exercise an 11 % underestimation of TSI was found at maximal peak work rates⁵⁴⁶.

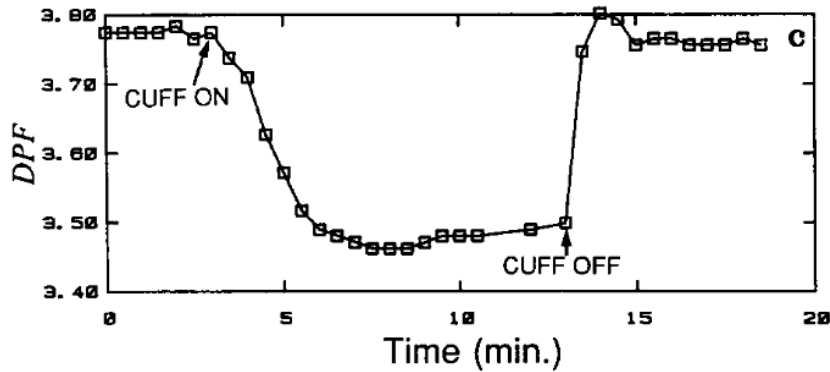


Figure 4-3: Evolution of DPF during ischaemia.

Inflation of the cuff (cuff on) induces ischaemia, leading to changes in the DPF value measured at 800 nm. Upon deflation of the cuff (cuff off), the DPF value returns to pre-inflation levels. From Ferrari et al. (1992)⁵⁶⁶.

The DPF changes during occlusion or exercise may be explained by several factors. First, during exercise, there is a significant increase in the scattering coefficient for lower wavelengths (690 nm), whereas higher wavelengths (830 nm) remain constant⁵⁴⁶. Since, scattering is dependent on the refractive index inside the media by the cells and intracellular organelle membranes, the increase in total[Hb+Mb] with occlusion or exercise modifies the content of the media and thus the refractive index. In addition to blood volume, RBC flux, and changes in anion and cation concentrations due to muscle contractions are also mentioned as factors affecting the scattering of the media during occlusion or exercise⁵⁴⁶. Moreover, since skeletal muscle consists of 75 % water, changes in scattering may also be attributed to the increase in tissue water during exercise⁵⁶⁷. Finally, exercise could elevate body and muscle temperatures, such that it will induce significant changes in optical density, or absorbance, by promoting changes in skin blood flow⁵⁶⁸.

Therefore, cautiousness is needed when interpreting the results of CW-NIRS using constant and not individualised DPF, since DPF is highly variable between subject, tissues and experimental conditions.

4.1.2.3 Adipose tissue thickness

NIRS devices measure the light absorption by the tissue underneath the optodes, to a depth of half the distance between the source and the receiver^{569,570}. The device is separated from the skeletal muscle by multiple tissue layers that may confound the NIRS measurements in the muscle, such as the skin or the subcutaneous adipose tissue (SAT). Since adipose tissue has a lower metabolic state compared to skeletal muscle, and that the inner optical characteristics of the adipose tissue medium are different from muscle, NIRS measurements will be underestimated if the fat layer is too thick^{571,572}. Most CW-NIRS devices available have inter-optode distances ranging between 20 and 40mm (Figure 4-2), thus the ATT may impact the NIRS estimations if the NIR light cannot reach the skeletal muscle, or if the fat layer is too large for precise measurements.

In a study involving 78 healthy volunteers (34 females and 44 males), with an average age of 28.2 ± 12.5 years, height of 177.6 ± 9.0 cm, and mass of 71.6 ± 11.4 kg, measurements of ATT and tissue oxygenation were conducted on the *flexor digitorum superficialis* muscle in the forearm⁵⁷². The NIRS device inter-optodes distance used was 35 mm, resulting in a maximal depth of 17.5 mm for measurement. Interestingly, when examining the NIRS data, it was found that in the case of the participant with the lowest ATT (1.4 mm), 92 % of the measured depth was attributed to skeletal muscle. In contrast, for the participant with the highest ATT (8.9 mm), this proportion dropped significantly to only 49 %⁵⁷². These observations highlight the variability between participants and confirms that ATT may confound the NIRS measurements. As expected, the muscle oxygen uptake ($m\dot{V}O_2$), measured during arterial occlusion, tend to decrease with the increasing ATT⁵⁷². These results confirm findings that showed a decreased sensitivity with the increasing ATT^{556,573,574}.

Nasseri *et al.* (2016)⁵⁵⁶ have investigated the effects of ATT on TSI % of four different NIRS devices using phantom blocks with four different thicknesses of

layers, reflecting what could be found in human ATT: 2.5mm, 5mm, 9mm and 16mm⁵⁵⁶. They have found that the thickness of adipose tissue layers significantly affected the sensitivity of the NIRS oximeter. However, due to their ability to record phase shift, FD oximeters were less affected by ATT compared to CW-NIRS.

Increasing ATT induces a decrease in [tHb] and an increase in TSI^{575,576}. To overcome these issues, most studies recruit participants with low ATT, excluding elderly or females that often show higher ATT than young males. Thus, results from these studies cannot be extrapolated to the whole population⁵⁷⁶.

Given that ATT affects NIRS estimations, could be attributed to the relationship between ATT and DPF, with a positive correlation found between ATT of the participant and the calculated DPF⁵⁷⁷. These findings are supported by Homma et al.⁵⁷⁰ who concluded that the shallower the ATT, the higher the optical density, suggesting that a higher proportion of NIR light signal is absorbed by skeletal muscle.

When using NIRS devices that provide absolute concentrations of [O₂Hb] and [HHb], such as FD- or TD-NIRS systems, it is recommended to apply a correction factor for ATT, to estimate more precisely the tissue oxygenation^{262,575}. Nonetheless, given the NIRS capacity and the influence of ATT on the signal, it is recommended to measure the participant's ATT to ensure the near-infrared light penetration depth, estimated as one half the source-detector separation, will sufficiently evaluate the studied muscle oxygenation²⁶². Therefore, recruiting participants with ATT lower than one quarter the longest source-receptor distance may reduce the inter-individual variability and the perturbations on the NIRS signal caused by ATT²⁶². However, it limits the NIRS investigations to an athletic, or lean population⁵⁷⁶ and questioned the reliability in a general population⁵⁴⁹.

4.2 ASSESSING CARDIOVASCULAR PARAMETERS

\dot{Q} is calculated as the product of the HR and the SV (Equation 1), that is the volume of blood ejected by the left ventricle at each heartbeat. Since \dot{Q} is a key parameter in the Fick equation, any alterations in haemodynamics can affect the calculated $\dot{V}O_2$. Consequently, assessing \dot{Q} during exercise is essential for monitoring cardiac function, estimating whole-body O_2 delivery and understanding training-induced cardiac adaptations.

While Fick's equation dates back to 1870^{87,578}, a direct measure of the \dot{Q} has only been available since 1970 using a flow-directed balloon-tipped catheter introduced into the heart and pulmonary artery⁵⁷⁹. However, this method is highly invasive just like other methods developed later such as the direct Fick method or the dilution techniques (thermodilution, dye dilution, lithium dilution). The problem with these techniques is the risk of severe complications such as pneumothorax, perforation of the heart chamber, valve damages^{580–582}. In addition, these techniques require extreme cautiousness and a highly skilled clinicians to be performed. While these techniques were considered as the "Gold standard" for \dot{Q} measurement, they are costly and present major limitations for participants' health. Therefore, a need has emerged for non-invasive, easy to use, accurate and reliable methods for \dot{Q} assessment.

To overcome the challenges of using invasive methods, non-invasive (or less invasive) methods of \dot{Q} monitoring have been developed. Among the multitude of developed methods^{583–585}, one can find the partial CO_2 rebreathing, inert gas rebreathing, pulse contour analysis, and electrical impedance cardiography. Both partial CO_2 and inert gas rebreathing techniques involves inhaling a known volume of gas and measuring its concentration in exhaled air to estimate \dot{Q} . Pulse contour analysis utilises the arterial pressure waveform to estimate SV by analysing changes in the contour of the pulse during each heartbeat. Finally, by recording changes in electrical conductivity across the thorax, electrical impedance cardiography

technique estimates changes in thoracic fluid content, from which \dot{Q} can be derived⁵⁸³⁻⁵⁸⁶. Three of these non- or less- invasive methods have been compared to the invasive modified Fick method during an incremental cycling exercise⁵⁸⁶. The results show that measurement of \dot{Q} during exercise differs up to 50 % between the four methods. The inert gas rebreathing techniques' lack of validation for use during exercise and provide underestimated values of \dot{Q} compared to other methods or to an established range of plausible values⁵⁸⁶. The modified Fick method has shown acceptable results but involves invasive data collection and methodological bias⁵⁸⁶. Pulse contour analysis method has been unable to produce satisfactory results in \dot{Q} measurement during exercise in hypoxia. In addition, this technique appears to underestimate the increase in \dot{Q} associated with heat stress, which during hypoxia, is linked to peripheral vasodilation^{586,587}. Finally, electrical impedance cardiography measured with PhysioFlow (PF-05 Lab1, Manatec Biomedical, Poissy, France), has been shown to report the highest \dot{Q} values and the highest intersubject variance between the four devices⁵⁸⁶. However, the PhysioFlow device has already been validated against a "gold standard" (direct Fick method) during exercise^{588,589}, although some studies suggest a low precision^{590,591} that may be caused by movement, respiratory artefacts or poor electrodes placement^{586,592}. The PhysioFlow device offers a great ease-of-use since it requires only six electrodes attached to the chest and linked to small device (Figure 4-4). In addition, this technique offers a validated non-invasive continuous monitoring of \dot{Q} during rest and exercise, which is not the case for other techniques used for \dot{Q} measurement.

The PhysioFlow has been tested against echocardiography, that is the most widely used equipment for non-invasive \dot{Q} monitoring⁵⁹³. Echocardiography has been found reliable and effective for \dot{Q} monitoring during strenuous exercise^{594,595}. The comparison between PhysioFlow and echocardiography, has shown weaknesses in SV and \dot{Q} estimation by the PhysioFlow compared to echocardiography⁵⁹³. However, echocardiography requires trained physicians and is far less portable than PhysioFlow device, which can be used easily on the field.

Therefore, the non-invasive measuring of \dot{Q} during exercise has remained complex and suffered from imprecision, but emerging technologies such as electrical impedance cardiography aim to address previously encountered issues^{584,591}. Better understanding of physiological responses and adaptations of \dot{Q} to exercise is nowadays possible outside of a clinical context^{157,596}.

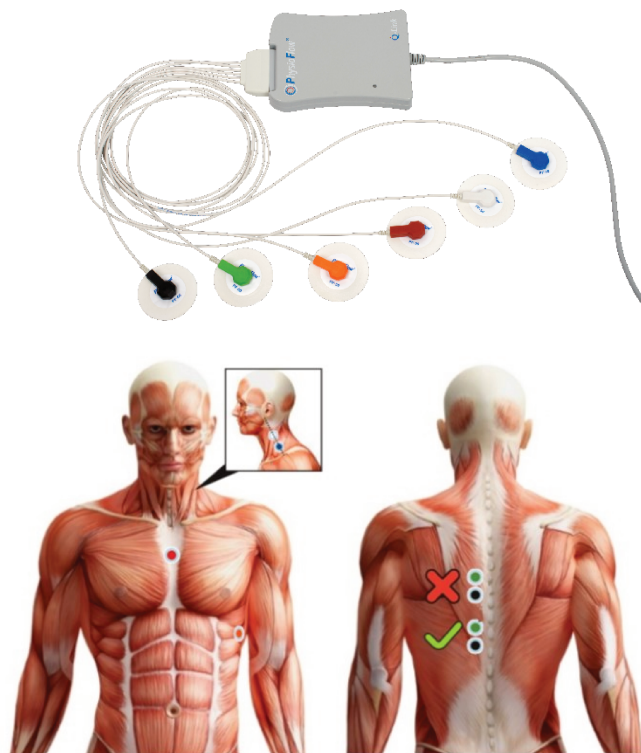


Figure 4-4: PhysioFlow Q-Link device and electrodes placement for exercise.

After having prepared the skin (shaving, cleaning, rubbing the skin), the electrodes could be placed on the participant. Two electrodes are placed on the neck (white and blue), one electrode (red) is placed on the middle of the sternum, one electrode (orange) is placed on the rib closest to V6 and the last two electrodes (green and black) are placed on the back of the participant. The green electrode must be placed at the xiphoid level, next to the spine.

4.3 ASSESSING CARDIO-RESPIRATORY PARAMETERS

In a series of fundamental papers published in 1923 and 1924, Hill and Lupton have reported the time course of $\dot{V}O_2$ during exercise and recovery, and introduced the concept of $\dot{V}O_{2max}$ ^{391,597-599}. In these papers, the expired air was collected in a Douglas bag strapped to the participants' back (Figure 4-5), involving practical challenges and indirect measure of $\dot{V}O_2$ ^{599,600}.

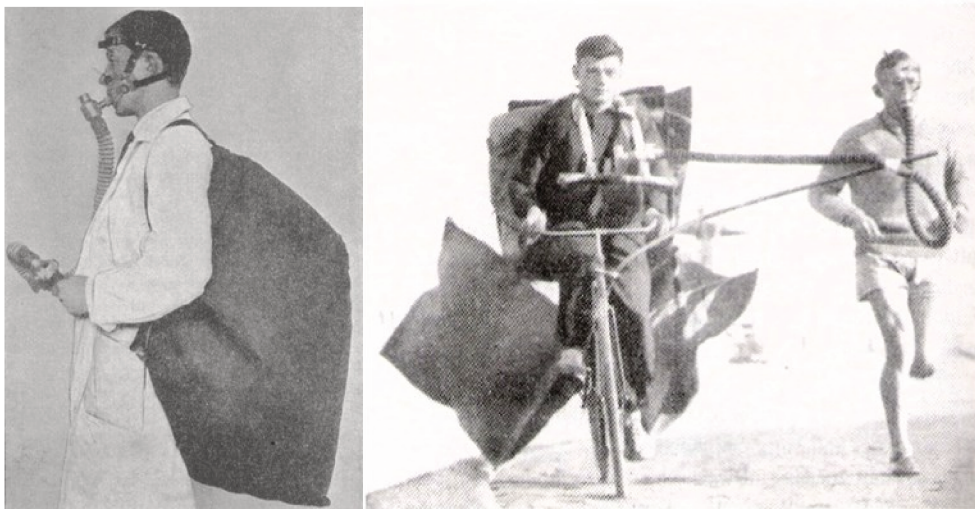


Figure 4-5: Illustration of the use of Douglas bags at rest or during exercise.

During exercise, the Douglas bag may be strapped to participant's back (left panel)⁵⁹⁹ or be carried on a bracket or by an investigator (right panel)⁶⁰¹. The gas content of the bag was analysed after the collection of data.

In the 1780s, French chemist Antoine Lavoisier has demonstrated that animals consume O_2 and produce CO_2 , establishing a direct relationship between CO_2 production and heat generation.^{7,602,603} By the end of 1780s, Lavoisier and Séguin were the first scientists to perform measurements of human O_2 consumption at rest and during exercise (foot-treadle)⁷.

Almost 100 years after Lavoisier's birth, the first closed-circuit measure of gas exchanges have been performed by Regnault and Reiset (1849), followed several years later by open-circuit approach⁶⁰⁴. Although the first bag collection of gases is

done by Prout (1813), the concept of redirecting expired gases to large twill bags lined with vulcanised rubber is first attributed to Douglas (1911) ^{604,605}.

Douglas bags have then been routinely used for many years and have served as a “Gold standard” for gas exchange measurement in exercise physiology ^{606,607}. After collection, gases are mixed in the impermeable bag and passed through a meter to estimate the volume of air. In addition, a sample of the bag content is analysed using a Haldane gas apparatus, a very first gas analyser. This technology has constantly continued to evolve, correcting technical and reliability issues, and could still be used as a “gold standard” to evaluate new gas measurement devices ⁶⁰⁴. However, newer approaches using real-time monitoring of gas exchanges have progressively replaced the use of Douglas bags in the field of exercise physiology.

Nowadays, gas analysers require less technical skills than Douglas bags ⁶⁰⁴ and can estimate $\dot{V}O_2$, $\dot{V}CO_2$ and \dot{V}_E with a better temporal resolution (breath-by-breath) ⁶⁰⁴. Recent metabolic carts use various methodologies measure these physiological variables: volume of expired gases can be measured with either volume-sensing (hot-wire anemometer) or flow-sensing (turbine) devices; O_2 is mainly analysed using fuel cells (Zirconia) or paramagnetic analysers; and CO_2 is estimated using infrared technologies (Non-Dispersive Infrared or infrared photometer) or thermal conductivity ⁶⁰⁸. In addition, most of the devices used today allow a breath-by-breath monitoring, meaning a real-time measurement. But some devices offer the possibility of choosing between mixing chamber or breath-by-breath monitoring methods, which both show reliable results during exercise ⁶⁰⁸, although mixing chamber systems have been reported to be more accurate at high volumes (during strenuous effort) ^{609,610}.

The evolution in gas analyser devices has led to accurate and reliable mobile or portable technologies, offering real-time monitoring of essential cardio-respiratory variables ⁶⁰⁸. While technical parameters could affect $\dot{V}O_{2max}$ assessment (humidity, temperature, device, sampling interval) ^{608,611–613}, or $\dot{V}O_{2max}$ attainment

⁶¹⁴⁻⁶¹⁶, its assessment remains essential for evaluating participants cardiorespiratory fitness, training intensity prescription and responsiveness to training. Indeed, analysing expired gases enables the calculation of the respiratory exchange ratio (RER) ^{617,618}, ventilatory thresholds ^{383,384,619,620} and $\dot{V}O_2$ kinetics ^{315,317,621}. Together with $\dot{V}O_{2max}$, these metrics are used to characterise the maximal or submaximal effort and monitor the physiological adaptations to training ^{315,622}.

4.4 CONCLUSION

Monitoring the cardiovascular and respiratory responses and adaptations to training is essential to better understand the action of BFR. The utilisation and significance of NIRS in monitoring skeletal muscle oxygenation are extensively documented in literature, with NIRS devices being widely employed in both resting and exercise contexts due to their moderate cost, compact size, and wireless connectivity ^{27,518,623}. However, there is a lack of studies on their reliability in the general population. The monitoring of muscle oxygenation using NIRS, could be associated with an assessment of the cardiovascular system. In order to monitor the cardiovascular function, several techniques have been developed throughout the years, but most of them are invasive. The development of the cardiac impedance technology (PhysioFlow), provide a non-invasive and reliable alternative of other techniques, while being particularly adapted for exercise ⁵⁸⁹. Finally, the non-invasive assessment of the different steps of O₂ transport could be completed by gas exchanges analysis. The effects of BFR training on respiratory responses and adaptations could be monitored through a gas analyser, allowing a breath-by-breath analysis contrary to the old Douglas bags technique. By combining those three methods, a large spectrum of the O₂ transport system could be assessed, offering the opportunity to better understand the physiological responses to BFR.

AIM OF THE THESIS



The theoretical framework of the present thesis demonstrates that induced-ischaemia, whether at rest using IPC or during exercise with BFR, causes acute or chronic effects on the components of the O₂ transport pathway. Additionally, methodological variations between studies, especially on the pressure selected tend to emphasise the heterogeneous findings. Therefore, this thesis aims to answer the following questions:

1. How can the effects of induced ischaemia on haemodynamic response be evaluated?
2. How are the components of the O₂ transport pathway affected by an induced ischaemia, at rest or during exercise?
3. What are the acute and chronic effects of different pressures used on cardiovascular and respiratory responses?

Two complementary protocols, IPC and BFR, were employed to answer these questions. Before investigating the specific effects of ischaemia, it was essential to establish the reliability of the device used for assessment. Consequently, the first study evaluated the reliability of muscle oxygenation responses using NIRS, across different pressures. The field of IPC presents heterogeneous results regarding beneficial effects for performance, with one of the main variations between studies being the cuff pressure used to induce arterial occlusion. To address this, a second

study examined the acute muscle oxygenation response to ischaemia induced by both absolute and personalised pressures. Finally, the last objective of this thesis was to investigate the effects of incorporating BFR to an aerobic training programme performed at high-intensity. Therefore, a third study investigated the effects of HIIT programme performed with varying levels of BFR on cardiovascular, haemodynamic and respiratory parameters.

Overall, this thesis aims to elucidate the role of cuff pressure in the acute and chronic cardiovascular, haemodynamic and respiratory responses to ischaemia, whether at rest or during exercise.

SCIENTIFIC CONTRIBUTIONS



STUDY 1:

Intra- and Inter-Day Reliability of the NIRS Portamon Device after Three Induced Muscle Ischemias

PUBLISHED ARTICLE

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Intra- and Inter-Day Reliability of the NIRS Portamon Device after Three Induced Muscle Ischemias. *Sensors* 2022, 22, 5165. <https://doi.org/10.3390/s22145165>

Introduction: NIRS is an innovative and non-invasive technology employed to explore muscular oxygenation. Its utility and significance in monitoring skeletal muscle O₂ levels have been extensively detailed in various literature reviews alongside advancements in technology and oximeters^{27,535,623,624}. NIRS investigations have demonstrated strong reliability during both static and dynamic exercises, as well as at rest following arterial occlusions above SBP⁶²⁵⁻⁶³⁰. Reliability has been investigated for intra-day and inter-day measurements, yet in most studies, the 95 % confidence interval (CI-95%) of the intraclass correlation coefficient (ICC) was omitted, potentially changing the interpretation of the level of reliability⁶³¹. The reliability of maximum and minimum NIRS values post-occlusion, indicating deoxygenation amplitude during occlusion and subsequent reoxygenation, is rarely reported. Hence, this study aims to investigate the intra-day and inter-day reliability of NIRS-derived parameters, alongside the associated maximum and minimum responses, following three blood flow occlusions at varying pressure levels while in a rested state.

Methods: 53 young males (mean \pm SD: 23 \pm 3 years, 75 \pm 12 kg, 178 \pm 7 cm, 13.8 \pm 4.1 % body fat, 7.8 \pm 5.7 hours of physical activity per week) volunteered to participate and randomly attributed to an experimental group (G1, G2, or G3). Depending on the experimental group, a cuff was inflated three times at the left upper arm to 50 mmHg (G1), SBP + 50 mmHg (G2) or 250 mmHg (G3). Participants visited the laboratory twice, within a 24 h to 72 h period. During the first visit, anthropometric measurements were recorded (stature, body mass, and adipose tissue thickness), while limb lengths were measured to ascertain NIRS device placement. In both sessions, the same protocol was followed that consisted of a baseline period followed by 3 x 7 min occlusion phases interspersed with three reperfusion phases where the cuff was deflated. Maximums, minimums and reoxygenation rate were assessed after each occlusion phase, using a Portamon (Artinis, Elst, Netherlands) device placed on left brachioradialis. Reliability was assessed with ICC and CI-95% values, coefficient of variation (CV) and standard error of measurement (SEM).

Results: Within-session results showed a good to excellent reliability for maximums and minimums of TSI, O₂Hb, HHb and tHb, and a mean coefficient of variation of 2.58 \pm 1.82 % and 5.14 \pm 3.81 % for maximum and minimums, respectively. However, between-session results did not show a good reliability for most of the measurement. In addition, the calculation of reoxygenation rate produced unreliable values for both intra- and inter-day assessments.

Conclusions: Multiple measurements of maximums and minimums within a single session appeared to be reliable which shows that only one measurement is necessary to assess these parameters. However, it is necessary to be cautious with comparison of maximums, minimums and reoxygenation rate values between-sessions. This lack of reliability could be due to the fact that ATT was not considered as an exclusion criterion. However, the participants were young, and mostly embrace a regular and substantial physical activity. Therefore, these results question the

usefulness of NIRS devices for monitoring muscle oxygenation on several occasions (for instance pre- and post-training) in the general population.

Article

Intra- and Inter-Day Reliability of the NIRS Portamon Device after Three Induced Muscle Ischemias

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Abstract: (1) Background: Near-infrared spectroscopy (NIRS) is an innovative and non-invasive technology used to investigate muscular oxygenation. The aim of this study is to assess the within- and between-session reliability of the NIRS Portamon (Artinis, Elst, Netherlands) device following three sets of induced muscle ischemia. (2) Methods: Depending on the experimental group (G1, G2 or G3), a cuff was inflated three times on the left upper arm to 50 mmHg (G1), systolic blood pressure (SBP) + 50 mmHg (G2) or 250 mmHg (G3). Maximum, minimum and reoxygenation rate values were assessed after each occlusion phase, using a Portamon device placed on the left brachioradialis. Reliability was assessed with intraclass correlation coefficient (ICC) value and ICC 95% confidence interval (CI-95%), coefficient of variation (CV) and standard error of measurement (SEM) (3) Results: Our results showed a good to excellent reliability for maximums and minimums within-session. However, the reoxygenation rate within sessions as well as measurements between sessions cannot predominantly show good reliability. (4) Conclusions: Multiple measurements of maximums and minimums within a single session appeared to be reliable which shows that only one measurement is necessary to assess these parameters. However, it is necessary to be cautious with a comparison of maximum, minimum and reoxygenation rate values between sessions.

Keywords: test–retest; arterial occlusion; blood flow restriction; ischemic preconditioning; hyperemia



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1. Introduction

Near-infrared spectroscopy (NIRS) is an innovative and non-invasive technology used to investigate muscular oxygenation through several indicators. By using near-infrared wavelengths (~700–900 nm), the light is able to penetrate biological tissues where the main absorbing chromophores in skeletal muscle are hemoglobin (Hb), myoglobin (Mb), and to a lesser extent, cytochrome oxidase (cytox) [1,2]. Thus, NIRS technology provides information on four variables, depending on the device used: oxy[Hb + Mb], deoxy[Hb + Mb], total[Hb + Mb] (i.e., the sum of oxy- and deoxy[Hb + Mb]) and tissue saturation [1]. The concept of recording muscle oxygenation light dates back to 1937 [3], and human skeletal oxygenation measurements to the end of the 1980s [4].

Due to their moderate cost, convenient small size and wireless connectivity, NIRS devices have been widely used at rest and during exercise [5–9]. Moreover, NIRS oximeters offer the advantage of acceptable signal-to-noise ratios, even during dynamic exercise [8]. Indeed, NIRS provides robust information on skeletal muscle oxidative capacity. NIRS-derived parameters such as the muscle reoxygenation rate after exercise [5,10,11], the muscular consumption of O₂ ($m\dot{V}O_2$) [12], as well as the size of the post-occlusive reactive hyperemia (PORH) [13] may be used to assess performance, vascular reactivity and training status. PORH is characterized by an increase in blood flow following an arterial occlusion, representing the significance of limb reperfusion after ischemia [14,15]. This physiological

reaction seems to be caused by the shear stress induced by arterial occlusion acting at the endothelial cell surface and metabolites released by the endothelium [15,16]. PORH is primarily used as a non-invasive method to assess peripheral microvascular function and cardiovascular morbidity and mortality [14].

The use and the interest of NIRS in monitoring skeletal muscle oxygenation have been well documented in the literature through multiple reviews as well as the evolution of the available technology and oximeters [1,7,8,17]. By using NIRS-derived reoxygenation rate parameters, it is possible to differentiate between trained and untrained participants [8], as well as healthy participants and diseased patients [18]. Thus, it is fundamental to assess the reliability of these measurements.

NIRS studies showed good to excellent reliability during static [19,20] and dynamic exercise [21–23], as well as at rest [22,24] following arterial occlusions over the systolic blood pressure (SBP). Reliability has been studied for intra-day measurements, between successive measurements of a single session, and for inter-day measurements, by assessing the reliability between measurements of separate sessions. Indeed, excellent reliability has been shown for both intra-day and inter-day (intraclass correlation coefficients (ICC) of 0.92 and 0.94, respectively) [24]. However, in most studies, the 95% confidence interval (CI-95%) was not reported, potentially leading to confusion by changing the interpretation of the level of reliability [25]. The reliability of maximum and minimum values of NIRS variables reached after arterial occlusion are rarely investigated, yet they provide information on the amplitude of the deoxygenation during occlusion and reoxygenation after cuff release [2].

Previous studies have investigated the muscle tissue oxygenation responses to different levels of occlusion pressure [26] as well as the effect of occlusion duration on muscular oxygenation [27]. However, the reliability of multiple NIRS-derived parameters between trials of a single session and between sessions, with different levels of occlusion pressure, has never been investigated to our knowledge. Thus, the present study aims to investigate the intra-day and inter-day reliability of NIRS-derived parameters, as well as the associated maximum and minimum responses, after three blood flow occlusions with different levels of pressure whilst in the rested state.

2. Materials and Methods

2.1. Participants

Following local institutional ethical approval (CER-Paris-Saclay-2020-006) and having provided written informed consent, 53 young males (mean \pm SD: 23 \pm 3 years, 75 \pm 12 kg, 178 \pm 7 cm, 13.8 \pm 4.1% body fat, 7.8 \pm 5.7 h of physical activity per week) volunteered to participate. The participants' characteristics, as well as the environmental conditions, are reported in Tables 1 and 2. Participants who reported diabetes, hypertension, previous cardiovascular events, vigorous physical activity, alcohol intake 24 h before the session, caffeine, or food intake two hours before the session were excluded from the study.

Table 1. Participant characteristics.

Group	G1	G2	G3	All
Occlusion pressure (mmHg)	50	164 \pm 8	250	
Age (year)	22 \pm 4	22 \pm 3	22 \pm 4	22 \pm 3
Mass (kg)	77 \pm 15	73 \pm 13	76 \pm 7	75 \pm 12
Stature (cm)	177 \pm 6	176 \pm 7	182 \pm 8	178 \pm 7
Body fat (%)	14.5 \pm 5.4	16.6 \pm 5.5	12.6 \pm 2.7	14.6 \pm 4.8
SBP (mmHg)	115 \pm 7	116 \pm 10	121 \pm 9	118 \pm 9
DBP (mmHg)	66 \pm 7	71 \pm 6	68 \pm 7	69 \pm 7
ABI	1.04 \pm 0.08	1.01 \pm 0.12	1.03 \pm 0.06	1.03 \pm 0.09

Legend: Values are reported as mean \pm SD; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ABI: Ankle to Brachial Index.

Table 2. Environmental conditions between sessions.

Group	G1	G2	G3	All
Temperature S1 (°C)	21 ± 1	22 ± 2	21 ± 2	21 ± 2
Temperature S2 (°C)	21 ± 1	22 ± 2	21 ± 1	21 ± 1
Humidity level S1 (%)	35 ± 13	43 ± 14	40 ± 13	40 ± 13 ↓
Humidity level S2 (%)	40 ± 15	48 ± 14	45 ± 15	45 ± 14 ↓

Legend: Values are reported as mean ± SD; S1: session 1; S2: session 2; ↓: significant difference between S1 and S2 ($p < 0.05$).

Participants visited the laboratory twice within a 24 h to 72 h period. During the first visit, anthropometric measurements were recorded (stature, body mass, and adipose tissue thickness), while limb lengths were measured to ascertain NIRS device placement.

2.2. Study Design

Using an experimental design, participants were randomly assigned to one of three groups with different occlusion pressures: 50 mmHg (G1), SBP + 50 mmHg (G2) and 250 mmHg (G3) [28,29]. The protocol is described in Figure 1 and consists of a baseline period followed by 3 × 7 min occlusion phases interspersed with three reperfusion phases where the cuff was deflated.

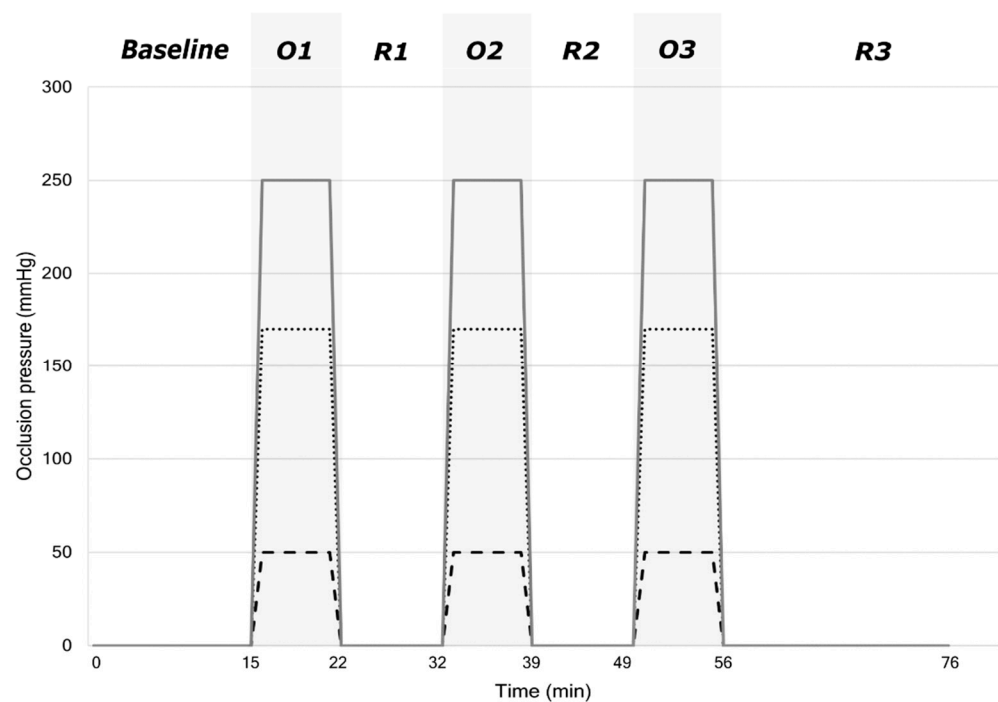


Figure 1. Participants were lying on a medical couch for the duration of the session in the dark and asked to avoid any movement which could disturb the signal. After a 15 min baseline period, the cuff placed on the left arm was inflated to induce three occlusion phases (O1, O2, O3) each of seven minutes. Each occlusion phase was followed by a reperfusion period (R1 = 10 min, R2 = 10 min, R3 = 20 min).

2.3. Measurements

2.3.1. Skinfolts

Triplicate measurements of skinfold thickness were attained from four-sites (non-dominant subscapular, bicipital, tricipital, and suprailiac) using a Harpenden Skinfold Caliper (Baty International, Wantage, United Kingdom). Skinfold thickness was calculated as the sum of the average values of the four sites. Fat percentage was determined with the correlation table provided by the manufacturer. Vastus lateralis skinfold thickness

(13.7 ± 6.6 mm) was also assessed before placing the NIRS instrument to avoid disturbance linked to adipose tissue thickness.

2.3.2. Ankle-Brachial Index (ABI)

The ABI was calculated as the ratio of the highest SBP value of posterior and dorsal tibial arteries and the highest SBP value of the brachial artery. Values are reported in Table 1. The assessment order was the following: right brachial artery; right tibial posterior and anterior arteries; left tibial posterior and anterior arteries; left brachial artery; right brachial artery [30]. SBP was assessed with a blood pressure monitor (Easy 3, Holtex+, Aix-en-Provence, France) and a manual stethoscope (Classic III, 3M Littman Stethoscopes, Maplewood, MN, USA) for the first measurement of the right brachial artery and with a mini-Doppler (Sonotrax Lite, Edan Instruments Inc., Shenzhen, China) for subsequent measures.

2.3.3. Near-Infrared Spectroscopy (NIRS)

A wireless NIRS device (PortaMon, Artinis, Elst, The Netherlands), connected with Bluetooth with a sampling rate of 10 Hz, was used on the left arm. This was dual-wavelength (760 and 850 nm), with three pairs of LEDs spaced 30, 35, and 40 mm from the receiving continuous-wave NIRS system using the modified Lambert–Beer law. It calculates the absolute concentration of tissue oxy-, deoxy- and total hemoglobin (O_2Hb , HHb , tHb , respectively). Tissue saturation index (TSI), expressed in % and reflecting the dynamic balance between O_2 supply and consumption, was calculated as stated in Equation (1):

$$TSI = [O_2Hb] / ([O_2Hb] + [HHb]) \times 100 \quad (1)$$

On the arm, the NIRS device was placed on the brachioradialis muscle, at two-thirds on the line from the styloid process to the central point between the lateral and medial epicondyles (Figure 2) in order to make device placement consistent for all participants [31].

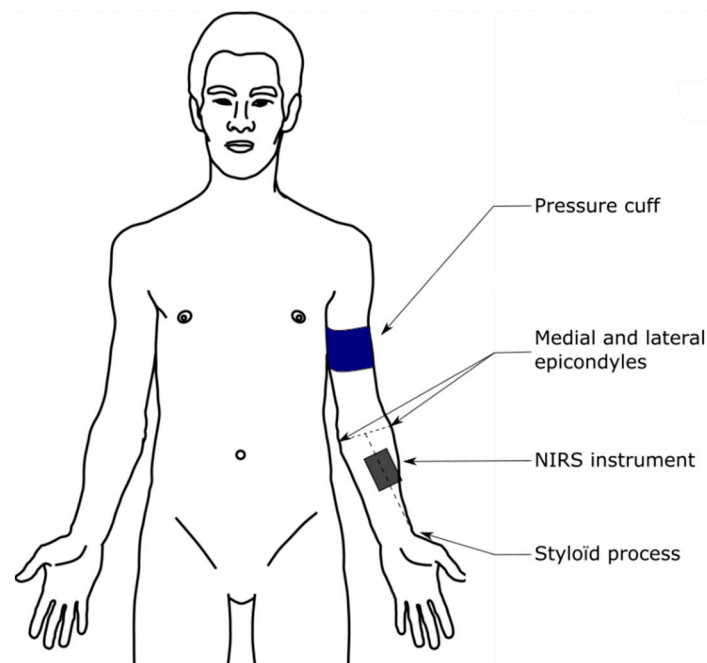


Figure 2. NIRS device placement illustration.

The device was adhered to the limb with double-sided auto-adhesive tape (Coheban, 3M, Cergy-Pontoise, France) and wrapped in black cloth and an elastic bandage to prevent any disturbance due to light interference or unintentional movement.

2.4. Data Analysis

NIRS data were acquired at 10 Hz, and the signal was smoothed using a 10th-order low-pass zero-phase Butterworth filter (cut-off frequency 0.8 Hz) using Pandas software library functions for Python (Python 3.8.8, Python Software Foundation, <https://www.python.org>, accessed on 19 October 2021) [32]. To avoid any disturbance linked to the beginning of the protocol, $TSI_{baseline}$, $HHb_{baseline}$ and $O_2HB_{baseline}$ were calculated as the average of the last minute before the first occlusion.

The difference (ΔTSI , ΔHHb and ΔO_2HB) between the maximum reached corresponding to the hyperemia spike during the reoxygenation (for TSI_{max} and O_2HB_{max}) and the minimum reached during the occlusion (TSI_{min} and O_2HB_{min}) and inversely for HHb_{max} and HHb_{min} were calculated (Figure 3).

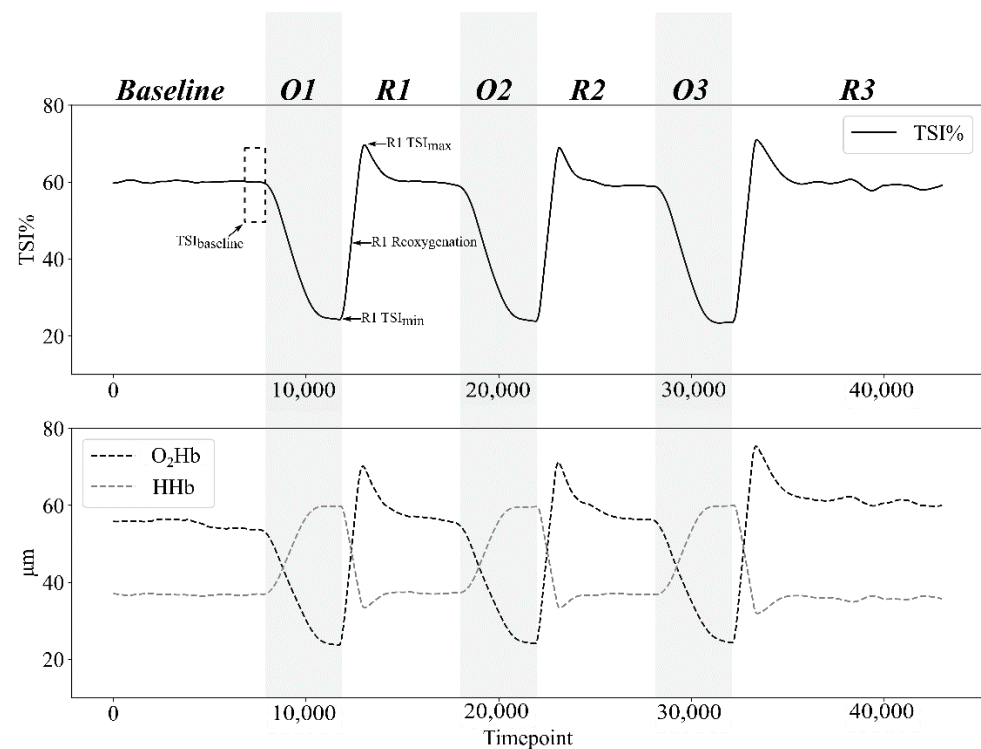


Figure 3. Example of tissue saturation index (TSI%), oxyhemoglobin concentration ($[O_2Hb]$) and deoxyhemoglobin concentration ($[HHb]$) responses during the whole protocol for a single participant in group 3 (G3: 250 mmHg). The dashed rectangle represents the last minute of the baseline, which is averaged to provide the baseline value of each parameter. When the cuff is inflated during occlusion phases (O1, O2, O3), both TSI% and $[O_2Hb]$ decrease until their minimum, whereas $[HHb]$ increases to its maximum. When the cuff is deflated at the beginning of reperfusion phases (R1, R2, R3), TSI% and $[O_2Hb]$ rise until their maximum above the baseline value (hyperemia spike), whereas $[HHb]$ reach its minimum.

Hemoglobin difference (HB_{diff}) was calculated as the difference between oxygenated hemoglobin (O_2Hb) and deoxygenated hemoglobin (HHb).

Reoxygenation rate was calculated for each parameter (TSI_{reoxy_rate} , HHb_{reoxy_rate} , $O_2HB_{reoxy_rate}$ and $HB_{diff_reoxy_rate}$) as the upslope ($r^2 = 0.968 \pm 0.016$) of TSI, $[HHb]$, $[O_2HB]$ and HB_{diff} between the start and the end of the reoxygenation curve, as well as the intercept and the coefficient of determination (r^2) (Figure 3). The start, the end, and the highest velocity value (V_{peak}) of the reoxygenation curve were calculated using automatic peak detection Python routine, with a 5% threshold for both start and end, applied to speed values. Speed values were obtained by derivative collected data and smoothed using a 10th-order low-pass zero-phase Butterworth filter (cut-off frequency 0.8 Hz) (Python 3.8.8).

2.5. Statistical Analysis

The three reperfusions per session are named R1, R2 and R3 for reperfusion 1, 2 and 3, respectively. Sessions 1 and 2 are named S1 and S2, respectively.

Student's *t*-tests were performed for the participants' characteristics and environmental conditions using JASP (Version 0.16.1.0, www.jasp-stats.org, accessed on 24 March 2022) after checking normality (Shapiro–Wilk test) and the equality of variances (Levene's test). The effect sizes (ES) are reported using Cohen's *d* and interpreted as small ($0.2 \geq d > 0.5$), medium ($0.5 \geq d > 0.8$), and large ($d \geq 0.8$) [33].

Intraclass correlation coefficient (ICC) and 95% confidence intervals (CI-95%) were calculated with the Pingouin package for Python using a two-way mixed-effects model with absolute agreement to compare repeated measures pairwise (Type 2, 1) [25]. Within-session ICC was calculated pairwise between R1–R2, R1–R3, R2–R3. Between-session ICC was calculated pairwise between the same event of each session: R1–R1, R2–R2, R3–R3. ICC [CI-95%] values less than 0.5 are indicative of poor reliability, values between 0.50 and 0.75 indicate moderate reliability, values between 0.75 and 0.90 indicate good reliability, and values greater than 0.90 indicate excellent reliability [25].

Standard error of measurement (SEM), an indicator of absolute reliability, was calculated as the standard deviation (SD) multiplied by the root mean square (RMS) of the difference of 1 and the ICC [34].

Coefficient of variation (CV) was used to assess the variability across multiple repeated measures. Within-participant CV was calculated as the mean of CV calculated over the three trials for each participant. Three CV between sessions were calculated for each participant for R1S1–R1S2, R2S1–R2S2 and R3S1–R3S2, respectively. A mean CV for each participant was then calculated, and the CV of each participant was averaged to obtain a mean between-session CV per group. Finally, a CV between participants was calculated for each trial and averaged.

3. Results

3.1. Participants' Characteristics and Environmental Conditions

Non-significant differences were observed across all variables of participants' characteristics. Comparisons of temperature and humidity level between sessions showed a significant difference between means only for humidity level ($t(30) = -2.580$; $p = 0.015$; $ES = -0.463$). Participant characteristics are reported in Table 1. Environmental conditions were monitored during each session and are reported in Table 2.

3.2. Within-Participant Reliability

3.2.1. Maximum and Minimum Responses

Results of within-participant variability assessed with CV are reported in Table 3. Results showed a lower mean CV between trials of each session across all variables for maximums ($2.58 \pm 1.82\%$) compared with minimums ($5.14 \pm 3.81\%$). The lowest variability for maximums was reached by TSI% ($1.23 \pm 0.62\%$), whereas the lowest variability was reached by HHb ($2.30 \pm 0.77\%$) for minimums.

Reliability between measurements assessed with ICC for minimums and maximums are reported in Tables 4 and 5, respectively. For both minimum and maximum values, ICC [ICC [CI-95%] showed a significant *p*-value for all conditions and all parameters ($p < 0.001$). Good to excellent reliability ($0.75 > ICC [CI-95\%] > 1$) was found in all conditions for maximums and minimums of G2 and G3, for TSI% and HHb only.

Table 3. Maximum and minimum variability.

Variable	Group	Mean	SD	Mean CV Btw. Trials (%)	Mean SD Btw. Trials (%)	CV Mean (%)	CV SD (%)
TSI _{min} (%)	G1	53.61	4.74	2.14	0.18	8.83	1.76
	G2	31.41	9.76	4.89	0.90	31.13	4.48
	G3	25.57	9.96	6.01	1.01	39.05	4.01
O ₂ Hb _{min} (μM)	G1	59.74	8.65	3.25	0.51	14.48	0.64
	G2	38.97	13.73	6.08	0.74	35.20	1.68
	G3	39.12	12.31	5.77	0.04	31.49	3.26
HHb _{min} (μM)	G1	31.41	4.13	3.39	0.14	13.14	2.14
	G2	32.59	5.30	1.78	0.14	16.27	1.14
	G3	33.60	6.51	1.32	0.22	19.39	2.96
tHb _{min} (μM)	G1	92.14	11.98	2.04	0.38	13.02	1.57
	G2	99.10	16.75	2.67	0.40	16.87	1.96
	G3	101.06	17.05	3.01	0.20	16.88	0.99
TSI _{max} (%)	G1	64.70	4.60	2.02	0.07	7.12	0.78
	G2	75.35	4.88	0.95	0.09	6.47	0.47
	G3	73.48	4.89	0.72	0.07	6.68	2.26
O ₂ Hb _{max} (μM)	G1	75.83	16.58	2.86	0.22	21.86	0.66
	G2	81.25	10.96	2.13	0.00	13.49	1.30
	G3	82.73	12.37	1.98	0.16	14.97	1.39
HHb _{max} (μM)	G1	50.90	8.02	3.11	0.91	15.74	2.92
	G2	63.14	9.88	1.85	0.26	15.62	1.76
	G3	64.94	9.52	1.94	0.31	14.65	0.35
tHb _{max} (μM)	G1	126.61	23.18	2.01	0.66	18.31	0.34
	G2	117.94	16.79	2.07	0.38	14.23	1.43
	G3	120.88	17.35	1.67	0.27	14.36	0.23

Legend: TSI: tissue saturation index; O₂Hb: oxyhemoglobin; HHb: deoxyhemoglobin, tHb: total hemoglobin; CV: coefficient of variation; SD: standard deviation; btw: between. CV mean and CV SD between trials are calculated for the same participant and averaged per group.

Table 4. Within-participant reliability for minimums.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI (%)	G1_S1_R1_R2	2.00	0.88 [0.62, 0.97] *	16.22
	G1_S1_R1_R3	1.56	0.92 [0.72, 0.98] *	22.43
	G1_S1_R2_R3	1.33	0.92 [0.66, 0.98] *	34.25
	G1_S2_R1_R2	0.61	0.97 [0.86, 0.99] **	100.52
	G1_S2_R1_R3	1.58	0.84 [0.5, 0.96] *	11.04
	G1_S2_R2_R3	1.49	0.87 [0.54, 0.96] *	12.57
	G2_S1_R1_R2	0.97	0.99 [0.97, 1.0] ***	228.17
	G2_S1_R1_R3	2.36	0.95 [0.86, 0.98] **	36.81
	G2_S1_R2_R3	1.99	0.96 [0.9, 0.99] **	51.42
	G2_S2_R1_R2	1.47	0.97 [0.92, 0.99] ***	71.26
	G2_S2_R1_R3	2.06	0.95 [0.85, 0.98] **	34.30
	G2_S2_R2_R3	1.27	0.98 [0.94, 0.99] ***	88.07
	G3_S1_R1_R2	1.16	0.99 [0.96, 1.0] ***	145.88
	G3_S1_R1_R3	1.43	0.98 [0.94, 0.99] ***	92.65
	G3_S1_R2_R3	1.36	0.98 [0.95, 0.99] ***	103.27
O ₂ Hb (μM)	G3_S2_R1_R2	1.74	0.97 [0.91, 0.99] ***	59.02
	G3_S2_R1_R3	1.88	0.96 [0.89, 0.99] **	47.02
	G3_S2_R2_R3	1.26	0.98 [0.94, 0.99] ***	101.32
	G1_S1_R1_R2	1.83	0.95 [0.79, 0.99] **	49.63
	G1_S1_R1_R3	2.96	0.87 [0.58, 0.97] *	14.09
	G1_S1_R2_R3	1.51	0.97 [0.88, 0.99] **	58.16
	G1_S2_R1_R2	2.40	0.92 [0.72, 0.98] *	22.69
	G1_S2_R1_R3	3.88	0.81 [0.43, 0.95]	10.95
	G1_S2_R2_R3	1.91	0.95 [0.61, 0.99] *	65.55

Table 4. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
	G2_S1_R1_R2	4.57	0.9 [0.74, 0.97] *	19.18
	G2_S1_R1_R3	3.81	0.93 [0.81, 0.98] **	26.74
	G2_S1_R2_R3	2.41	0.97 [0.92, 0.99] ***	65.26
	G2_S2_R1_R2	3.39	0.93 [0.73, 0.98] *	40.33
	G2_S2_R1_R3	2.50	0.96 [0.82, 0.99] **	65.90
	G2_S2_R2_R3	2.10	0.97 [0.92, 0.99] ***	69.04
	G3_S1_R1_R2	3.57	0.92 [0.78, 0.97] **	23.18
	G3_S1_R1_R3	2.08	0.98 [0.93, 0.99] ***	79.64
	G3_S1_R2_R3	3.03	0.94 [0.82, 0.98] **	28.75
	G3_S2_R1_R2	2.08	0.97 [0.91, 0.99] ***	56.58
	G3_S2_R1_R3	3.01	0.93 [0.8, 0.97] **	26.94
	G3_S2_R2_R3	3.07	0.93 [0.81, 0.98] **	27.33
HHb (μ M)	G1_S1_R1_R2	0.81	0.96 [0.84, 0.99] **	43.09
	G1_S1_R1_R3	1.24	0.87 [0.55, 0.97] *	16.55
	G1_S1_R2_R3	1.52	0.82 [0.45, 0.95]	10.07
	G1_S2_R1_R2	0.58	0.98 [0.94, 1.0] ***	110.64
	G1_S2_R1_R3	1.65	0.86 [0.54, 0.96] *	15.14
	G1_S2_R2_R3	1.42	0.9 [0.62, 0.97] *	22.49
	G2_S1_R1_R2	0.92	0.97 [0.92, 0.99] ***	73.30
	G2_S1_R1_R3	0.96	0.97 [0.92, 0.99] ***	66.34
	G2_S1_R2_R3	0.42	0.99 [0.98, 1.0] ***	309.94
	G2_S2_R1_R2	0.84	0.97 [0.91, 0.99] ***	83.32
	G2_S2_R1_R3	0.94	0.96 [0.84, 0.99] **	78.49
	G2_S2_R2_R3	0.50	0.99 [0.97, 1.0] ***	210.56
	G3_S1_R1_R2	0.33	1 [0.99, 1.0] ***	1321.88
	G3_S1_R1_R3	0.53	0.99 [0.98, 1.0] ***	339.18
G3_S1_R2_R3	0.58	0.99 [0.98, 1.0] ***	275.76	
G3_S2_R1_R2	0.61	0.99 [0.97, 1.0] ***	171.15	
G3_S2_R1_R3	0.71	0.98 [0.95, 0.99] ***	143.09	
G3_S2_R2_R3	0.45	0.99 [0.98, 1.0] ***	349.01	
tHb (μ M)	G1_S1_R1_R2	2.16	0.96 [0.86, 0.99] **	57.42
	G1_S1_R1_R3	2.28	0.95 [0.83, 0.99] **	38.64
	G1_S1_R2_R3	1.37	0.98 [0.94, 1.0] ***	144.38
	G1_S2_R1_R2	2.44	0.96 [0.86, 0.99] **	48.62
	G1_S2_R1_R3	3.52	0.92 [0.74, 0.98] *	24.80
	G1_S2_R2_R3	1.64	0.98 [0.93, 1.0] ***	120.85
	G2_S1_R1_R2	2.85	0.98 [0.93, 0.99] ***	77.17
	G2_S1_R1_R3	3.28	0.97 [0.91, 0.99] ***	60.21
	G2_S1_R2_R3	2.13	0.99 [0.96, 1.0] ***	141.39
	G2_S2_R1_R2	4.83	0.89 [0.7, 0.96] *	19.56
	G2_S2_R1_R3	3.51	0.94 [0.68, 0.98] *	55.99
	G2_S2_R2_R3	3.32	0.95 [0.86, 0.98] **	36.74
	G3_S1_R1_R2	3.85	0.95 [0.86, 0.98] **	36.88
	G3_S1_R1_R3	2.41	0.98 [0.95, 0.99] ***	104.67
G3_S1_R2_R3	3.95	0.94 [0.84, 0.98] **	31.51	
G3_S2_R1_R2	3.63	0.95 [0.87, 0.98] **	40.01	
G3_S2_R1_R3	3.83	0.94 [0.84, 0.98] **	33.40	
G3_S2_R2_R3	3.29	0.96 [0.88, 0.99] **	44.93	

Legend: G1: group 1; G2: group 2; G3: group 3; S1: session 1; S2: session 2; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); Degrees of freedom (df): 9, 14, 14 for G1, G2 and G3, respectively. $p < 0.001$ in all conditions. * Moderate reliability; ** Good reliability; *** Excellent reliability.

Table 5. Within-participant reliability for maximums.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI (%)	G1_S1_R1_R2	0.83	0.97 [0.89, 0.99] **	88.24
	G1_S1_R1_R3	1.82	0.85 [0.54, 0.96] *	14.10
	G1_S1_R2_R3	1.59	0.88 [0.6, 0.97] *	14.84
	G1_S2_R1_R2	0.59	0.98 [0.92, 0.99] ***	85.33
	G1_S2_R1_R3	1.70	0.84 [0.52, 0.96] *	12.73
	G1_S2_R2_R3	1.76	0.84 [0.49, 0.96]	13.01
	G2_S1_R1_R2	1.30	0.92 [0.79, 0.97] **	24.12
	G2_S1_R1_R3	0.97	0.96 [0.89, 0.99] **	47.76
	G2_S1_R2_R3	0.81	0.96 [0.88, 0.99] **	61.66
	G2_S2_R1_R2	1.05	0.96 [0.88, 0.99] **	44.38
	G2_S2_R1_R3	1.42	0.92 [0.8, 0.97] **	26.09
	G2_S2_R2_R3	0.60	0.98 [0.95, 0.99] ***	139.95
	G3_S1_R1_R2	0.65	0.99 [0.97, 1.0] ***	156.65
	G3_S1_R1_R3	0.72	0.98 [0.96, 0.99] ***	135.37
	G3_S1_R2_R3	0.40	1 [0.99, 1.0] ***	475.50
O ₂ Hb (μM)	G3_S2_R1_R2	0.76	0.96 [0.88, 0.99] **	47.45
	G3_S2_R1_R3	0.81	0.95 [0.87, 0.98] **	40.06
	G3_S2_R2_R3	0.39	0.99 [0.97, 1.0] ***	164.66
	G1_S1_R1_R2	2.79	0.97 [0.89, 0.99] **	65.72
	G1_S1_R1_R3	3.48	0.96 [0.84, 0.99] **	42.47
	G1_S1_R2_R3	1.48	0.99 [0.97, 1.0] ***	242.21
	G1_S2_R1_R2	1.23	0.99 [0.97, 1.0] ***	285.16
	G1_S2_R1_R3	3.04	0.96 [0.81, 0.99] **	71.85
	G1_S2_R2_R3	3.09	0.96 [0.76, 0.99] **	70.93
	G2_S1_R1_R2	1.88	0.98 [0.91, 0.99] ***	105.41
	G2_S1_R1_R3	2.43	0.96 [0.78, 0.99] **	69.59
	G2_S1_R2_R3	0.95	0.99 [0.97, 1.0] ***	370.10
	G2_S2_R1_R2	1.50	0.98 [0.82, 0.99] **	154.37
	G2_S2_R1_R3	2.50	0.94 [0.27, 0.99]	90.95
	G2_S2_R2_R3	1.51	0.98 [0.84, 0.99] **	143.04
HHb (μM)	G3_S1_R1_R2	1.51	0.99 [0.94, 1.0] ***	178.14
	G3_S1_R1_R3	2.22	0.97 [0.72, 0.99] *	133.93
	G3_S1_R2_R3	1.64	0.98 [0.94, 0.99] ***	150.76
	G3_S2_R1_R2	2.27	0.96 [0.89, 0.99] **	50.78
	G3_S2_R1_R3	2.34	0.96 [0.88, 0.99] **	53.42
	G3_S2_R2_R3	1.37	0.99 [0.96, 1.0] ***	144.53
	G1_S1_R1_R2	2.09	0.9 [0.26, 0.98]	42.70
	G1_S1_R1_R3	2.50	0.87 [0.5, 0.97] *	19.84
	G1_S1_R2_R3	1.52	0.95 [0.81, 0.99] **	34.38
	G1_S2_R1_R2	1.20	0.98 [0.93, 1.0] ***	122.78
	G1_S2_R1_R3	1.70	0.96 [0.71, 0.99] *	92.60
	G1_S2_R2_R3	1.33	0.98 [0.91, 0.99] ***	104.52
	G2_S1_R1_R2	1.59	0.98 [0.93, 0.99] ***	82.03
	G2_S1_R1_R3	1.52	0.98 [0.94, 0.99] ***	94.54
	G2_S1_R2_R3	1.06	0.99 [0.97, 1.0] ***	187.16
G2_S2_R1_R2	1.35	0.98 [0.82, 0.99] **	148.52	
G2_S2_R1_R3	1.39	0.97 [0.91, 0.99] ***	100.13	
G2_S2_R2_R3	0.58	1 [0.99, 1.0] ***	494.12	
tHb (μM)	G3_S1_R1_R2	1.44	0.98 [0.93, 0.99] ***	77.85
	G3_S1_R1_R3	1.11	0.99 [0.96, 0.99] ***	124.83
	G3_S1_R2_R3	1.25	0.98 [0.94, 0.99] ***	94.77
	G3_S2_R1_R2	1.42	0.98 [0.94, 0.99] ***	91.88
	G3_S2_R1_R3	1.61	0.97 [0.92, 0.99] ***	68.96
	G3_S2_R2_R3	1.56	0.97 [0.92, 0.99] ***	67.93
	G1_S1_R1_R2	3.22	0.98 [0.91, 0.99] ***	112.34
	G1_S1_R1_R3	4.78	0.96 [0.84, 0.99] **	48.67
	G1_S1_R2_R3	3.02	0.98 [0.93, 1.0] ***	103.70
	G1_S2_R1_R2	1.90	0.99 [0.97, 1.0] ***	319.43

Table 5. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
	G1_S2_R1_R3	2.43	0.99 [0.96, 1.0] ***	165.32
	G1_S2_R2_R3	3.51	0.98 [0.91, 0.99] ***	87.44
	G2_S1_R1_R2	4.47	0.94 [0.83, 0.98] **	30.94
	G2_S1_R1_R3	3.74	0.96 [0.88, 0.99] **	47.94
	G2_S1_R2_R3	1.89	0.99 [0.96, 1.0] ***	191.37
	G2_S2_R1_R2	2.11	0.98 [0.68, 1.0] *	252.60
	G2_S2_R1_R3	2.88	0.96 [0.43, 0.99]	153.16
	G2_S2_R2_R3	1.44	0.99 [0.97, 1.0] ***	271.99
	G3_S1_R1_R2	2.01	0.99 [0.95, 1.0] ***	167.18
	G3_S1_R1_R3	2.47	0.98 [0.77, 0.99] **	192.52
	G3_S1_R2_R3	2.05	0.98 [0.95, 0.99] ***	139.59
	G3_S2_R1_R2	2.99	0.97 [0.92, 0.99] ***	76.27
	G3_S2_R1_R3	2.62	0.98 [0.93, 0.99] ***	102.32
	G3_S2_R2_R3	2.82	0.97 [0.92, 0.99] ***	71.31

Legend: G1: group 1; G2: group 2; G3: group 3; S1: session 1; S2: session 2; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 9, 14, 14 for G1, G2 and G3, respectively. $p < 0.001$ in all conditions. * Moderate reliability; ** Good reliability; *** Excellent reliability.

3.2.2. Reoxygenation Rate

Reoxygenation rate within-participant variability results are reported in Table 6. In all conditions and for all parameters, G1 showed the lowest slope value ($0.50 \pm 0.16\% \cdot s^{-1}$) compared with G2 ($1.26 \pm 0.17\% \cdot s^{-1}$) and G3 ($1.62 \pm 0.51\% \cdot s^{-1}$). The greatest V_{peak} for TSI% was obtained in G3 ($2.87 \pm 0.67\% \cdot s^{-1}$), whereas G2 and G1 elicited, respectively, $2.24 \pm 0.66\% \cdot s^{-1}$ and $1.07 \pm 0.38\% \cdot s^{-1}$, respectively.

Table 6. Reoxygenation rate within-participant variability.

Variable	Group	Mean	SD	Mean CV Btw. Trials (%)	Mean SD Btw. Trials (%)	Mean CV (%)	Mean SD (%)
TSI _{Slope} (%·s ⁻¹)	G1	0.53	0.15	107.50	130.42	52.56	19.85
	G2	1.29	0.02	9.21	1.21	36.69	1.62
	G3	1.66	0.05	11.10	1.15	27.59	13.01
O ₂ Hb _{Slope} (μM·s ⁻¹)	G1	0.44	0.26	65.47	59.61	107.39	58.25
	G2	1.37	0.08	16.11	3.72	37.44	2.55
	G3	1.75	0.08	12.49	1.50	27.29	3.58
HHb _{Slope} (μM·s ⁻¹)	G1	0.52	0.13	17.51	9.62	58.26	0.76
	G2	1.07	0.14	12.17	2.63	32.73	3.85
	G3	1.31	0.06	13.48	1.14	25.56	3.91
tHb _{Slope} (μM·s ⁻¹)	G1	0.51	0.24	33.25	19.46	82.36	31.64
	G2	1.29	0.27	33.55	5.18	62.29	26.41
	G3	1.76	0.10	24.72	3.59	47.04	10.47
TSI _{Vpeak} (%·s ⁻¹)	G1	1.07	0.38	13.89	5.37	35.78	0.62
	G2	2.24	0.66	9.37	0.52	29.35	2.58
	G3	2.87	0.67	9.57	0.37	23.33	11.13
O ₂ Hb _{Vpeak} (μM·s ⁻¹)	G1	2.82	2.05	9.47	0.82	73.36	7.75
	G2	2.58	0.89	13.05	0.46	34.68	0.07
	G3	2.90	0.87	15.30	2.11	30.18	0.62
HHb _{Vpeak} (μM·s ⁻¹)	G1	2.11	0.95	7.09	0.46	45.16	2.13
	G2	1.74	0.57	10.76	0.02	33.08	2.20
	G3	2.02	0.59	11.06	0.36	29.61	2.95
tHb _{Vpeak} (μM·s ⁻¹)	G1	4.85	2.97	6.92	2.03	61.38	4.21
	G2	2.37	0.81	19.42	2.20	34.15	2.45
	G3	2.39	0.72	21.27	2.10	30.38	8.36

Legend: TSI: tissue saturation index; O₂Hb: oxyhemoglobin; HHb: deoxyhemoglobin, tHb: total hemoglobin. CV: coefficient of variation; SD: standard deviation; btw: between. CV mean and CV SD between trials are calculated for the same participant and averaged per group.

Despite the mean ICC value showing good reliability for TSI% (0.79 ± 0.18) and a p -value < 0.001 in all conditions, ICC [CI-95%] results of slope and Vpeak were not able to reach the moderate threshold of 0.5, in most conditions or parameters (Tables 7 and 8).

Table 7. Within-participant reliability for slope of reoxygenation.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI (%·s ⁻¹)	G1_S1_R1_R2	0.25	0.38 [−0.20, 0.80]	2.48
	G1_S1_R1_R3	0.25	0.37 [−0.22, 0.80]	2.37
	G1_S1_R2_R3	0.09	0.75 [0.21, 0.94]	6.49
	G1_S2_R1_R2	0.07	0.91 [0.63, 0.98] *	28.22
	G1_S2_R1_R3	0.13	0.67 [0.02, 0.92]	4.60
	G1_S2_R2_R3	0.10	0.74 [0.25, 0.93]	6.91
	G2_S1_R1_R2	0.09	0.96 [0.87, 0.99] **	55.42
	G2_S1_R1_R3	0.15	0.89 [0.54, 0.97] *	27.53
	G2_S1_R2_R3	0.11	0.94 [0.81, 0.98] **	42.94
	G2_S2_R1_R2	0.12	0.94 [0.46, 0.98]	63.09
	G2_S2_R1_R3	0.14	0.92 [0.19, 0.98]	66.09
	G2_S2_R2_R3	0.12	0.94 [0.83, 0.98] **	32.28
	G3_S1_R1_R2	0.17	0.73 [−0.08, 0.94]	24.98
	G3_S1_R1_R3	0.19	0.63 [−0.09, 0.90]	14.93
	G3_S1_R2_R3	0.11	0.82 [0.53, 0.94] *	10.05
O ₂ Hb (μM·s ⁻¹)	G3_S2_R1_R2	0.18	0.91 [0.74, 0.97] *	21.78
	G3_S2_R1_R3	0.23	0.82 [0.50, 0.94] *	11.85
	G3_S2_R2_R3	0.17	0.92 [0.76, 0.97] **	22.97
	G1_S1_R1_R2	0.08	0.95 [0.80, 0.99] **	39.40
	G1_S1_R1_R3	0.18	0.72 [0.16, 0.93]	7.82
	G1_S1_R2_R3	0.13	0.83 [0.43, 0.96]	13.19
	G1_S2_R1_R2	0.10	0.93 [0.72, 0.98] *	25.10
	G1_S2_R1_R3	0.14	0.86 [0.53, 0.97] *	15.09
	G1_S2_R2_R3	0.12	0.91 [0.63, 0.98] *	25.71
	G2_S1_R1_R2	0.34	0.67 [0.22, 0.88]	6.29
	G2_S1_R1_R3	0.42	0.56 [0.03, 0.84]	4.89
	G2_S1_R2_R3	0.15	0.91 [0.67, 0.97] *	28.21
	G2_S2_R1_R2	0.16	0.88 [0.49, 0.96]	23.29
	G2_S2_R1_R3	0.24	0.76 [0.05, 0.93]	14.68
	G2_S2_R2_R3	0.18	0.88 [0.63, 0.96] *	18.25
HHb (μM·s ⁻¹)	G3_S1_R1_R2	0.02	0.8 [−0.03, 0.95]	24.74
	G3_S1_R1_R3	0.02	0.69 [−0.08, 0.92]	15.13
	G3_S1_R2_R3	0.01	0.92 [0.77, 0.98] **	26.16
	G3_S2_R1_R2	0.03	0.69 [0.14, 0.90]	7.95
	G3_S2_R1_R3	0.02	0.78 [0.13, 0.94]	14.44
	G3_S2_R2_R3	0.02	0.88 [0.66, 0.96] *	15.05
	G1_S1_R1_R2	0.06	0.93 [0.74, 0.98] *	31.39
	G1_S1_R1_R3	0.13	0.67 [0.05, 0.91]	7.17
	G1_S1_R2_R3	0.09	0.81 [0.28, 0.95]	13.33
	G1_S2_R1_R2	0.05	0.98 [0.92, 1.00] ***	97.22
	G1_S2_R1_R3	0.06	0.97 [0.84, 0.99] **	71.73
	G1_S2_R2_R3	0.06	0.97 [0.88, 0.99] **	63.89
	G2_S1_R1_R2	0.11	0.9 [0.71, 0.97] *	20.99
	G2_S1_R1_R3	0.15	0.82 [0.53, 0.94] *	10.84
	G2_S1_R2_R3	0.12	0.88 [0.68, 0.96] *	15.34
G2_S2_R1_R2	0.13	0.84 [0.56, 0.95] *	13.40	
G2_S2_R1_R3	0.16	0.76 [0.19, 0.93]	11.96	
G2_S2_R2_R3	0.13	0.86 [0.62, 0.95] *	14.29	
G3_S1_R1_R2	0.19	0.74 [0.34, 0.91]	7.84	
G3_S1_R1_R3	0.25	0.55 [0.05, 0.84]	4.35	
G3_S1_R2_R3	0.19	0.72 [0.32, 0.91]	6.15	
G3_S2_R1_R2	0.14	0.75 [0.37, 0.92]	8.15	
G3_S2_R1_R3	0.19	0.71 [0.12, 0.91]	9.08	

Table 7. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
tHb ($\mu\text{M}\cdot\text{s}^{-1}$)	G3_S2_R2_R3	0.17	0.74 [0.36, 0.91]	7.01
	G1_S1_R1_R2	0.09	0.93 [0.73, 0.98] *	26.50
	G1_S1_R1_R3	0.16	0.7 [0.17, 0.92]	6.62
	G1_S1_R2_R3	0.13	0.8 [0.37, 0.95]	9.14
	G1_S2_R1_R2	0.05	0.98 [0.93, 1.0] ***	103.74
	G1_S2_R1_R3	0.12	0.89 [0.58, 0.97] *	20.10
	G1_S2_R2_R3	0.14	0.87 [0.54, 0.97] *	14.17
	G2_S1_R1_R2	0.26	0.91 [0.75, 0.97] *	22.62
	G2_S1_R1_R3	0.26	0.92 [0.77, 0.98] **	29.02
	G2_S1_R2_R3	0.22	0.93 [0.81, 0.98] **	28.05
	G2_S2_R1_R2	0.34	0.74 [0.30, 0.91]	9.04
	G2_S2_R1_R3	0.30	0.83 [0.45, 0.95]	15.42
	G2_S2_R2_R3	0.24	0.86 [0.63, 0.95] *	12.87
	G3_S1_R1_R2	0.19	0.91 [0.24, 0.98]	53.41
	G3_S1_R1_R3	0.53	0.11 [−0.25, 0.54]	1.35
	G3_S1_R2_R3	0.52	0.1 [−0.41, 0.59]	1.24
G3_S2_R1_R2	0.48	0.76 [0.41, 0.92]	7.94	
G3_S2_R1_R3	0.41	0.81 [0.50, 0.94] *	10.23	
G3_S2_R2_R3	0.24	0.93 [0.79, 0.98] **	25.95	

Legend: G1: group 1; G2: group 2; G3: group 3; S1: session 1; S2: session 2; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 8, 13, 12 for G1, G2 and G3, respectively. $p < 0.001$ in all conditions. * Moderate reliability; ** Good reliability; *** Excellent reliability.

Table 8. Within-participant reliability for Vpeak of reoxygenation rate.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI% ($\%\cdot\text{s}^{-1}$)	G1_S1_R1_R2	0.10	0.92 [0.69, 0.98] *	21.72
	G1_S1_R1_R3	0.20	0.51 [−0.24, 0.87]	2.84
	G1_S1_R2_R3	0.18	0.66 [0.01, 0.91]	4.48
	G1_S2_R1_R2	0.07	0.97 [0.88, 0.99] **	68.24
	G1_S2_R1_R3	0.16	0.82 [0.38, 0.96]	9.12
	G1_S2_R2_R3	0.11	0.9 [0.61, 0.98] *	16.53
	G2_S1_R1_R2	0.20	0.89 [0.23, 0.97]	40.40
	G2_S1_R1_R3	0.30	0.8 [0.12, 0.94]	17.29
	G2_S1_R2_R3	0.16	0.94 [0.81, 0.98] **	33.87
	G2_S2_R1_R2	0.18	0.93 [0.61, 0.98] *	43.94
	G2_S2_R1_R3	0.23	0.89 [0.30, 0.97]	33.26
	G2_S2_R2_R3	0.17	0.94 [0.82, 0.98] **	30.46
	G3_S1_R1_R2	0.31	0.58 [−0.09, 0.89]	14.37
	G3_S1_R1_R3	0.30	0.59 [−0.09, 0.89]	14.57
	G3_S1_R2_R3	0.09	0.96 [0.87, 0.99] **	45.44
	G3_S2_R1_R2	0.36	0.84 [0.54, 0.95] *	13.70
G3_S2_R1_R3	0.24	0.9 [0.69, 0.97] *	23.71	
G3_S2_R2_R3	0.29	0.87 [0.63, 0.96] *	13.78	
O ₂ Hb ($\mu\text{M}\cdot\text{s}^{-1}$)	G1_S1_R1_R2	0.46	0.95 [0.82, 0.99] **	41.27
	G1_S1_R1_R3	0.37	0.97 [0.88, 0.99] **	65.49
	G1_S1_R2_R3	0.15	0.99 [0.98, 1.0] ***	322.77
	G1_S2_R1_R2	0.21	0.99 [0.95, 1.0] ***	147.02
	G1_S2_R1_R3	0.16	0.99 [0.97, 1.0] ***	303.78
	G1_S2_R2_R3	0.23	0.99 [0.94, 1.0] ***	152.72
	G2_S1_R1_R2	0.34	0.87 [0.03, 0.97]	42.00
	G2_S1_R1_R3	0.51	0.75 [0.02, 0.93]	14.39
	G2_S1_R2_R3	0.26	0.93 [0.79, 0.98] **	33.23
	G2_S2_R1_R2	0.34	0.85 [0.55, 0.95] *	14.94
	G2_S2_R1_R3	0.38	0.84 [0.04, 0.96]	29.73
	G2_S2_R2_R3	0.33	0.86 [0.62, 0.95] *	15.05

Table 8. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
HHb ($\mu\text{M}\cdot\text{s}^{-1}$)	G3_S1_R1_R2	0.45	0.74 [0.11, 0.93]	11.55
	G3_S1_R1_R3	0.57	0.7 [−0.07, 0.92]	14.50
	G3_S1_R2_R3	0.47	0.74 [0.37, 0.91]	7.22
	G3_S2_R1_R2	0.42	0.79 [−0.06, 0.96]	44.60
	G3_S2_R1_R3	0.49	0.66 [−0.09, 0.91]	13.41
	G3_S2_R2_R3	0.26	0.91 [0.72, 0.97] *	19.06
	G1_S1_R1_R2	0.22	0.94 [0.79, 0.99] **	38.02
	G1_S1_R1_R3	0.20	0.96 [0.83, 0.99] **	45.48
	G1_S1_R2_R3	0.10	0.99 [0.95, 1.00] ***	159.37
	G1_S2_R1_R2	0.12	0.98 [0.91, 1.00] ***	148.02
	G1_S2_R1_R3	0.18	0.97 [0.87, 0.99] **	58.41
	G1_S2_R2_R3	0.16	0.97 [0.88, 0.99] **	61.26
	G2_S1_R1_R2	0.19	0.89 [0.66, 0.97] *	22.05
	G2_S1_R1_R3	0.25	0.84 [0.32, 0.96]	19.66
	G2_S1_R2_R3	0.11	0.96 [0.80, 0.99] **	76.43
	G2_S2_R1_R2	0.21	0.85 [0.17, 0.96]	26.33
	G2_S2_R1_R3	0.21	0.85 [−0.04, 0.97]	51.73
	G2_S2_R2_R3	0.16	0.92 [0.78, 0.97] **	23.74
	G3_S1_R1_R2	0.24	0.86 [0.28, 0.96]	23.63
	G3_S1_R1_R3	0.25	0.86 [0.03, 0.97]	37.29
	G3_S1_R2_R3	0.10	0.97 [0.91, 0.99] ***	67.75
	G3_S2_R1_R2	0.34	0.66 [0.07, 0.89]	7.67
	G3_S2_R1_R3	0.26	0.74 [0.04, 0.93]	12.90
	G3_S2_R2_R3	0.18	0.87 [0.64, 0.96] *	14.27
tHb ($\mu\text{M}\cdot\text{s}^{-1}$)	G1_S1_R1_R2	0.66	0.95 [0.83, 0.99] **	42.79
	G1_S1_R1_R3	0.53	0.97 [0.89, 0.99] **	70.64
	G1_S1_R2_R3	0.23	0.99 [0.97, 1.00] ***	288.36
	G1_S2_R1_R2	0.28	0.99 [0.96, 1.00] ***	202.64
	G1_S2_R1_R3	0.17	1 [0.99, 1.00] ***	534.65
	G1_S2_R2_R3	0.29	0.99 [0.95, 1.00] ***	189.00
	G2_S1_R1_R2	0.35	0.83 [0.42, 0.95]	15.31
	G2_S1_R1_R3	0.65	0.56 [0.10, 0.83]	3.82
	G2_S1_R2_R3	0.45	0.77 [0.43, 0.92]	7.38
	G2_S2_R1_R2	0.58	0.44 [−0.13, 0.78]	2.46
	G2_S2_R1_R3	0.36	0.84 [0.59, 0.95] *	11.65
	G2_S2_R2_R3	0.54	0.58 [0.08, 0.84]	3.59
	G3_S1_R1_R2	0.49	0.62 [0.17, 0.87]	4.49
	G3_S1_R1_R3	0.53	0.6 [−0.10, 0.89]	10.31
	G3_S1_R2_R3	0.67	0.4 [−0.09, 0.76]	2.56
	G3_S2_R1_R2	0.46	0.49 [−0.11, 0.83]	6.04
G3_S2_R1_R3	0.59	0.27 [−0.14, 0.66]	2.26	
G3_S2_R2_R3	0.49	0.46 [−0.13, 0.80]	2.56	

Legend: G1: group 1; G2: group 2; G3: group 3; S1: session 1; S2: session 2; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 8, 13, 12 for G1, G2 and G3, respectively. $p < 0.01$ in all conditions. * Moderate reliability; ** Good reliability; *** Excellent reliability.

3.2.3. Maximums and Minimums

Results of maximum and minimum variability between sessions, assessed with CV, are reported in Table 9. When averaged across groups, CV of HHb ($3.70 \pm 2.95\%$) and TSI% ($2.43 \pm 2.48\%$) show the lowest variability for minimums and maximums, respectively. Averaged across all groups and variables, minimums and maximums showed a CV of $7.08 \pm 7.17\%$ and $4.30 \pm 3.95\%$, respectively.

Table 9. Between-session variability for minimums and maximums.

Variable	Group	Mean_S1	SD_S1	Mean_S2	SD_S2	Mean CV Btw. Sessions (%)	SD of CV Btw. Sessions (%)
TSI _{min} (%)	G1	54.55	5.39	52.67	3.85	3.33	1.99
	G2	30.79	10.45	32.02	8.87	9.11	8.34
	G3	24.75	10.30	26.38	9.49	15.05	12.17
O ₂ Hb _{min} (μM)	G1	60.75	8.33	58.72	8.49	4.11	2.82
	G2	40.54	14.42	37.41	12.58	9.91	5.45
	G3	38.54	12.84	39.70	11.36	13.03	9.55
HHb _{min} (μM)	G1	31.78	3.61	31.04	4.44	3.60	2.77
	G2	32.44	5.50	32.75	5.03	3.14	2.11
	G3	33.26	7.13	33.94	5.85	4.33	3.75
tHb _{min} (μM)	G1	93.55	11.03	90.74	12.63	3.48	2.55
	G2	101.70	18.43	96.49	14.63	5.28	3.15
	G3	99.30	17.26	102.82	16.37	6.03	4.24
TSI _{max} (%)	G1	64.02	4.78	65.39	4.15	2.50	1.29
	G2	75.74	4.61	74.95	5.03	1.58	1.21
	G3	72.68	5.99	74.29	3.73	3.25	3.62
O ₂ Hb _{max} (μM)	G1	77.89	17.22	73.76	15.67	7.45	6.08
	G2	82.13	11.77	80.38	10.05	3.02	1.90
	G3	81.07	12.89	84.40	11.69	5.10	4.37
HHb _{max} (μM)	G1	50.67	6.78	51.13	9.05	7.37	4.62
	G2	64.32	10.78	61.95	8.87	3.18	2.30
	G3	63.43	9.09	66.45	9.82	4.99	3.87
tHb _{max} (μM)	G1	128.42	23.01	124.81	23.05	7.42	5.39
	G2	119.27	17.95	116.62	15.37	3.17	1.78
	G3	118.53	16.76	123.22	17.74	5.06	4.55

Legend: TSI: tissue saturation index; O₂Hb: oxyhemoglobin; HHb: deoxyhemoglobin, tHb: total hemoglobin; CV: coefficient of variation; SD: standard deviation; btw: between. CV mean and CV SD between trials are calculated for the same participant and averaged per group.

ICC [CI-95%] results for between-session analyses are reported in Tables 10 and 11. Results indicate a lack of reliability for both maximums and minimums between sessions, with the majority of CI-95% below the 0.5 threshold in all conditions. An example of the reliability of maximums both between- and within-session is provided by an analysis of the limits of agreement (Figure 4).

Table 10. Between-session reliability of minimums.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI (%)	G1_R1	3.15	0.64 [0.08, 0.90]	4.51
	G1_R2	3.03	0.59 [0.02, 0.88]	5.07
	G1_R3	2.44	0.7 [0.21, 0.91]	6.36
	G2_R1	5.33	0.71 [0.33, 0.89]	5.72
	G2_R2	5.10	0.71 [0.33, 0.89]	5.64
	G2_R3	5.35	0.69 [0.30, 0.89]	5.29
	G3_R1	7.69	0.44 [−0.08, 0.77]	2.52
	G3_R2	7.73	0.39 [−0.15, 0.74]	2.22
	G3_R3	7.30	0.39 [−0.15, 0.75]	2.21
O ₂ Hb (μM)	G1_R1	4.13	0.77 [0.35, 0.94]	8.13
	G1_R2	4.10	0.75 [0.29, 0.93]	8.56
	G1_R3	3.83	0.8 [0.38, 0.95]	8.40
	G2_R1	6.57	0.79 [0.41, 0.93]	10.95
	G2_R2	6.79	0.76 [0.42, 0.91]	7.01
	G2_R3	6.02	0.78 [0.48, 0.92]	8.39
	G3_R1	11.06	0.26 [−0.30, 0.68]	1.66
	G3_R2	10.05	0.24 [−0.32, 0.67]	1.60
	G3_R3	10.49	0.24 [−0.33, 0.67]	1.60

Table 10. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
HHb (μM)	G1_R1	1.88	0.8 [0.40, 0.95]	8.77
	G1_R2	2.37	0.7 [0.17, 0.92]	5.39
	G1_R3	1.72	0.77 [0.35, 0.94]	8.13
	G2_R1	1.97	0.86 [0.65, 0.95] *	13.22
	G2_R2	1.71	0.89 [0.71, 0.96] *	16.41
	G2_R3	1.94	0.86 [0.63, 0.95] *	12.29
	G3_R1	3.14	0.76 [0.42, 0.91]	6.92
	G3_R2	3.06	0.78 [0.46, 0.92]	7.69
	G3_R3	2.91	0.8 [0.50, 0.93] *	8.59
tHb (μM)	G1_R1	5.47	0.79 [0.40, 0.94]	8.98
	G1_R2	5.45	0.79 [0.38, 0.94]	9.74
	G1_R3	4.95	0.81 [0.43, 0.95]	9.29
	G2_R1	9.56	0.7 [0.27, 0.89]	6.93
	G2_R2	8.92	0.7 [0.33, 0.89]	5.78
	G2_R3	8.78	0.73 [0.38, 0.90]	6.62
	G3_R1	12.78	0.48 [−0.01, 0.79]	2.87
	G3_R2	10.98	0.55 [0.07, 0.82]	3.34
	G3_R3	11.30	0.53 [0.05, 0.81]	3.20

Legend: G1: group 1; G2: group 2; G3: group 3; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 9, 14, 14 for G1, G2 and G3, respectively. $p < 0.05$ in all conditions. * Moderate reliability.

Table 11. Between-session reliability of maximums.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI (%)	G1_R1	2.56	0.7 [0.22, 0.92]	6.44
	G1_R2	1.99	0.8 [0.41, 0.95]	9.09
	G1_R3	3.09	0.53 [−0.07, 0.85]	3.29
	G2_R1	2.51	0.78 [0.48, 0.92]	8.41
	G2_R2	2.14	0.76 [0.43, 0.91]	7.22
	G2_R3	1.98	0.82 [0.55, 0.93] *	10.08
	G3_R1	4.81	0.07 [−0.45, 0.55]	1.14
	G3_R2	4.96	0.05 [−0.46, 0.54]	1.11
	G3_R3	4.81	0.05 [−0.47, 0.54]	1.10
O ₂ Hb (μM)	G1_R1	11.17	0.55 [−0.05, 0.86]	3.45
	G1_R2	10.37	0.58 [0.02, 0.88]	3.90
	G1_R3	10.59	0.57 [−0.08, 0.87]	3.44
	G2_R1	4.58	0.84 [0.59, 0.94] *	11.48
	G2_R2	4.09	0.85 [0.63, 0.95] *	13.26
	G2_R3	4.22	0.84 [0.60, 0.94] *	11.28
	G3_R1	8.25	0.53 [0.07, 0.81]	3.37
	G3_R2	8.24	0.54 [0.08, 0.82]	3.43
	G3_R3	8.17	0.58 [0.12, 0.83]	3.72
HHb (μM)	G1_R1	6.36	0.36 [−0.38, 0.80]	2.01
	G1_R2	6.00	0.42 [−0.28, 0.82]	2.34
	G1_R3	6.76	0.26 [−0.50, 0.75]	1.62
	G2_R1	4.31	0.81 [0.46, 0.93]	12.05
	G2_R2	3.43	0.87 [0.67, 0.95] *	15.48
	G2_R3	3.73	0.86 [0.64, 0.95] *	14.69
	G3_R1	6.57	0.58 [0.14, 0.83]	3.86
	G3_R2	5.80	0.61 [0.19, 0.85]	4.34
	G3_R3	5.73	0.6 [0.17, 0.84]	4.23
tHb (μM)	G1_R1	16.76	0.45 [−0.22, 0.83]	2.57
	G1_R2	15.97	0.49 [−0.18, 0.85]	2.79
	G1_R3	17.30	0.43 [−0.29, 0.83]	2.37
	G2_R1	6.95	0.83 [0.58, 0.94] *	11.88
	G2_R2	6.37	0.85 [0.62, 0.95] *	12.38
	G2_R3	6.18	0.85 [0.63, 0.95] *	12.70
	G3_R1	13.59	0.4 [−0.11, 0.75]	2.31
	G3_R2	12.62	0.45 [−0.04, 0.77]	2.67
	G3_R3	12.52	0.47 [−0.03, 0.78]	2.73

Legend: G1: group 1; G2: group 2; G3: group 3; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 9, 14, 14 for G1, G2 and G3, respectively. $p < 0.05$ in all conditions. * Moderate reliability.

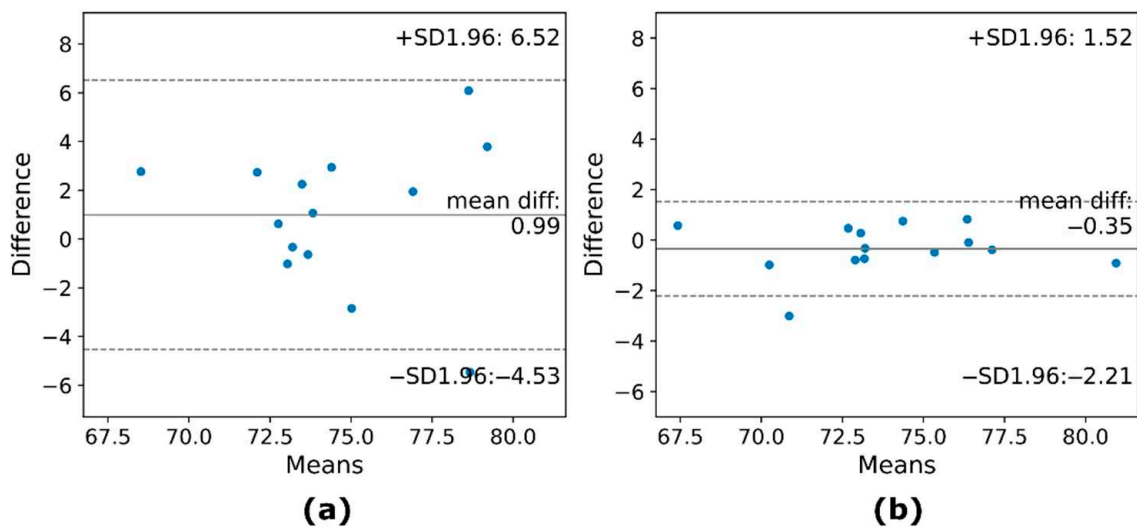


Figure 4. Examples of Bland–Altman plots of the reliability across trials for TSI_{max} of participants belonging to the group 2 (G2): (a) between sessions (bias = 0.99, 95% CI = -4.53 to 6.52) (b) and within sessions (bias = -0.35 , 95% CI = -2.21 to 1.52). The within-session trial comparison (b) shows a lower dispersion around the mean compared to between-session trial comparison (a).

3.2.4. Reoxygenation Rate

Between-session variability of the reoxygenation rate parameters is reported in Table 12. For both slope and V_{peak} , G1 showed CVs averaged across all variables of $41.60 \pm 51.77\%$ and $22.21 \pm 32.79\%$, respectively, which are higher than the CVs reported for G2 ($22.21 \pm 32.79\%$ and $13.07 \pm 9.62\%$, respectively) and G3 ($12.56 \pm 16.40\%$ and $8.67 \pm 4.71\%$, respectively).

Table 12. Between-session variability of reoxygenation rate.

Variable	Groupe	Mean_S1	SD_S1	Mean_S2	SD_S2	Mean of CV Btw. Sessions (%)	SD of CV Btw. Sessions (%)
TSI_{Slope} ($\% \cdot s^{-1}$)	G1	0.45	0.22	0.62	0.35	28.65	35.03
	G2	1.18	0.34	1.00	0.33	11.98	8.68
	G3	1.37	0.33	1.30	0.30	9.25	8.76
O_2Hb_{Slope} ($\mu M \cdot s^{-1}$)	G1	0.29	0.33	0.66	0.39	65.91	73.73
	G2	1.47	0.52	1.36	0.47	15.55	18.79
	G3	1.72	0.39	1.80	0.49	9.21	3.67
HHb_{Slope} ($\mu M \cdot s^{-1}$)	G1	0.37	0.30	0.70	0.37	22.64	13.54
	G2	1.11	0.89	1.47	0.66	13.67	6.42
	G3	1.77	0.47	1.82	0.91	10.04	8.43
tHb_{Slope} ($\mu M \cdot s^{-1}$)	G1	0.42	0.22	0.62	0.21	49.19	59.65
	G2	1.34	0.46	1.29	0.48	47.65	56.06
	G3	1.64	0.27	1.72	0.57	21.73	29.31
$TSI_{V_{peak}}$ ($\% \cdot s^{-1}$)	G1	0.95	0.28	1.18	0.38	16.40	9.67
	G2	2.25	0.62	2.27	0.68	12.33	10.76
	G3	2.79	0.42	2.91	0.80	10.20	6.51
$O_2Hb_{V_{peak}}$ ($\mu M \cdot s^{-1}$)	G1	3.17	2.13	2.51	1.98	17.47	18.02
	G2	2.74	0.94	2.54	0.86	12.13	10.81
	G3	2.87	0.87	3.04	0.82	6.76	2.94
$HHb_{V_{peak}}$ ($\mu M \cdot s^{-1}$)	G1	2.21	0.96	2.02	0.96	14.05	10.13
	G2	1.88	0.58	1.65	0.54	11.60	7.67
	G3	1.98	0.61	2.07	0.49	7.32	3.97
$tHb_{V_{peak}}$ ($\mu M \cdot s^{-1}$)	G1	5.31	3.08	4.47	2.89	15.07	15.56
	G2	2.41	0.84	2.40	0.74	16.23	9.26
	G3	2.42	0.71	2.51	0.52	10.40	3.95

Legend: TSI: tissue saturation index; O_2Hb : oxyhemoglobin; HHb : deoxyhemoglobin, tHb : total hemoglobin; CV: coefficient of variation; SD: standard deviation; btw: between. CV mean and CV SD between trials are calculated for the same participant and averaged per group.

The results of the reoxygenation rate analyses of reliability are reported in Tables 13 and 14. For TSI%, HHb and tHb, reliability could not elicit any reliable pairwise measurements of slope or Vpeak. For O₂Hb Vpeak, only three conditions showed moderate reliability.

Table 13. Between sessions reliability for the slope of reoxygenation rate.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI _{Slope} (%·s ⁻¹)	G1_R1	0.29	0.38 [−0.15, 0.80]	3.00
	G1_R2	0.17	0.34 [−0.17, 0.78]	2.60
	G1_R3	0.14	0.45 [−0.12, 0.83]	3.18
	G2_R1	0.22	0.75 [0.40, 0.91]	7.06
	G2_R2	0.22	0.79 [0.45, 0.93]	7.86
	G2_R3	0.28	0.65 [0.21, 0.87]	4.59
	G3_R1	0.35	0.43 [−0.10, 0.78]	2.55
	G3_R2	0.37	0.43 [−0.17, 0.79]	2.39
	G3_R3	0.26	0.62 [0.12, 0.87]	4.06
O ₂ Hb _{Slope} (μM·s ⁻¹)	G1_R1	0.33	0.42 [−0.13, 0.82]	4.42
	G1_R2	0.36	0.31 [−0.19, 0.76]	2.35
	G1_R3	0.33	0.23 [−0.19, 0.70]	2.04
	G2_R1	0.31	0.7 [0.28, 0.89]	5.38
	G2_R2	0.25	0.74 [0.37, 0.91]	7.36
	G2_R3	0.27	0.72 [0.35, 0.90]	6.61
	G3_R1	0.24	0.7 [0.27, 0.90]	5.53
	G3_R2	0.33	0.56 [0.06, 0.84]	3.57
	G3_R3	0.25	0.69 [0.24, 0.89]	5.14
HHb _{Slope} (μM·s ⁻¹)	G1_R1	0.20	0.6 [−0.01, 0.89]	5.56
	G1_R2	0.20	0.6 [0.00, 0.89]	5.23
	G1_R3	0.15	0.67 [0.11, 0.91]	5.94
	G2_R1	0.20	0.65 [0.02, 0.89]	8.09
	G2_R2	0.24	0.56 [0.09, 0.83]	4.35
	G2_R3	0.24	0.56 [0.10, 0.83]	3.78
	G3_R1	0.24	0.42 [−0.17, 0.78]	2.35
	G3_R2	0.21	0.63 [0.17, 0.87]	4.47
	G3_R3	0.19	0.74 [0.35, 0.91]	6.54
tHb _{Slope} (μM·s ⁻¹)	G1_R1	0.31	0.4 [−0.14, 0.81]	3.47
	G1_R2	0.34	0.3 [−0.20, 0.75]	2.26
	G1_R3	0.28	0.29 [−0.16, 0.74]	2.52
	G2_R1	0.45	0.73 [0.35, 0.90]	7.02
	G2_R2	0.59	0.37 [−0.09, 0.73]	2.50
	G2_R3	0.58	0.52 [0.06, 0.81]	3.54
	G3_R1	0.42	0.75 [0.37, 0.92]	6.80
	G3_R2	0.69	0.31 [−0.29, 0.73]	1.87
	G3_R3	0.50	0.42 [−0.16, 0.78]	2.39

Legend: G1: group 1; G2: group 2; G3: group 3; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 8, 13, 12 for G1, G2 and G3, respectively. $p < 0.05$ in all conditions.

Table 14. Between sessions reliability for Vpeak of reoxygenation rate.

Variable	Condition	SEM	ICC [CI-95%]	F
TSI _{Vpeak} (%·s ⁻¹)	G1_R1	0.31	0.4 [−0.18, 0.81]	2.62
	G1_R2	0.28	0.48 [−0.10, 0.84]	3.47
	G1_R3	0.23	0.43 [−0.13, 0.82]	3.43
	G2_R1	0.42	0.56 [0.04, 0.83]	3.35
	G2_R2	0.48	0.45 [−0.11, 0.79]	2.55
	G2_R3	0.50	0.45 [−0.12, 0.79]	2.52
	G3_R1	0.55	0.36 [−0.18, 0.74]	2.16
	G3_R2	0.53	0.46 [−0.13, 0.80]	2.57
	G3_R3	0.43	0.39 [−0.22, 0.77]	2.18

Table 14. Cont.

Variable	Condition	SEM	ICC [CI-95%]	F
O ₂ Hb _{Vpeak} ($\mu\text{M}\cdot\text{s}^{-1}$)	G1_R1	1.61	0.46 [−0.20, 0.85]	2.74
	G1_R2	1.11	0.67 [0.12, 0.91]	5.37
	G1_R3	1.26	0.59 [−0.06, 0.89]	3.74
	G2_R1	0.42	0.77 [0.44, 0.92]	7.61
	G2_R2	0.64	0.46 [−0.04, 0.79]	2.76
	G2_R3	0.57	0.66 [0.25, 0.88]	5.00
	G3_R1	0.28	0.9 [0.71, 0.97] *	20.76
	G3_R2	0.39	0.8 [0.45, 0.93]	10.36
	G3_R3	0.33	0.87 [0.62, 0.96] *	13.14
HHb _{Vpeak} ($\mu\text{M}\cdot\text{s}^{-1}$)	G1_R1	0.71	0.46 [−0.27, 0.85]	2.60
	G1_R2	0.50	0.69 [0.11, 0.92]	5.19
	G1_R3	0.63	0.56 [−0.13, 0.88]	3.38
	G2_R1	0.33	0.65 [0.19, 0.87]	5.89
	G2_R2	0.30	0.74 [0.38, 0.91]	7.24
	G2_R3	0.33	0.68 [0.26, 0.89]	6.42
	G3_R1	0.32	0.74 [0.34, 0.91]	6.31
	G3_R2	0.27	0.77 [0.43, 0.92]	8.36
	G3_R3	0.22	0.81 [0.49, 0.94]	9.06
tHb _{Vpeak} ($\mu\text{M}\cdot\text{s}^{-1}$)	G1_R1	2.31	0.46 [−0.23, 0.84]	2.64
	G1_R2	1.58	0.68 [0.14, 0.92]	5.42
	G1_R3	1.86	0.59 [−0.07, 0.89]	3.70
	G2_R1	0.71	0.31 [−0.26, 0.72]	1.87
	G2_R2	0.71	0.14 [−0.44, 0.62]	1.31
	G2_R3	0.55	0.7 [0.28, 0.89]	5.37
	G3_R1	0.32	0.75 [0.35, 0.92]	6.49
	G3_R2	0.50	0.57 [0.10, 0.84]	4.11
	G3_R3	0.35	0.78 [0.42, 0.93]	7.63

Legend: G1: group 1; G2: group 2; G3: group 3; R1: first reperfusion; R2: second reperfusion; R3: third reperfusion. Standard error of measurement (SEM) is expressed in arbitrary units (u.a.). Intraclass correlation coefficient (ICC) type: (2, 1); df: 8, 13, 12 for G1, G2 and G3, respectively. $p < 0.001$ in all conditions. * Moderate reliability.

4. Discussion

The present study aims to assess the reliability of NIRS measurements of maximum, minimum and reoxygenation rate values following three occlusions. The main finding of this study is that NIRS assessment of maximums and minimums was highly reliable across trials of a single session but was not reliable on two separate occasions. Moreover, if the 95% CI of the ICC is reported, the slope of reoxygenation and V_{peak} cannot be considered reliable parameters both within and between sessions.

4.1. Maximums and Minimums Reliability

Between sessions, our results are in agreement with the findings of Lacroix et al. (2012) for O₂Hb and tHb maximums [35]. Their study focused on forearm oxygenation response to occlusion. However, they had a unique 5 min occlusion at 100 mmHg over the SBP (mean: 219 ± 7 mmHg), close to our G3 pressure (250 mmHg). Lacroix et al. (2012), found an ICC of 0.63 and 0.31, whereas we reported an ICC of 0.53 and 0.40 for O₂Hb and tHb maximums, respectively (Table 11). Unfortunately, 95% CI of ICC was not reported and the O₂Hb maximum was considered as good, while our result reported with CIs is considered as not reliable (ICC = 0.53 [0.07, 0.81]). Additionally, the authors also reported similar between-session CVs for O₂Hb (6.68%) compared to 5.10% in the current study.

Our findings indicate lower results for minimums and for maximums of TSI in G3 ($25.57 \pm 9.96\%$ and $73.48 \pm 4.89\%$, respectively), compared to de Oliveira et al. (2021), who found a TSI of $44.00 \pm 10.39\%$ and $79.98 \pm 5.11\%$ for the minimum and maximum, respectively. Indeed, the authors assessed the StO₂ on a similar population of young healthy adults, utilizing the same equipment (PortaMon, Artinis, Elst, Netherlands) and occlusion pressure (250 mmHg) but on a different forearm muscle (flexor carpi radialis)

and with a 5 min occlusion period. These differences, particularly for TSI_{\min} responses, could be attributed to the 2 min occlusion difference between studies since the duration of occlusion induces differences in microcirculatory responses [36] and significant differences in maximums and minimums of $TSI\%$ [27]. It may also result from the spatial heterogeneity of tissue responses, which makes comparison between different muscles difficult [1,37]. Moreover, differences between findings from this study for minimums recorded at brachioradialis and those at the lower limb after 5 min of occlusion at 250 mmHg ($46.2 \pm 7.5\%$) [24] could be further explained by limb-specific variation in arterial function [38].

To our knowledge, our study is the first to investigate the intra-day and day-to-day pairwise reliability of NIRS maximums and minimums after three arterial occlusions. Since the minimum and maximum values directly influence the calculation of other parameters, such as the slope of reoxygenation or the amplitude [39,40] or the maximal physiological range [23], and have an effect on the results of index calculation, such as the area under the curve (AUC), it is essential to know the reliability of those parameters. Thus, these should be ascertained both between successive measurements within a single session and also between separate occasions, such as pre- and post-intervention. Our findings highlighted the highly acceptable reliability after arterial occlusions of maximums and minimums for intra-day measurements but suggest caution when comparing values between sessions.

4.2. Reliability of Reoxygenation Rates

The reoxygenation rate, or reperfusion rate, has been widely investigated, and our findings are consistent with the literature [24,27,41]. In their study, Soares et al. (2019) showed that after 5 min occlusion at 250 mmHg at the arm, the NIRS reperfusion slope calculated for the forearm (flexor digitorum superficialis muscle) showed a value of $1.77 \pm 0.60\% \cdot s^{-1}$ for $TSI\%$ [41], which is slightly higher than our findings for G3 ($1.37 \pm 0.33\% \cdot s^{-1}$). On the flexor carpi radialis muscle, a reoxygenation rate of $1.24 \pm 0.41\% \cdot s^{-1}$ was found after 5 min occlusion at 250 mmHg [42], and, interestingly, results on leg measurements match our findings on forearm measurements, with 1.32 ± 0.38 [24] and $1.60 \pm 0.80\% \cdot s^{-1}$ [27] for tibialis anterior.

When compared to results found in trained participants ($2.83 \pm 0.48\% \cdot s^{-1}$) [40], our results seem to be much lower and inconsistent with previous findings, despite similar population demographics and characteristics. However, in our study, walking was considered a physical activity, contrary to the work of McLay et al. (2016). Thus, it is noteworthy that our population elicits results closer to a reperfusion slope for an untrained group ($1.26 \pm 0.28\% \cdot s^{-1}$) than for a trained group [40].

The reliability of the reperfusion slope of NIRS-derived parameters has not been widely investigated in the literature. McLay et al. (2016) reported an excellent intraday ICC (0.92) with a CV of $9 \pm 4\%$ [24]. We reported ICC values of 0.91, 0.82 and 0.92 for pairwise measurements between the three occlusions in session 2. However, we reported a higher CV of $27.59 \pm 13.01\%$. Interestingly, between-session slope measurements have been reported to be highly reliable with ICCs of 0.88 [27] and 0.94 [24] between sessions. Those findings are high when compared to our results of 0.43, 0.43 and 0.62. Moreover, we are unable to ratify a level of good reliability between sessions for our measurements, since the 95% CI reported all elicit a lower value under the threshold of 0.50. In this way, we proffer cautiousness with a comparison of reperfusion slope measurements, particularly between sessions.

The higher variability found for intra-day reoxygenation rate measurement may be explained by the extended durations of the successive occlusion and reperfusion phases, leading to a 76 min long protocol. This duration and a resting state may induce changes in skin temperature or in blood pressure levels [43], which could affect the results.

Our findings showed a dissonance with results in the literature regarding inter-day reliability. This dissonance may be due to room temperature changes between two sessions for the same participant. Even if no significant difference was found between S1 and S2, the temperature may vary between both sessions, inducing changes in both blood pressure

level and muscle oxygenation kinetics [44,45]. Another explanation may result from the blood volume changes during successive occlusions and across each session [46]. Indeed, during ischemia, the muscle blood flow increases, which affects the quantity of oxy- and deoxyhemoglobin measured with the NIRS device. Thus, these blood volume changes may bring confusion in slope measurements, and some researchers suggest correcting the NIRS signal with blood volume changes [12]. However, deoxyhemoglobin is supposed to be less sensitive to blood volume changes [47], but our findings showed that HHb was not more reliable between sessions than other parameters.

This discrepancy between our findings and that of the literature is emphasized by the way in which ICCs are reported in reliability studies focusing on NIRS-derived parameters. Following recent guidelines on selecting and reporting ICCs [25], we indicated reliability according to the lower bound value of 95% CI. However, the level of reliability (“poor”, “moderate”, “good” and “excellent”) seems to differ among guidelines [48]. In the present study, 50% of within-session and no between-session measurements of reperfusion slope for TSI% are considered reliable according to the guidelines [25]. However, if ICC was reported without the 95% CI, 89% of the within-session and 22% of the between-session measurements would have been reported as reliable. This huge difference, particularly for within-session measurements, highlights the significance of reporting the 95% CI in reliability studies.

4.3. The Reliability When Assessing Tissue Oxygenation

NIRS technology is also used to investigate cerebral oxygenation (fNIRS). In healthy participants, fNIRS recordings of changes in cerebral oxygenation indicate good reliability (ICC = 0.83) [49]. Initial drop amplitude recorded during an orthostatic test showed good reliability for O₂Hb (ICC = 0.83) and for TSI% (ICC = 0.99) [50]. Once again, confidence intervals are not reported in these studies. Moreover, a good to excellent test–retest reliability is found for O₂Hb and HHb in response to postural changes [51].

The inter-day reliability of cutaneous microvascular perfusion responses assessed using integrating-probe laser Doppler flowmetry showed acceptable reliability (ICC = 0.60; CV = 13%) PORH after 5 min occlusion [52]. When assessed with laser speckle contrast analysis (LSCI), PORH indicated a good inter-day reliability (ICC = 0.76; CV = 8%) [53].

4.4. Limitations

The main limitation of this study may be the inability to control the room temperature, as microcirculation is sensitive to skin temperature [54]. However, no significant differences between groups or sessions have been found.

5. Conclusions

In conclusion, the present study demonstrated that maximum and minimum values reached after 3 periods of 7 min occlusions are highly reliable within a single session, thus it may not be necessary to perform multiple occlusions to obtain reliable values of maximums and minimums. However, NIRS-derived parameters such as the slope of reperfusion or V_{peak} during reperfusion cannot show reliable reliability when ICC is reported with 95% CI. It is thus important to be cautious when comparing values between sessions, particularly when comparing pre- and post-intervention values.

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STUDY 2:

Effects of occlusion pressure on haemodynamic responses recorded by Portamon near-infrared spectroscopy device across two visits

PUBLISHED ARTICLE

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Effects of occlusion pressure on haemodynamic responses recorded by Portamon near-infrared spectroscopy device across two visits.

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Introduction: IPC has been shown to be beneficial for medical purposes and has demonstrated beneficial effects on performance enhancement. However, prolonged exposure to an occlusion of the blood flow may induce deleterious adverse effects^{632,633}. These adverse effects arise from tissue compression beneath the inflated cuff or from tissue ischaemia⁶³³. The review of O'Brien & Jacobs (2021) shows considerable heterogeneity in the pressure levels used to induce ischaemia⁴¹. Two primary methods for determining cuff pressure are observed in the literature: absolute or individualised based on SBP⁴¹. Absolute pressures typically range from 180 mmHg to 250 mmHg (180, 185, 200, 220, 225, 240, 250 and >300 mmHg)⁴¹. Conversely, individualised pressures range from SBP value and SBP + 50 mmHg (SBP, 120 % of SBP, and SBP + 10, 15, 20, 30, 40, or 50 mmHg)⁴¹. However, using an absolute pressure may induce heterogeneous haemodynamic changes between

participants due to individual characteristics (such as SBP) ²⁷³. In addition, recent studies have suggested that SBP + 50 mmHg should be preferred when using individualised pressure to reach complete blood flow occlusion ^{489,634}. Consequently, selecting absolute or individualised pressure for blood flow occlusion may lead to variations in haemodynamic disturbances. NIRS is a viable method for assessing peripheral haemodynamic responses both at rest and during exercise and quantifying the magnitude of deoxygenation induced by either absolute (250 mmHg) or individualised pressures (SBP + 50 mmHg) ²⁶¹. Thus, this study aimed to evaluate peripheral haemodynamic responses assessed by NIRS during partial or complete, absolute or individualised blood flow occlusions.

Methods: 46 young males (mean \pm SD: 23 \pm 3 years, 75 \pm 12 kg, 178 \pm 7 cm, 13.8 \pm 4.1 % body fat, 7.8 \pm 5.7 h of physical activity per week) volunteered to participate and randomly attributed to an experimental group (G1, G2, or G3). Depending on the experimental group, a cuff was inflated three times at the left upper arm to 50 mmHg (G1), SBP + 50 mmHg (G2) or 250 mmHg (G3). Participants visited the laboratory twice, within a 24 h to 72 h period. During the first visit, anthropometric measurements were recorded (stature, body mass, and adipose tissue thickness), while limb lengths were measured to ascertain NIRS device placement. In both sessions, the same protocol consisted of a baseline period followed by 3 x 7 min occlusion phases interspersed with three reperfusion phases (10 min) where the cuff was deflated. Maximums, minimums, and reoxygenation rate were assessed after each occlusion phase, using a Portamon device placed on left brachioradialis. Area Under the Curve (AUC) was calculated for each occlusion using absolute values (TSI_{AUC} , $[O_2Hb]_{AUC}$, $[HHb]_{AUC}$, ΔHb_{AUC}), in order to assess the magnitude of oxygenation variations during deoxygenation and reoxygenation ^{627,635,636}. Mixed analysis of variance (mixed ANOVA) with Bonferroni post hoc correction were used to determine the difference in tissue deoxygenation and reperfusion between groups.

Results: Results showed a significantly lower magnitude of deoxygenation (TSI_{AUC}) for G1 compared to G2 ($P < .001$) and G3 ($P < .001$), without differences between G2 and G3. The deoxygenation was faster for G2 and G3 compared to G1 ($P < 0.001$), but no differences were shown between G2 and G3. However, G3 showed a significantly faster reoxygenation only for tissue saturation index (TSI_{slope}) compared to G2 ($P = 0.010$), but without differences in the speed of recovery of deoxyhaemoglobin ($[HHb]_{slope}$), or in the magnitude of PORH. Besides TSI reoxygenation speed, G2 and G3 elicit comparable resting haemodynamic responses measured by NIRS.

Conclusion: Data showed greater, faster deoxygenation induced by suprasystolic pressures compared to G1 but cannot show differences between both groups with occlusion pressures over the SBP (G2 and G3). The reoxygenation was faster for suprasystolic pressures compared to G1, with findings indicating that greater pressure (250 mmHg vs. SBP+50 mmHg) used to occlude blood flow may induce a faster reoxygenation response in the 30s window following cuff deflation. However, this difference between G2 and G3 was not found in the TSI_{AUC} indicating that both suprasystolic pressure induce comparable deoxygenation. Therefore, most of the findings do not show significant differences between G2 and G3, questioning the usefulness of relying on higher absolute pressure. It is then recommended that individualised pressure based on SBP should be used for inducing ischaemia during IPC.



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Effects of occlusion pressure on hemodynamic responses recorded by near-infrared spectroscopy across two visits

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Ischemic Preconditioning (IPC) has emerged as a promising approach to mitigate the impact of hypoxia on physiological functions. However, the heterogeneity of occlusion pressures for inducing arterial occlusion has led to inconsistent hemodynamic outcomes across studies. This study aims to evaluate the peripheral hemodynamic responses to partial and total blood-flow occlusions on the left arm at rest, using absolute or individualized pressures, on two occasions. Thirty-five young males volunteered to participate in this study. IPC procedure (3 × 7-min) was performed on the left upper arm with cuff pressures at 50 mmHg (G1), 50 mmHg over the systolic blood pressure (SBP + 50 mmHg) (G2) or 250 mmHg (G3). NIRS-derived parameters were assessed for each occlusion and reperfusion phase in the brachioradialis. Results showed a significantly lower magnitude of deoxygenation (TSIAUC) for G1 compared to G2 (-1959.2 ± 1417.4 vs. -10908.1 ± 1607.5 , $P < 0.001$) and G3 (-1959.2 ± 1417.4 vs. -11079.3 ± 1828.1 , $P < 0.001$), without differences between G2 and G3. However, G3 showed a significantly faster reoxygenation only for tissue saturation index (TSI_{slope}) compared to G2 (1.3 ± 0.1 vs. 1.0 ± 0.2 , $P = 0.010$), but without differences in the speed of recovery of deoxyhemoglobin [(HHb) slope], or in the magnitude of post-occlusive hyperemia (PORH). Besides TSI reoxygenation speed, G2 and G3 elicit comparable resting hemodynamic responses measured by NIRS. Thus, this study highlights the practicality and effectiveness of using relative occlusion pressures based on systolic blood pressure (SBP) rather than relying on excessively high absolute pressures.

KEYWORDS

remote ischemic preconditioning, arterial occlusion, muscle oxygenation, blood flow restriction, muscle ischemia, NIRS

1 Introduction

Ischemic Preconditioning (IPC) is a clinical procedure that protects organs against ischemia-reperfusion injury by inducing transient cycles of arterial blood flow occlusion and reperfusion (Kloner, 2009; O'Brien and Jacobs, 2021). Originally used to prevent irreversible injuries of coronary artery occlusion (Murry et al., 1986); IPC is one of the most powerful and reproducible phenomena in cardioprotection (Kloner, 2009). However, the original invasive procedure consists of inflating and deflating a balloon placed in the artery or clamping and unclamping the artery, with the possibility of further complications, such

as atherosclerotic emboli in the coronaries (Kloner, 2009). To address these risks, the effects of repeated remote, non-invasive brief ischemia's have been investigated previously to overcome the invasive procedural shortcomings. In this approach, a pressure cuff is applied to the upper or lower limb and inflated above systolic blood pressure (SBP) to occlude the remote vascular bed (Przyklenk et al., 1993). This method avoids the complications of invasive IPC procedures while being equally effective (Kloner, 2009; Przyklenk et al., 1993). Safe and cost-effective, IPC has been transposed to sport sciences to prevent the deleterious effects of hypoxia on performance (Marocolo et al., 2016). Effects of IPC on neuromuscular, endothelial and oxidative functions, including enhancing angiogenesis (Gu et al., 2021; Michiels, 2003; Rajendran et al., 2013) and cutaneous microcirculation (Gerovasili et al., 2009; Gomez et al., 2008; Kolbenschlag et al., 2017; Kraemer et al., 2011; Rosenberry and Nelson, 2020; Sogorski et al., 2021), have been widely documented (Paradis-Deschênes, 2021). Although IPC's effectiveness in clinical guidelines has been well established, the significant effects of IPC on performance are still debated (Marocolo et al., 2016), particularly regarding assessment methods (Marocolo et al., 2019; Paradis-Deschênes, 2021).

Mechanisms of IPC currently need to be better understood. The literature suggests that the beneficial effects of IPC operate through neuronal pathways, humoral substances released into the systemic circulation or peripheral hemodynamic responses (Kloner, 2009; O'Brien and Jacobs, 2021; Paradis-Deschênes et al., 2016). Between 3 and 5 cycles of 5–10 min are recommended to induce positive effects of IPC on performance and avoid deleterious adverse effects (Johnsen et al., 2016; Kolbenschlag et al., 2017; Sogorski et al., 2021), such as skin or muscle injuries, or “hyper-conditioning” (Kam et al., 2001; Whittaker and Przyklenk, 2014). Thus, inflating cuffs to the required pressure is essential to preserve participant integrity. In the literature, two different methods of determining cuff pressure are used: absolute or individualized based on systolic blood pressure (SBP) (O'Brien and Jacobs, 2021). For absolute pressures, the same pressure is applied for each participant implying that the selected pressure is high enough to occlude the blood flow for every participant. Absolute pressures often range between 180 and 250 mmHg (O'Brien and Jacobs, 2021), for the upper limb, which represents between 130% and 210% of the SBP for a typical 120 mmHg SBP (Hardy et al., 2021). Alternatively, upper limb arterial occlusion pressure could be based on SBP, when using the same cuffs for BP estimation and IPC intervention (Loenneke et al., 2015). Individualized pressures used for upper limb arterial occlusion range between 15 and 50 mmHg over the SBP, to induce arterial occlusion (O'Brien and Jacobs, 2021). However, inflating the cuff only up to 15 mmHg over the SBP (SBP + 15 mmHg) value may be insufficient to achieve the occlusion of certain deep arterioles (Reid et al., 1983; Sharma et al., 2014). Due to factors such as limb positioning and non-uniform cuff inflation, it is necessary to inflate the cuff to a minimum pressure of 50 mmHg over the SBP (SBP + 50 mmHg) to reach complete blood flow occlusion (Reid et al., 1983; Sharma et al., 2014). The use of individualized pressures for IPC appears to be safer for the participant by reducing the pressure used in a normotensive population (Reid et al., 1983), but may also allow complete occlusion for participants with high SBP. The same approach of choosing individualized pressures based on SBP is also recommended in Blood Flow Restriction (BFR) training

(Loenneke et al., 2015; McEwen et al., 2019). The recommended pressure for BFR training ranges from 40% to 80% of AOP (Patterson et al., 2019), with 40% of AOP showing similar effects on muscle deoxygenation and activation as 80% of AOP (Gray et al., 2023). Thus, selecting an absolute or an individualized pressure to occlude the blood flow, may induce variations in the magnitude of hemodynamic disturbances. Both at rest and during exercise, near-infrared spectroscopy (NIRS) could be used to assess peripheral hemodynamic responses (Van Beekvelt, 2002). NIRS provides non-invasive estimations of haemoglobin concentration changes, giving information on muscular tissue oxygenation (Ferrari et al., 2011; Perrey and Ferrari, 2018). The reperfusion rate after ischemia allows differentiation between trained and untrained (Perrey and Ferrari, 2018) as well as healthy and diseased populations (Hamaoka et al., 2007). Additionally, sex-related differences emerge when desaturation levels are matched, with men exhibiting greater PORH than women, potentially due to phenotypic characteristics (Keller et al., 2023). These sex-based differences in muscle oxygenation responses, as measured by NIRS, could highlight variations across different groups (Keller et al., 2023). During exercise, muscular deoxygenation and oxygen extraction after the IPC procedure have been investigated (Patterson et al., 2015; Tanaka et al., 2016). NIRS technology was found to be reliable for intra-session measurements, but cautiousness is needed when comparing measurements between sessions at rest (Desanlis et al., 2022).

Heterogeneous results regarding the ergogenic aid of IPC have been reported in the literature, attributed to a lack of standardization in IPC protocols, especially concerning occlusion pressure (O'Brien and Jacobs, 2021). IPC has been found effective in enhancing athletic performance, $\dot{V}O_2 \max$, and increasing blood flow and muscle oxygenation during exercise in some studies, but did not yield significant changes in other studies (Marocolo et al., 2016; Marocolo et al., 2019). It may then be of interest to further explain any peripheral dynamic responses assessed by NIRS. When the cuff is inflated and occlude the blood flow totally (arterial occlusion), the O_2 is consumed leading to decrease in O_2 Hb and TSI and a concomitant increase in HHb (Van Beekvelt, 2002). After a few minutes of occlusion (≈ 3 –5 min), the NIRS signals plateau, showing the maximal deoxygenation reached (Barstow, 2019; Ferrari et al., 1992; Grassi and Quaresima, 2016). This minimum of TSI is not to confound with a true zero (TSI = 0%), that would require much time and/or the addition of exercise (Barstow, 2019). Upon deflation, the muscle tissue is rapidly reperfused allowing for calculation of reoxygenation slope (Rosenberry and Nelson, 2020; Van Beekvelt, 2002). The reoxygenation reaches a maximum of TSI that exceeds the baseline value and could be quantitatively calculated using the area under the curve (AUC) method (Agbangla et al., 2017; Barstow, 2019). However, muscle microcirculation is affected not only by arterial occlusion but also by partial blood flow occlusion at rest, which significantly reduces muscle oxygenation (Cherouveim et al., 2021). Thus, this study aimed to evaluate the peripheral hemodynamic responses assessed by NIRS to partial or complete, absolute or individualized blood-flow occlusions on the left arm at rest. We hypothesized that complete arterial occlusion would induce greater deoxygenation and reoxygenation during and after the occlusion but without significant differences between absolute or individualized arterial

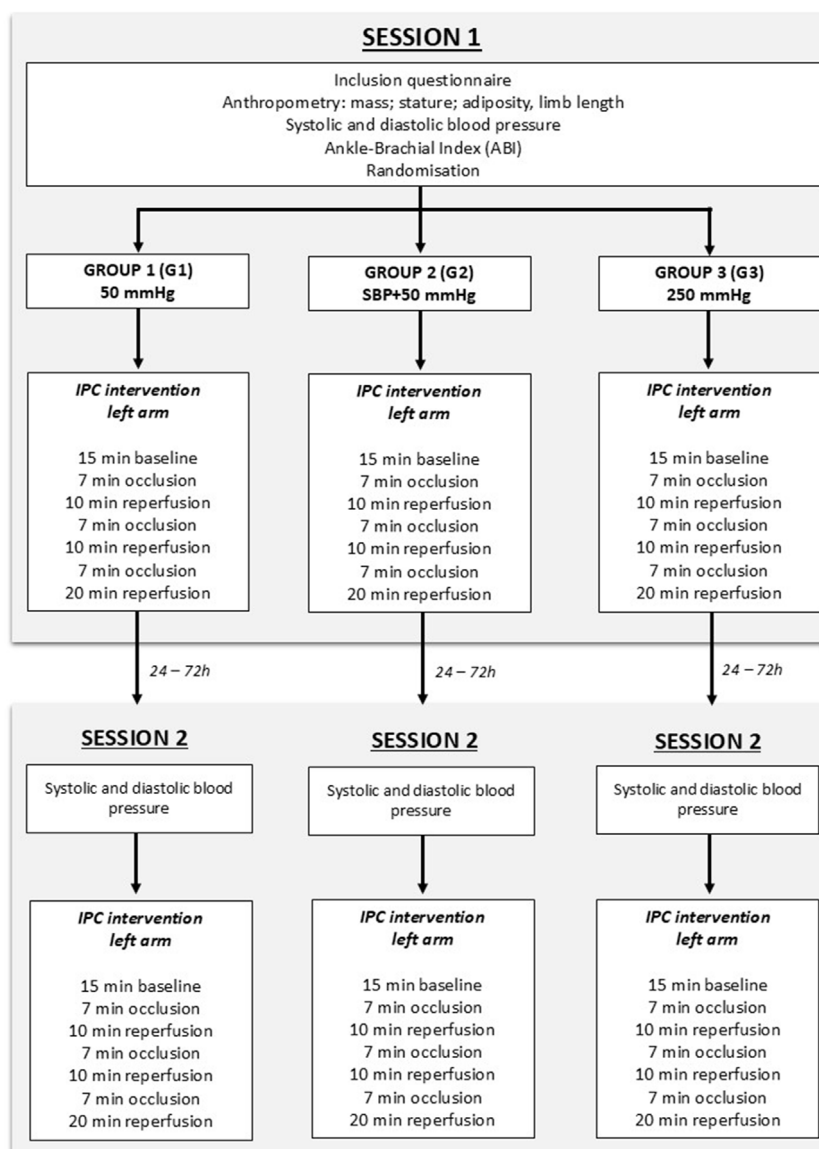


FIGURE 1

Flowchart of the study design. During the protocol, the participant was supine on a medical couch, with the arm at the level of the heart. The baseline consisted of 15 min of rest, without any occlusion, to establish resting values.

occlusion pressure since both pressures are high enough to induce complete arterial occlusion.

2 Methods

2.1 Participants

This study was supported by local institutional ethical approval (CER-Paris-Saclay-2020-006), and written informed consent was provided by all participants. Forty-six young males volunteered to participate. Only males were asked to participate in order to limit experimental bias linked to a sex-related effect in microvascular responses (Chambliss and Shaul, 2002; Kao and Sun, 2015). Fluctuations in circulating oestrogen

levels throughout the menstrual cycle impact both vasodilation and blood pressure (Dubey et al., 2002; Mendelsohn and Karas, 2005; Reckelhoff, 2001). Due to the necessity of ensuring consistency between sessions and the inability to evaluate oestrogen levels in our participants, females were excluded from this study.

Participants who reported diabetes, hypertension, previous cardiovascular events, and any unordinary vigorous physical activity 24 h before the session (e.g., competition), were excluded from the study. Participants were asked to avoid alcohol intake 24 h before the sessions and caffeine or food intake 2 h before any session. Participants made two separate visits to the laboratory (Figure 1), within 24–72 h. All participants were scheduled for their visits between 9:00 a.m. and 18:30 p.m., ensuring consistency in the timing of both visits for each participant.

TABLE 1 Description of environmental and participants' characteristics.

Group	G1 (50 mmHg)	G2 (SBP + 50 mmHg)	G3 (250 mmHg)	Total
n	9	14	12	35
Age (years)	22 ± 4	22 ± 3	22 ± 4	22 ± 3
Stature (cm)	177 ± 6	176 ± 7	183 ± 8	178 ± 7
Mass (kg)	77 ± 15	73 ± 13	76 ± 7	75 ± 12
Body fat (%)	14.5 ± 5.4	16.6 ± 5.5	12.5 ± 2.8	15 ± 5
Physical activity (h/wk)	5 ± 2	9 ± 7	10 ± 8	8 ± 6.4
Aerobic (%)	75.0	57.1	41.7	55.9
Mixed (%)	12.5	28.6	41.7	29.4
Resistance (%)	12.5	14.3	16.7	14.7
SBP (mmHg)	115 ± 7	116 ± 10	122 ± 9	118 ± 9
DBP (mmHg)	66 ± 7	71 ± 6	68 ± 7	69 ± 7
ABI	1.04 ± 0.08	1.03 ± 0.07	1.03 ± 0.06	1.03 ± 0.07
Temperature (°C)	21.0 ± 1.3	21.8 ± 1.4	20.8 ± 1.7	21.2 ± 1.5
Humidity (%)	38 ± 14	45 ± 13	42 ± 15	43 ± 14
Occlusion pressure	50	166 ± 10	250	

Values are reported as mean ± SD; SBP, systolic blood pressure; DBP, diastolic blood pressure; ABI, ankle to brachial index; G1, group 1; G2, group 2; G3, group 3. Physical activity type (aerobic, resistance, mixed) is reported as a frequency table based on the classification of Howley (2001). The term mixed is used when the participant practices both aerobic- and resistance-based activities.

Participants were asked by the investigator to provide the type and duration of weekly physical activity, including walking as defined by the World Health Organization (WHO, 2024). Physical activity type (aerobic, resistance, mixed) is reported in Table 1 as a frequency table based on the classification of Howley (2001). The term “mixed” refers to participants who engage in both aerobic and resistance activities. No physical fitness examination was conducted.

2.2 Study design

During the first session (“S1”), anthropometric measurements (stature, body mass, adiposity, and limb length) and blood pressure were assessed. Then, participants were randomly assigned to one of three groups with different occlusion pressures applied to the left arm: 50 mmHg (G1) (to occlude venous return (Wilkinson and Webb, 2001)), 50 mmHg over the systolic blood pressure (SBP + 50 mmHg, (G2)) and 250 mmHg (G3) (O’Brien and Jacobs, 2021; Sogorski et al., 2021). The protocol consisted of a 15-min baseline period, followed by 3 min × 7 min occlusion phases interspersed with three reperfusion phases of 10, 10 and 20 min where the cuff was deflated (Desanlis et al., 2022), as depicted in Figure 1. Occlusions were induced using a manual sphygmomanometer (Easy 3, Holtex +, Aix-en-Provence, France) and manually adjusted to maintain the selected pressure during the allotted 7-min. Inflation and deflation durations are approximately 5–8 s. During the whole protocol, the participant was supine on a medical couch, with the arm in a horizontal position at the same level than the heart, to prevent limb position effects on blood flow (Jasperse et al., 2015; Krishnan et al., 2011).

During the second session (S2), participants received the same intervention as in S1, at the same time of day and with the same occlusion pressure, to ensure consistency between sessions. Humidity and temperature were assessed at the beginning of each session by a digital and wireless weather station (Mtraining, France).

2.3 Measurements

2.3.1 Skinfolts

Triplicate skinfold thickness measurements were attained from four sites (nondominant subscapular, bicipital, tricipital, and suprailiac) by a single researcher responsible for all skinfolds assessment using Harpenden Skinfold Caliper (Baty International, Wantage, United Kingdom), according to ISAK standards. Skinfold thickness was calculated as the sum of the average values of the four sites. Fat percentage was determined with the correlation table provided by the manufacturer.

2.3.2 Ankle-brachial index (ABI)

ABI was assessed in the supine position by a trained investigator in all participants to verify their peripheral cardiovascular health status (Clark et al., 2011; Sacks et al., 2003). ABI was calculated according to the recommendations of Aboyans et al. (2012) (Equation 1). SBP was assessed with a manual sphygmomanometer (Easy 3, Holtex +, Aix-en-Provence, France) and a manual stethoscope (Classic III, 3M Littman Stethoscopes, Maplewood, United States) for the first measurement of the right brachial artery and with a mini-doppler (Sonotrax Lite, Edan Instruments Inc., Shenzhen, China) for subsequent measures.

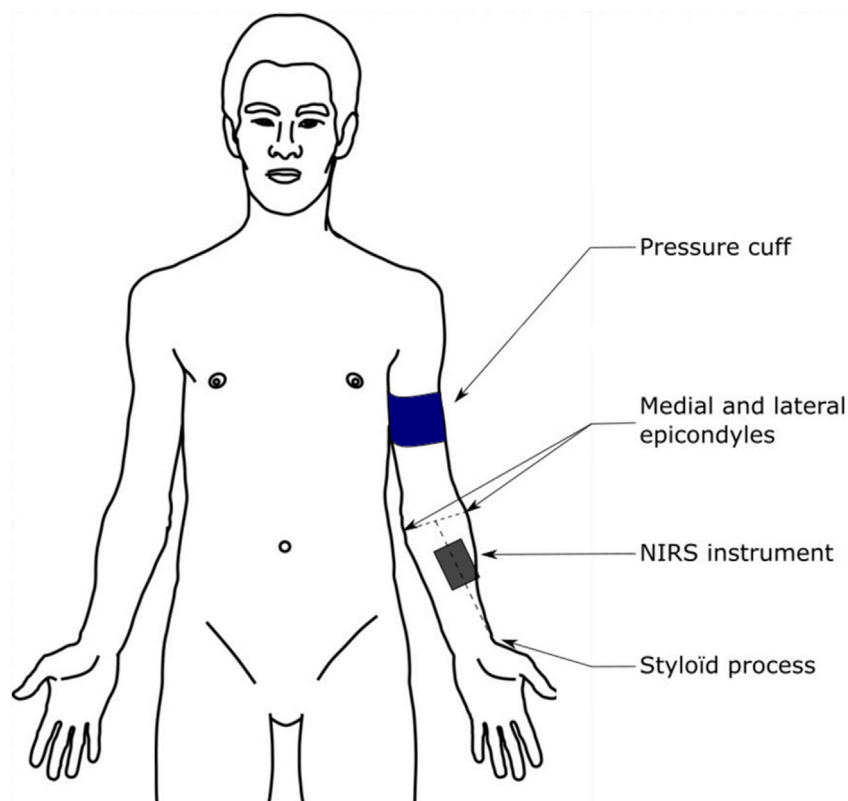


FIGURE 2
Illustration of the NIRS device placement. Reproduced with permission from Desantis et al. (2022).

$$ABI = \frac{\text{Highest ankle SBP (mmHg)}}{\text{Highest brachial SBP (mmHg)}} \quad (1)$$

2.3.3 Near-infrared spectroscopy (NIRS)

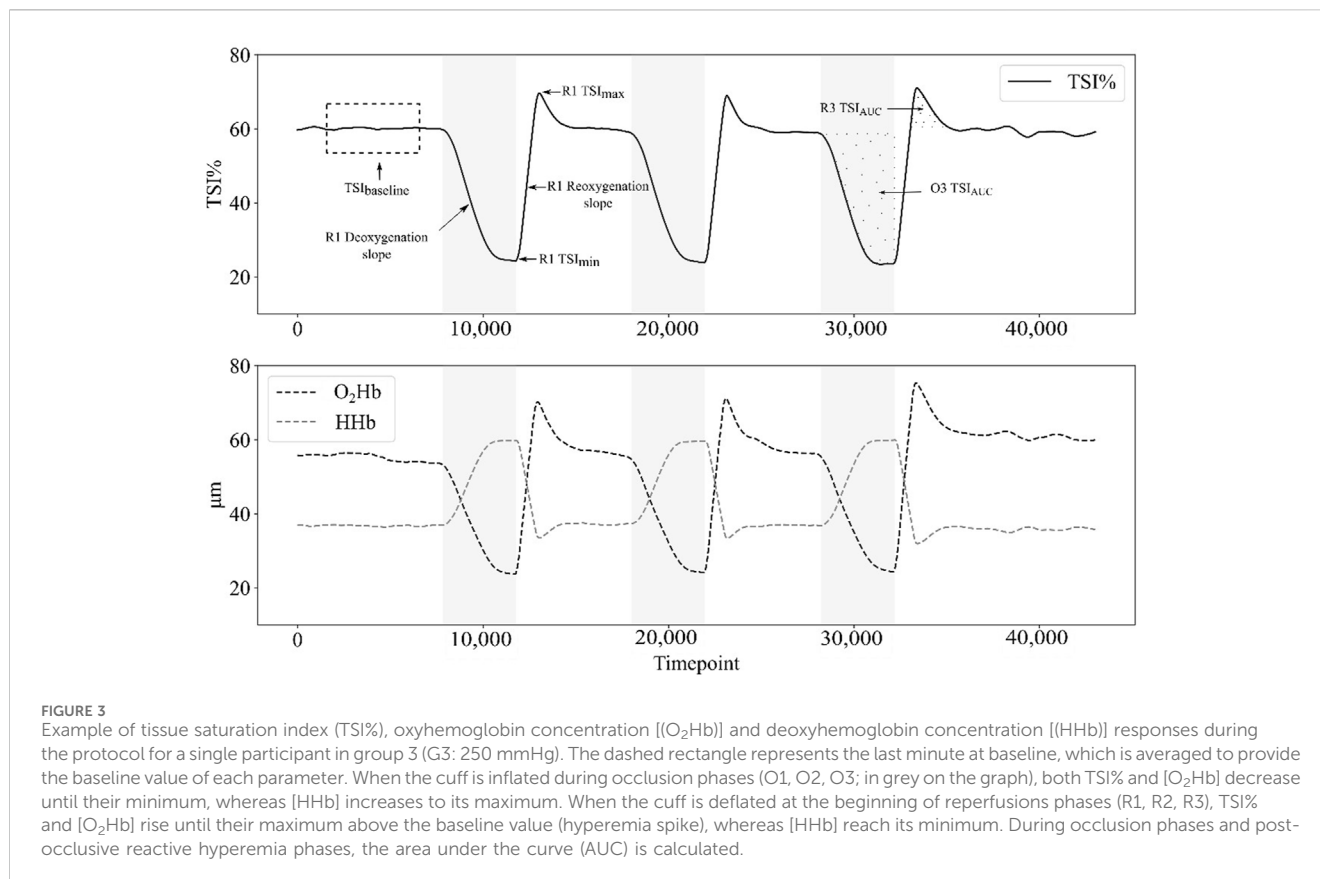
A wireless NIRS device (PortaMon, Artinis, Elst, Netherlands), connected with Bluetooth with a sampling rate of 10 Hz, was located on the left arm. This is dual-wavelength device (760 and 850 nm), with three pairs of LEDs spaced 30, 35, and 40 mm from the receiver, continuous-wave NIRS system using the modified Lambert-Beer law. It calculates the concentration changes of tissue oxy-, deoxy- and total haemoglobin (O_2Hb , HHb , tHb , respectively). The tissue saturation index (TSI), expressed in percentage (%) and reflecting the dynamic balance between dioxygen (O_2) supply and consumption, is calculated as stated in Equation 2:

$$TSI = [O_2Hb] / ([O_2Hb] + [HHb]) \times 100 \quad (2)$$

The NIRS device was placed on the brachioradialis muscle, at two-thirds on the line from the styloid process to the central point between the lateral and medial epicondyles (Figure 2), to have consistent device placement for all participants (Barbero et al., 2012). The device was adhered to the limb with double-sided auto-adhesive tape (Cohesion, 3M, Cergy-Pontoise, France) and wrapped in black cloth and an elastic bandage to prevent any disturbance due to light interference or unintentional movement.

2.4 Data analysis

NIRS data was smoothed using a 10th-order low-pass zero-phase Butterworth filter (cut-off frequency 0.8 Hz) using Pandas software library functions for Python (Python 3.8.8, Python Software Foundation, <https://www.python.org>) (McKinney, 2018). All data were normalized on the average of the ten central min of the baseline (i.e., $t = 150 - t = 750$ s) and expressed as the magnitude difference with respect to baseline values. Hemoglobin difference (ΔHb), indicating oxygenated blood (Cunniffe et al., 2017), was calculated as the difference between the oxygenated haemoglobin (O_2Hb) and deoxygenated haemoglobin (HHb). Maximum [TSI_{max} , (O_2Hb) $_{max}$, (HHb) $_{max}$] and minimum values [TSI_{min} , (O_2Hb) $_{min}$, (HHb) $_{min}$] were calculated for each occlusion/reperfusion cycle. Area Under the Curve (AUC) was calculated for each occlusion using absolute values [TSI_{AUC} , (O_2Hb) $_{AUC}$, (HHb) $_{AUC}$, ΔHb_{AUC}], in order to assess the magnitude of oxygenation variations during deoxygenation and reoxygenation (Agbangla et al., 2017; de Oliveira et al., 2021; Manfredini et al., 2009). The AUC for reoxygenation was calculated from the zero-crossing closest to each of the three hyperemia spikes to the first crossing of 63% deoxygenation after each spike. Repeated occlusions may alter microcirculation, causing oxygenation to remain elevated above baseline (Kraemer et al., 2011). The 63% threshold was chosen to ensure consistent comparisons between participants. The time constant (τ) was calculated for [HHb] as the time taken from the beginning of each occlusion to reach 63% of the maximal [HHb] value. The



NIRS-derived parameters from the three cycles were averaged to obtain a single value per parameter for each session, as intra-session muscle oxygenation assessments using CW-NIRS are reliable (Desanlis et al., 2022).

Deoxy – and reoxygenation slopes were calculated for each parameter [TSI_{slope} , $(HHb)_{slope}$, $(O_2Hb)_{slope}$ and ΔHb_{slope}] as the slope of TSI, $[HHb]$, $[O_2Hb]$, and ΔHb during the first 30 s of the deoxy – or reoxygenation curve (Figure 3) (Papadopoulos et al., 2018). The start, the end, and the peak value of the deoxy – or reoxygenation curve were calculated using an automatic peaks detection Python routine, with a 10% threshold for both start and end (Python 3.8.8). The data analyzed in this study is part of a larger dataset previously published to assess intra- and inter-session NIRS device reliability (Desanlis et al., 2022). This study provides original findings on muscle oxygenation responses to arterial occlusion.

2.5 Statistical analysis

Data are presented as means \pm standard deviations (SD). Outliers deviating from mean \pm 2 SD were removed from the dataset (Bhambhani et al., 2006; Southern et al., 2014). After testing for normality (Shapiro-Wilk's test), the homogeneity of variance (Levene's test) and the sphericity (Mauchly's test), mixed analysis of variance (mixed ANOVA) with Bonferroni *post hoc* correction were used to determine the difference between groups. One-way ANOVA was used to compare demographical

data between groups. The magnitude of effects was calculated with Cohen's *d* and interpreted as small ($0.2 \geq d > 0.5$), medium ($0.5 \geq d > 0.8$), and large ($d \geq 0.8$) (Cohen, 1988). Non-parametric analysis of variance (Kruskal-Wallis test) with Dunn's *post hoc* were used when *p*-value of Shapiro-Wilk's and Levene's tests were lower than significance threshold. Effect sizes for non-parametric *post hoc* were given by the rank biserial correlation (*r*) for the Mann-Witney test (Kerby, 2014), as *r* = 0 for an absence of relationship between the two groups, and *r* = 1 for the strongest difference in ranks between groups. Statistical analysis was performed using JASP 0.17.1.0 (JASP Team, 2023). Statistical significance was set at $P < 0.05$.

3 Results

3.1 Environmental and participant descriptions

Four participants were excluded due to technical issues. Seven participants were excluded during data analysis due to inconsistent NIRS responses (i.e., tissue saturation index reaching 0%; prolonged degraded signal quality). Thus, thirty-five young males were included in the study.

Non-significant differences were observed across all variables of participants' characteristics (Table 1). No significant humidity differences were found between groups in S1 ($p = 0.33$) or S2 ($p = 0.54$) and between sessions (S1: $41\% \pm 14\%$, S2: $45\% \pm 15\%$,

TABLE 2 Changes in NIRS-derived parameters during occlusion.

Variable	Session	A	B	A: mean ± SD	B: mean ± SD	P _{bonf}	Effect size
TSI _{min} (%)	S1	G1	G2	-7.688 ± 5.500	-31.797 ± 5.914	<0.001*	d = 4.067
	S1	G1	G3	-7.688 ± 5.500	-34.482 ± 5.487	<0.001*	d = 4.520
	S1	G2	G3	-31.797 ± 5.914	-34.482 ± 5.487	0.735	d = 0.453
	S2	G1	G2	-11.782 ± 3.295	-28.094 ± 5.271	<0.001*	d = 3.109
	S2	G1	G3	-11.782 ± 3.295	-34.589 ± 6.367	<0.001*	d = 4.347
	S2	G2	G3	-28.094 ± 5.271	-34.589 ± 6.367	0.015*	d = 1.238
[O ₂ Hb] _{min} (mmol/L)	S1	G1	G2	16.571 ± 13.588	-19.308 ± 11.005	<0.001*	r = 1.000
	S1	G1	G3	16.571 ± 13.588	-17.828 ± 6.941	<0.001*	r = 1.000
	S1	G2	G3	-19.308 ± 11.005	-17.828 ± 6.941	1.000	r = 0.024
	S2	G1	G2	12.972 ± 10.05	-17.318 ± 7.561	<0.001*	r = 1.000
	S2	G1	G3	12.972 ± 10.05	-19.708 ± 4.982	<0.001*	r = 1.000
	S2	G2	G3	-17.318 ± 7.561	-19.708 ± 4.982	1.000	r = -0.051
[HHb] _{max} (mmol/L)	S1	G1	G2	18.924 ± 6.367	29.145 ± 4.823	<0.001*	d = -1.899
	S1	G1	G3	18.924 ± 6.367	28.509 ± 4.893	<0.001*	d = -1.780
	S1	G2	G3	29.145 ± 4.823	28.509 ± 4.893	1.000	d = 0.118
	S2	G1	G2	17.837 ± 4.428	26.196 ± 3.218	<0.001*	d = -2.025
	S2	G1	G3	17.837 ± 4.428	29.186 ± 4.43	<0.001*	d = -2.749
	S2	G2	G3	26.196 ± 3.218	29.186 ± 4.43	0.199	d = -0.724

Values are reported as mean ± SD; TSI, tissue saturation index (%); O₂Hb and HHb, oxy- and deoxyhemoglobin; A and B, names of the first and the second group of the condition in the pairwise t-test; *, significant difference between groups; Effect sizes, Cohen's d and rank biserial correlation (r).

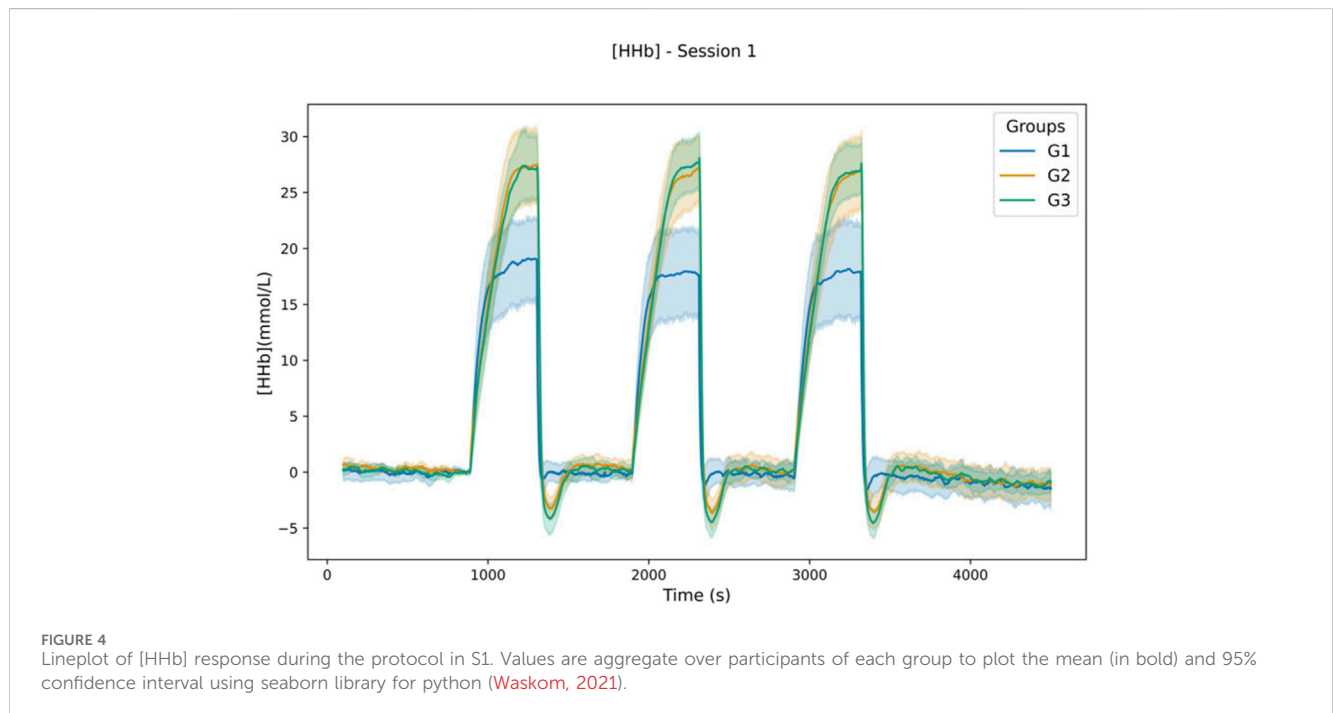


TABLE 3 Quantity of deoxygenation during occlusion.

Variable	Session	A	B	A: mean \pm SD	B: mean \pm SD	P_{bonf}	Effect size
TSI _{AUC} (a.u.)	S1	G1	G2	-1959.235 \pm 1417.399	-10908.097 \pm 1607.469	<0.001*	d = 5.231
	S1	G1	G3	-1959.235 \pm 1417.399	-11079.344 \pm 1828.094	<0.001*	d = 5.331
	S1	G2	G3	-10908.097 \pm 1607.469	-11079.344 \pm 1828.094	1.000	d = 0.100
	S2	G1	G2	-3563.863 \pm 1040.148	-10066.303 \pm 2367.666	<0.001*	d = 3.173
	S2	G1	G3	-3563.863 \pm 1040.148	-10784.103 \pm 2156.969	<0.001*	d = 3.523
	S2	G2	G3	-10066.303 \pm 2367.666	-10784.103 \pm 2156.969	1.000	d = 0.350
$\Delta\text{Hb}_{\text{AUC}}$ (a.u.)	S1	G1	G2	1130.178 \pm 2501.658	-15513.846 \pm 5187.309	<0.001*	d = 4.437
	S1	G1	G3	1130.178 \pm 2501.658	-13839.073 \pm 2034.38	<0.001*	d = 3.99
	S1	G2	G3	-15513.846 \pm 5187.309	-13839.073 \pm 2034.38	0.854	d = -0.446
	S2	G1	G2	-1540.371 \pm 2053.732	-14855.142 \pm 4222.848	<0.001*	d = 3.967
	S2	G1	G3	-1540.371 \pm 2053.732	-14757.584 \pm 2655.487	<0.001*	d = 3.938
	S2	G2	G3	-14855.142 \pm 4222.848	-14757.584 \pm 2655.487	1.000	d = -0.029

Values are reported as mean \pm SD; TSI, tissue saturation index (%); $\Delta[\text{Hb}]$, haemoglobin difference; AUC, area under the curve; a.u., arbitrary unit; A and B, names of the first and the second group of the condition in the pairwise t-test; *, significant difference between groups; Effect sizes, Cohen's d and rank biserial correlation (r).

TABLE 4 Speed and length of deoxygenation after occlusion.

Variable	Session	A	B	A: mean \pm SD	B: mean \pm SD	P_{bonf}	Effect size
TSI τ (s)	S1	G1	G2	77.275 \pm 39.991	128.728 \pm 19.664	<0.001*	d = -1.924
	S1	G1	G3	77.275 \pm 39.991	148.247 \pm 21.93	<0.001*	d = -2.654
	S1	G2	G3	128.728 \pm 19.664	148.247 \pm 21.93	0.253	d = -0.730
	S2	G1	G2	89.763 \pm 15.633	116.794 \pm 23.457	0.040*	d = -1.202
	S2	G1	G3	89.763 \pm 15.633	131.397 \pm 25.059	<0.001*	d = -1.851
	S2	G2	G3	116.794 \pm 23.457	131.397 \pm 25.059	0.368	d = -0.649
TSI _{slope} (%/s)	S1	G1	G2	-0.044 \pm 0.069	-0.145 \pm 0.027	0.006*	r = 0.852
	S1	G1	G3	-0.044 \pm 0.069	-0.153 \pm 0.034	0.002*	r = 0.829
	S1	G2	G3	-0.145 \pm 0.027	-0.153 \pm 0.034	1.000	r = 0.103
	S2	G1	G2	-0.087 \pm 0.048	-0.141 \pm 0.034	0.010*	d = 1.332
	S2	G1	G3	-0.087 \pm 0.048	-0.159 \pm 0.031	<0.001*	d = 1.759
	S2	G2	G3	-0.141 \pm 0.034	-0.159 \pm 0.031	0.889	d = 0.427
[HHb] τ (s)	S1	G1	G2	57.759 \pm 14.909	140.633 \pm 16.781	<0.001*	d = -3.791
	S1	G1	G3	57.759 \pm 14.909	146.542 \pm 26.458	<0.001*	d = -4.062
	S1	G2	G3	140.633 \pm 16.781	146.542 \pm 26.458	1.000	d = -0.270
	S2	G1	G2	68.733 \pm 6.088	140.767 \pm 16.436	0.002*	r = -1.000
	S2	G1	G3	68.733 \pm 6.088	148.989 \pm 22.158	<0.001*	r = -1.000
	S2	G2	G3	140.767 \pm 16.436	148.989 \pm 22.158	1.000	r = -0.212

Values are reported as mean \pm SD; TSI, tissue saturation index (%); HHb, deoxyhemoglobin; τ , time constant; A and B, names of the first and the second group of the condition in the pairwise t-test; *, significant difference between groups; Effect sizes, Cohen's d and rank biserial correlation (r).

TABLE 5 Changes in TSI% after cuff deflation.

Variable	Session	A	B	A: mean \pm SD	B: mean \pm SD	P_{bonf}	Effect size
TSI _{max} (%)	S1	G1	G2	1.933 \pm 2.01	9.54 \pm 3.274	0.006*	r = -0.981
	S1	G1	G3	1.933 \pm 2.01	12.219 \pm 1.485	<0.001*	r = -1.000
	S1	G2	G3	9.54 \pm 3.274	12.219 \pm 1.485	0.349	r = -0.497
	S2	G1	G2	1.831 \pm 1.627	10.179 \pm 2.33	<0.001*	d = -4.739
	S2	G1	G3	1.831 \pm 1.627	10.734 \pm 1.046	<0.001*	d = -5.055
	S2	G2	G3	10.179 \pm 2.33	10.734 \pm 1.046	1.000	d = -0.315

Values are reported as mean \pm SD; TSI, tissue saturation index (%); AUC, area under the curve; A and B, names of the first and the second group of the condition in the pairwise t-test; *, significant difference between groups; Effect sizes, Cohen's d and rank biserial correlation (r).

$p = 0.095$). No significant temperature differences were found between groups in S1 ($p = 0.24$) or S2 ($p = 0.20$) and between sessions (S1: $21.2^{\circ}\text{C} \pm 1.8^{\circ}\text{C}$, S2: $21.3\% \pm 1.5\%$, $p = 0.96$).

3.2 Effects of occlusion pressure on muscle deoxygenation

NIRS-derived parameters showed that using absolute (G3) or individualized (G2) occlusion pressures over SBP induced significantly greater deoxygenation compared to venous occlusion (G1), in terms of maximum values reached during occlusion, as reported in Table 2. A significant difference was shown for TSI_{min} in both sessions between G1 and G2, G1 and G3, and between G2 and G3 only for session 2. Regarding [O₂Hb], a significant difference was found using Dunn's *post hoc* comparisons between G1 and G2 ($p < .001$), G1 and G3 ($p < .001$), but not between G2 and G3 in both sessions. [HHb]_{max} during occlusions showed a significant difference for both sessions between G1 and G2 ($p < .001$), between G1 and G3 ($p < .001$), but not between G2 and G3 (Figure 4).

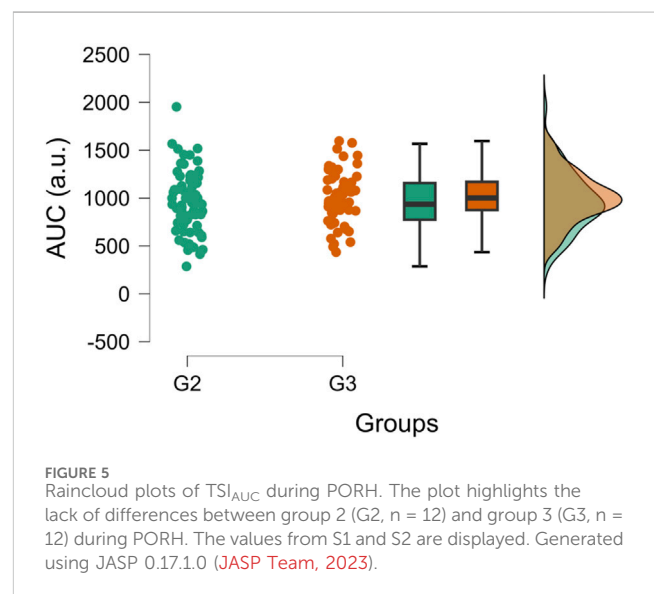
This greater deoxygenation during occlusion for participants of G2 and G3 is also confirmed by the higher area under the curve (AUC) values, in TSI_{AUC} and $\Delta\text{Hb}_{\text{AUC}}$, for G2 and G3 compared to G1, without significant differences between G2 and G3, as reported in Table 3.

The deoxygenation, while greater for G2 and G3, was also faster, as indicated by the large difference in [HHb] time constant (τ) [(HHb) τ], between G2 and G1, and between G3 and G1 (Table 4). The TSI τ during occlusion showed significant differences with large effect sizes in both sessions between G1 and G2, G1 and G3, but not between G2 and G3 (Table 4).

As reported in Table 4, the faster deoxygenation for G2 and G3 was confirmed by steeper TSI_{slopes} between G1 and G2, and between G1 and G3, without significant differences between G2 and G3.

3.3 Effects of occlusion pressure on muscle reoxygenation

After cuff deflation, muscle oxygenation of the occluded arm increased rapidly and overcame the baseline level for a short duration. This post-occlusive reactive hyperemia (PORH) is affected by the pressure applied during occlusion since a



significant effect of group on $\Delta\text{TSI}_{\text{max}}$ reached during hyperemia spike was found for both sessions between G1 and G2, G1 and G3, but not between G2 and G3, with higher PORH values for higher pressures (Table 5). In addition, no significant differences were found between G2 and G3 during PORH for TSI_{AUC} (G2 = 961.425 ± 296.556 a.u.; G3 = 1007.45 ± 228.827 a.u.; $p = 0.559$) (Figure 5) and TSI τ (G2 = 15.267 ± 6.064 s; G3 = 14.779 ± 4.899 s; $p = 0.697$).

Regarding the speed of reperfusion during PORH, G2 and G3 pressures tend to induce faster reperfusion than G1. [HHb]_{slope} during reperfusion was significantly steeper for G2 compared to G1, and for G3 compared to G1, without significant differences between G2 and G3 (Table 6). However, a tendency of a steeper slope for G3 compared to G2 could be highlighted in session 2. When TSI_{slopes} of both sessions are averaged for G2 and G3 and compared with a t-test, a significant difference between groups is shown (1.264 ± 0.147 vs. 1.044 ± 0.209 , $p = 0.010$, $d = -1.184$).

4 Discussion

The aim of this study was to evaluate the peripheral hemodynamic responses to partial and complete, absolute, or

TABLE 6 Reperfusion speed after cuff deflation.

Variable	Session	A	B	A: mean \pm SD	B: mean \pm SD	P_{bonf}	Effect size
TSI _{slope} (%/s)	S1	G1	G2	0.265 \pm 0.103	1.119 \pm 0.228	0.003*	r = -1.000
	S1	G1	G3	0.265 \pm 0.103	1.282 \pm 0.182	<0.001*	r = -1.000
	S1	G2	G3	1.119 \pm 0.228	1.282 \pm 0.182	0.598	r = -0.397
	S2	G1	G2	0.336 \pm 0.122	0.936 \pm 0.265	0.008*	r = -0.968
	S2	G1	G3	0.336 \pm 0.122	1.258 \pm 0.130	<0.001*	r = -1.000
	S2	G2	G3	0.936 \pm 0.265	1.258 \pm 0.130	0.079	r = -0.701
[HHb] _{slope} (%/s)	S1	G1	G2	-0.392 \pm 0.137	-0.931 \pm 0.124	<0.001*	d = 3.628
	S1	G1	G3	-0.392 \pm 0.137	-0.946 \pm 0.154	<0.001*	d = 3.730
	S1	G2	G3	-0.931 \pm 0.124	-0.946 \pm 0.154	1.000	d = 0.102
	S2	G1	G2	-0.398 \pm 0.12	-0.854 \pm 0.168	<0.001*	d = 3.154
	S2	G1	G3	-0.398 \pm 0.12	-0.937 \pm 0.131	<0.001*	d = 3.729
	S2	G2	G3	-0.854 \pm 0.168	-0.937 \pm 0.131	0.485	d = 0.574

Values are reported as mean \pm SD; TSI, tissue saturation index (%); HHb, deoxyhemoglobin; A and B, names of the first and the second group of the condition in the pairwise t-test; *, significant difference between groups; Effect sizes, Cohen's d and rank biserial correlation (r).

individualized blood-flow occlusions on the arm in the rested state. The results support our hypothesis that the magnitude of hemodynamic changes during occlusion and reperfusion are greater for groups with complete arterial occlusion, without differences between absolute or individualized pressure group.

4.1 Effects of occlusion pressure on deoxygenation

Data showed greater deoxygenation induced by suprasystolic pressures compared to G1 but cannot show differences between both groups with occlusion pressures over the SBP (G2 and G3). This greater deoxygenation reached for G2 and G3 is characterized by higher values of [HHb]_{AUC}, [HHb]_{max} (Figure 3) and lower values of TSI_{min} compared to G1, with no differences between G2 and G3, except for TSI_{min} in S2. In addition, the deoxygenation rate is not different between G2 and G3, while it is significantly faster for both groups than for G1.

These present results support the hypothesis that the hemodynamic response is driven by occlusion pressure when comparing partial and complete occlusions. However, no systematic differences were found between G2 and G3, indicating that no dose-response effect could be elicited for pressures over the SBP. The hemodynamic response seems similar between suprasystolic pressures. Thus, similar effects on deoxygenation could be induced at a much lower intensity than IPC pressures established in the literature (i.e., between 200 and 250 mmHg) (O'Brien and Jacobs, 2021).

Our findings are attractive since a dose-response effect was previously found in both upper and lower limbs with occlusion pressures of 140, 160 and 180 mmHg (Cunniffe et al., 2017), with greater deoxygenation characterized by a greater drop in [O₂Hb] after occlusion with higher pressures. However, the authors reported that 140 and 160 mmHg pressures were insufficient to induce complete arterial occlusion in the upper limb for all participants

(Cunniffe et al., 2017; Sharma et al., 2014). Thus, the results reported in the study from Cunniffe et al. (2017) support our findings since the authors found greater deoxygenation for complete (180 mmHg) and partial arterial occlusion (140 and 160 mmHg).

Since arm arterial occlusion depends on SBP, which is highly variable between individuals, and arm circumference in a lesser extent (Loenneke et al., 2015), using absolute values for IPC cannot guarantee complete arterial occlusion (Pickering et al., 2005; Sharma et al., 2014). Thus, it was proposed to use occlusion pressures based on the SBP. Some studies used individualized occlusion pressures ranging from 10 to 50 mmHg over the SBP (O'Brien and Jacobs, 2021). However, SBP measurements could show significant shortcomings, leading to the misclassification of patients (Pickering et al., 2005). Moreover, the first Korotkoff sound, attributed to systolic pressure, tends to underestimate the systolic pressure recorded by direct intra-arterial measurement up to 31 mmHg (Hunyor et al., 1978; Pickering et al., 2005). Thus, choosing an individualized pressure of less than 50 mmHg > SBP cannot guarantee complete arterial occlusion as well.

Even with an absence of a detectable pressure pulse, Mouser et al. (2017) reported a minor blood flow in several participants, which could be attributed to enhanced BP caused by solicitation of muscle metaboreflex and mechanoreflex or blood reaching occluded tissues through deep arteries (Brown et al., 2018; Spranger et al., 2015). Moreover, subtle movements of the participant could reduce pressure underneath the cuff leading to incomplete occlusion (Brown et al., 2018). Light postural changes could be caused by discomfort induced by cuff pressure. Previous studies have shown that IPC induces a discomfort in both upper and lower limbs (ratings of pain on a 0–10 scale during IPC: 4 \pm 0.7 and 4.36 \pm 1.29 for the arm (Lalonde and Curnier, 2015; Sharma et al., 2014); 7 \pm 0.4 for the legs (Sharma et al., 2014)). Thus, it is possible that the discomfort caused by the arterial occlusion led to postural changes during the protocol, although the participants were asked to stay immobile.

4.2 Effects of occlusion pressure on reoxygenation speed

Cuff deflation induces a fast reoxygenation – or reperfusion – phase caused by restored blood inflow. Our data showed that this phase was faster for G2 and G3 than for G1, with steeper reperfusion slopes. No significant differences in TSI_{slope} for reoxygenation rate were found between G2 and G3 when sessions were compared independently but a significant difference was found when measurements of the two sessions were averaged for G2 and G3. A greater pressure used to occlude blood flow may induce a faster reoxygenation response in the 30 s window following cuff deflation, which could be due to an increased blood flow after deflation.

It has been shown that IPC decreases blood pH and increases blood lactate concentration ($[La^-]$), during the occlusion, at much lower occlusion pressures than pressures used in the current study (Sharma et al., 2014). An enhanced clearance, or a lower production of $[La^-]$, has been shown during exercise preceded by IPC (Bailey et al., 2012b), which could result from better endothelial protection induced by IPC (Gu et al., 2021; Rajendran et al., 2013). Better endothelial protection enhances vascular function, which regulates the blood flow to remove and transport lactate. Increased blood flow could also arise from increased adenosine levels and the opening of ATP-sensitive potassium (K_{ATP}) channels under hypoxia conditions (Bailey et al., 2012a; Riksen et al., 2004; Rosenberry and Nelson, 2020), that belongs to the main mediators of PORH (Mahé et al., 2012). Indeed, inwardly rectifying potassium channels and $Na^+/K^+-ATPase$ are the main determinants of the magnitude of PORH in humans (Creclius et al., 2013). After multiple ischemia-reperfusion cycles, it has been shown that capillary blood flow was significantly increased (Kraemer et al., 2011).

Interestingly, the difference in reperfusion speed between G2 and G3 could also result from greater vasodilation inducing a higher blood flow (Enko et al., 2011). In addition, the velocity of red blood cells (calculated by assessing the distance of red blood cells movement in each frame) is increased after the IPC procedure in an animal model (Tapuria et al., 2009), which could explain the result found in the present study. However, this higher red blood cells velocity has not been demonstrated in muscle (Adanali et al., 2002).

Even though the TSI_{slope} of G3 was steeper than the slope of G2, it is noteworthy that this difference in our result is only shown for TSI but not for $[HHb]$ or $[O_2Hb]$. Since TSI is calculated from $[O_2Hb]$, which is known to be sensitive to blood flow changes, this parameter may not be the most useful to characterize reoxygenation. Thus, it would be fallacious to conclude that reoxygenation is faster when higher pressures are used after a complete arterial occlusion. These findings need to be investigated in future studies to confirm or not our results.

4.3 Effects of occlusion pressure on PORH

Arterial occlusion induces a transient phase of fast reperfusion known as post-occlusive reactive hyperemia (PORH) when the cuff is deflated after a sufficient time. The PORH represents the magnitude of limb reperfusion after ischemia (Rosenberry and Nelson, 2020), and PORH assessment using NIRS has been largely reported in the literature (Rosenberry and Nelson, 2020). Our study compared the TSI_{AUC} of PORH between G2 and G3. No differences were found between both groups. Similarly, while

G2 showed longer PORH phases in both sessions than G3, this difference was not significant. Thus, although reoxygenation seems to be faster for G3 than G2, the magnitude of the reperfusion, characterized by AUC, is not significantly different between groups (Figure 5). Therefore, this conclusion supports the necessity of being cautious when concluding about the speed of reperfusion of TSI between G3 and G2. It is then necessary to not only look at the speed of reperfusion but also take into account the other parameters such as the AUC, and maximums or minimums reached during occlusion and reperfusion phases.

These findings are consistent with previous results that could not show differences in PORH values between 140, 160 and 180 mmHg, sweeping any dose-response effect away (Cunniffe et al., 2017). Occlusion in this study led to PORH even with much lower pressures than traditionally used. It is also consistent with our results that cannot show significant differences between the two groups with complete arterial occlusion.

5 Limitations

A limitation of this study is that room temperature was not controlled, which could affect skin temperature (Hom et al., 2004). However, no significant differences between groups or sessions have been found. In addition, as stated previously, few studies using occlusion pressures over the SBP reported incomplete arterial occlusion. The gold standard to assess the absence of pulse is the Doppler ultrasound (Brown et al., 2018), which was not used in our study. However, the pressures selected in the current study are consistent with the literature (O'Brien and Jacobs, 2021) and high enough to overcome SBP measurement errors (Pickering et al., 2005) in young healthy participants, or decreases in cuff pressure due to subtle postural changes (Brown et al., 2018).

Another limitation of this study is the lack of ATT assessment at the NIRS site. Including participants with a fat layer greater than one-quarter the distance between the NIRS receptor and the most distant emitter could have affected the signal (Barstow, 2019). This may explain the inconsistent NIRS responses observed in seven participants.

As mentioned in the introduction, typical ischemic preconditioning (IPC) interventions involve periods of ischemia lasting between 5 and 10 min (O'Brien and Jacobs, 2021). In this study, the duration of ischemia periods was set at 7 min, which was also found in only one other study conducted on humans (Bushell et al., 2002). Initially, this specific duration was chosen to investigate the effects of occlusion pressure on heart rate variability (HRV) and blood pressure variability (BPV), requiring a minimum of 256 points for analysis (Souza Neto et al., 2003). However, due to a technical issue during data collection, HRV and BPV cannot be presented in this article.

6 Conclusion

This study presents compelling evidence that individualized occlusion pressures (SBP + 50 mmHg), within the healthy population, yield hemodynamic responses measured by NIRS, that are not significantly different from those seen with traditionally higher absolute pressures (>200 mmHg) reported in the literature. With the exception of the reoxygenation rate, no

significant differences were observed between G2 and G3. Using more personalized IPC protocols can benefit athletes and patients by improving performance, blood flow, and oxygen delivery while offering a safer approach than using high absolute pressures (Marocolo et al., 2016; Marocolo et al., 2019; Paradis-Deschênes et al., 2016). The use of NIRS devices to monitor muscle oxygenation allows for precise, real-time, and non-invasive interventions, applicable in research, clinical, and training contexts (Perrey, 2022; Perrey and Ferrari, 2018). Overall, these insights support the standardization of IPC protocols, making them more consistent and reliable across various applications (O'Brien and Jacobs, 2021). These findings highlight the practicality and effectiveness of using individualized occlusion pressures based on systolic blood pressure (SBP) rather than relying on excessively high absolute pressures. By challenging the conventional practice, this approach offers an alternative for optimizing occlusion pressure protocols while ensuring participant safety in research and clinical settings.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.5281/zenodo.7920956>.

Ethics statement

The studies involving humans were approved by CER POLETHIS, Université Paris-Saclay. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing–original draft,

Writing–review and editing. DG: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing–original draft, Writing–review and editing. CF: Writing–original draft, Writing–review and editing. CC: Conceptualization, Investigation, Writing–original draft, Writing–review and editing. FC: Conceptualization, Methodology, Supervision, Validation, Writing–original draft, Writing–review and editing. MG: Conceptualization, Methodology, Resources, Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STUDY 3:

Cardiovascular and respiratory adaptations to HIIT + BFR

The recent systematic review of Chua *et al.* (2022) included only seven studies about the chronic adaptations to HIIT + BFR on aerobic performance⁸³. Most of the studies found significant increases in $\dot{V}O_{2max}$ in the HIIT + BFR group, but only three studies have elicited a superiority of HIIT + BFR compared to HIIT in increasing $\dot{V}O_{2max}$ ^{454,637,638}. Increases in $\dot{V}O_{2max}$ have been found with as few as 8 sessions of training over 4 weeks^{637,638}. The discrepancy in the findings may arise from heterogeneous methodologies of inducing BFR. Indeed, methodological aspects are crucial with BFR since the disturbance in blood flow depends on the amount of pressure in the cuff, but also on the cuff's and individual's characteristics²⁸. It is recommended that occlusion pressure should be individualised using LOP rather than arbitrary to improve results consistency and participant safety²⁷³. Predictive equations of LOP have been established based on limb circumference and cuff width^{273,446,508}. Among the seven studies in the review of Chua *et al.* (2022)⁸³, two used postexercise BFR, which does not restrict blood flow during exercise, implying different physiological mechanisms^{637,638}. Of the five remaining studies, none used LOP to select the occlusion pressure, as recommended by McEwen *et al.* (2019)²⁷³. A recent review recommends using pressure ranging between 40 % and 80 % of LOP, when combined with aerobic exercise²⁸. However, findings in the literature tend to suggest that pressure above 40 % of the LOP may not be necessary to induce physiological adaptations for resistance exercise with BFR. Yet, the effects of occlusion pressure during HIIT+BFR are still unclear⁴²³. Therefore, this study aimed

to investigate the effectiveness of HIIT + BFR at 40 % or 60 % of LOP in inducing cardiovascular and respiratory adaptations after 4 weeks of training.

Methods: 27 healthy and active participants (11 females, 16 males; 19 ± 7 years; 175 ± 8 cm; 67 ± 8 kg; 17 ± 8 % of body fat; 9 ± 5 h of physical activity per week) were allocated to one of three training groups: G1 (HIIT+20 mmHg; SHAM), G2 (HIIT+BFR at 40 % LOP), and G3 (HIIT+BFR at 60 % LOP). Participants have done 12 training sessions over 4 weeks using a cycle ergometer. Each group followed the same training consisting of 4 to 7 sets of 90 s at 90 % of the power reached at $\dot{V}O_{2max}$ interspersed with 120 s of recovery. The cuffs were placed on both thighs and deflated during recovery bouts. Cardiovascular and respiratory parameters (\dot{Q} , muscle oxygenation and gas exchanges), anthropometric data and blood composition (Hct, Hb, blood lactate and glucose concentrations) were recorded pre- and post-training intervention. These parameters were assessed at rest, during a graded exercise test, or through a submaximal test composed of 2 x 6 min constant load trials in the moderate and heavy exercise domains.

Results: Training resulted in a significant reduction in body mass ($P = .03$), fat mass ($P < .001$), and body fat percentage ($P < .001$) across all groups, with no significant differences observed between the groups. Significant reductions in Hct ($P < .001$) and Hb ($P = 0.04$) and an increase in post-exercise $[La^-]$ were found after training, with no difference between groups. MAP ($P < .001$) and TTE ($P < .001$) increased without differences between groups, but $\dot{V}O_{2max}$ was not changed in any of the groups ($P > .05$). Training induced a significant rightward shift of the GET ($P < .001$) and the RCP ($P < .001$), with no differences between groups. \dot{Q}_{max} and TSI remained unchanged after training ($P > .05$). In the submaximal test, a decrease in HR ($P < .001$) was observed after training, with no differences between groups. Additionally, a reduced $\dot{V}O_2$ ($P < .001$) and $\dot{V}O_2$ ($P < .001$) was observed in both baseline bouts of the submaximal test for G3 only.

Conclusion: In the present study, the three groups increased their MAP and TTE, but not their maximal cardiovascular and respiratory parameters (\dot{Q} , TSI, or $\dot{V}O_{2max}$). combined with the present HIIT protocol, adding BFR does not seem to be a potent stimulus for maximal cardiovascular and respiratory adaptations. However, the results from the submaximal test suggest that HIIT+BFR (especially at 60 % of LOP) may lead to greater training efficiency. Further investigations are warranted to optimise the integration of BFR and HIIT protocols for optimal training adaptations.

The effects of four weeks of high-intensity interval training coupled with blood flow restriction on maximal and submaximal physiological adaptations.

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Key words: KAATSU, occlusion pressure, interval training, aerobic exercise, endurance, muscular efficiency.

INTRODUCTION

High-intensity interval training (HIIT) is characterized by alternating phases of exercise performed at intensities exceeding 90 % of maximal oxygen uptake ($\dot{V}O_{2max}$) with intervals of active or passive recovery ²⁷⁹. The primary objective of HIIT is to maximise the duration spent near $\dot{V}O_{2max}$, thereby inducing a maximal stress on the O₂ transport systems and providing an optimal stimulus for improving $\dot{V}O_{2max}$ ²⁷⁹. HIIT has been shown to be efficient in enhancing the cardiorespiratory system ($\dot{V}O_{2max}$, \dot{Q}_{max}), metabolic function, and endurance performance ^{456,639–642}. HIIT is considered a robust, time-efficient approach to induce physiological adaptations compared to moderate-intensity continuous training (MICT) ^{642–644}. The physiological adaptations to training are highly dependent on the activity and corresponding pattern of motor unit recruitment ⁶⁴⁵, with a large impact of frequency, intensity and volume of work performed ⁶⁴⁶. High-intensity contractions

require a high level of muscle fibre recruitment and solicit type 2 fibres that induce metabolic disturbances and promoting mitochondrial biogenesis and metabolism⁶⁴⁷.

Blood Flow Restriction (BFR) training is a method consisting of inflating a compressive cuff on the arms or legs during exercise to reduce the blood flow to exercising muscle⁶⁴⁸. BFR exercise, by reducing the O₂ delivery to the active muscles¹³⁰, also induced earlier recruitment of type 2 muscle fibres compared to exercise without BFR^{649–651}. By occluding the venous return, BFR training induces a blood pooling and an accumulation of metabolites that leads to an earlier onset of neuromuscular fatigue¹³⁰. Consequently, the recruitment of higher threshold motor units participates to the physiological adaptations following BFR training^{130,652}. When combined with low-intensity aerobic exercise (AE), BFR has shown delayed onset of blood lactate accumulation (OBLA)⁶⁵³, reduction in heart rate during exercise (HR)⁶⁵⁴, increase in stroke volume (SV) during exercise⁶⁵⁴, improvements in performance^{81,414,655}, and greater increases $\dot{V}O_{2max}$ compared to training without BFR^{81,301,653}. During high-intensity exercise, venous return increases¹¹⁹, leading to a greater volume of blood in the left ventricle, which stretches the myocardium and activates mechanoreceptors in cardiac muscle cells¹¹⁸. This activation triggers a signalling pathway that induces a cardiac hypertrophy and greater \dot{Q} after several weeks or months of training^{118,120,656}. HIIT is effective to induce central adaptations, but also peripheral adaptations^{147,656}. Additionally, combining BFR with endurance training results in greater changes muscle oxygenation compared to endurance training alone^{74,276}. Therefore, HIIT coupled with BFR may provide a sufficient stimulus to induce central adaptations, but greater peripheral adaptations than HIIT alone. Thus, combining HIIT and BFR could potentially offer a novel approach to optimise athletic performance and physiological adaptations.

To date the BFR literature has focused on the effects of low-intensity resistance exercise (RE) or aerobic exercise (AE), with interest growing regarding the

acute^{16,69,362} and chronic cardiovascular responses of HIIT coupled with BFR^{74,276,310,356,657–659}. Chronic adaptations of HIIT paired to BFR elicit an improvement in $\dot{V}O_{2max}$, performance and exercise economy, but the superiority of HIIT paired to BFR compared to HIIT alone is equivocal due to a lack of standardization in the protocols⁴²³.

Methodological factors must be considered when using BFR, since the disturbance in blood flow depends on the amount of pressure in the cuff, and cuff and participant's characteristics²⁸. BFR pressure should be based on the minimum amount of pressure required to interrupt the blood flow in the limb, known as the limb occlusion pressure (LOP). To avoid issues related to arterial occlusion, including pain and equipment limitations^{273,431,446,508}, equations have been established to estimate the LOP based on limb circumference and cuff width^{273,446,508}. Findings in the literature suggest that pressure above 40 % of the LOP may not be necessary to induce physiological adaptations for RE with BFR⁸⁴. But a recent study showed a pressure effect in the $\dot{V}O_2$ kinetics responses during a cycling task in the heavy domain³⁴⁷. Consequently, the pressure could have a great impact on cardiovascular responses during short exercise duration such as high-intensity exercise and needs further investigations⁴²³.

Therefore, the primary objective of this study was to test the efficacy of combining HIIT with BFR in inducing haemodynamic and cardiorespiratory adaptations, compared to HIIT alone. Additionally, the study aimed to compare the effects of two different BFR pressures applied during HIIT sessions.

Given the promising results of early work on HIIT coupled with BFR⁴²³, it was hypothesized that $\dot{V}O_{2max}$ and time to exhaustion would experience greater improvements for HIIT + BFR groups than HIIT alone. Additionally, there should be an absence of differences between HIIT + BFR groups, suggesting that high BFR pressures may not be needed for achieving physiological adaptations.

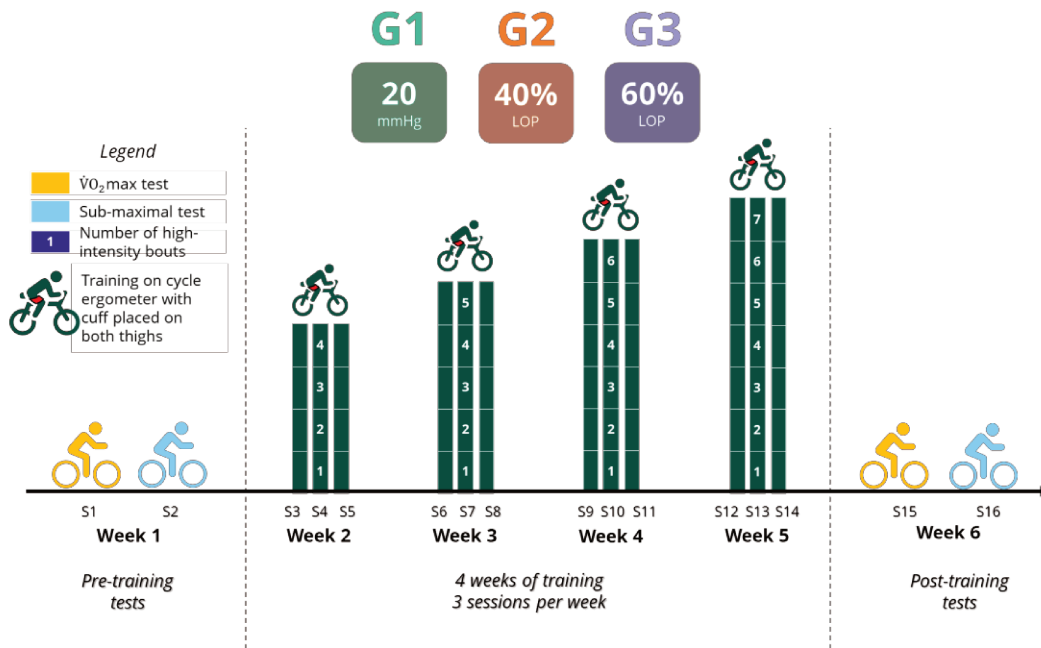
MATERIALS AND METHODS

1. Participants

Following local institutional ethical approval (SES_STAFF_19-47) and having provided written informed consent, 27 participants were recruited (11 females, 16 males). Participants who reported diabetes, hypertension, previous cardiovascular events, or any injury were not included in the study. Participants had to complete a Pre-Exercise Health Questionnaire ⁶⁶⁰, which the lead investigator certified. Participants were asked to avoid vigorous physical activity and alcohol intake 24 h before assessment sessions.

2. Study Design

After the initial $\dot{V}O_{2max}$ assessment session, participants were allocated to one of three groups based on their $\dot{V}O_{2max}$, ensuring that the mean $\dot{V}O_{2max} \pm SD$ of each group was paired to avoid statistically significant differences between them. The three groups correspond to different occlusion pressures used during training sessions: 20 mmHg (SHAM - G1), 40 % of LOP (G2) and 60 % of LOP (G3). Participants had to undertake a pre- and post- training cycle incremental test, a pre-training cycle submaximal test, a familiarisation session, eleven training sessions (Study 3 – Figure 1).



Study 3 - Figure 1: Schematic representation of the study design.

Pre- and post-training maximal and submaximal tests were achieved during the first and the last week, respectively. Participants were split into three BFR groups: G1 (20 mmHg), G2 (40 % of LOP) and G3 (60 % of LOP). Each group followed the same training for 4 weeks (4 to 7 sets of 90s at 90 % of the power reached at $\dot{V}O_{2max}$ with BFR + 120s of recovery). The cuffs were placed on both thighs and deflated during recovery bouts.

a. Materials

The same equipment was used during both maximal and submaximal assessments. Expired air was collected using a breath-by-breath metabolic cart (Cortex MetaLyzer 3B, Leipzig, Germany), which was calibrated before each test using a 3 litres syringe and a certified reference gas (15 % O_2 , 5 % CO_2). Haemodynamic data (*i.e.* \dot{Q} , SV and HR) were recorded using the PhysioFlow (PF05L1, Manatec, Petit-Ebersviller, France), with a sampling rate of 250 Hz, a beat-by-beat measure and a 30 beats period for calibration. Electrodes leads for the PhysioFlow were attached following the manufacturer guidelines for exercise. Changes in both vastus lateralis oxygenation were assessed using two Portamon (Artinis, Elst,

Netherlands) near-infrared spectroscopy (NIRS) devices sampling at 10 Hz. Both NIRS devices were placed at the two thirds the distance between the iliac spine and the lateral side of the patella ⁶⁶¹. Adipose tissue thickness (ATT) was assessed on both thighs at the NIRS location site, since ATT may affect NIRS data ⁶²⁴. ATT was assessed twice, before the pre- and post-training $\dot{V}O_{2max}$ tests (Study 3 – Figure 1). For each session, the measure was done three times using Harpenden skinfold calliper (Baty International, Wantage, United Kingdom) and averaged, following ISAK guidelines. Blood lactate and glucose concentrations were estimated from finger capillary blood ($\approx 20 \mu\text{L}$) (Biosen C-Line, EKF-diagnostic, Germany). Haemoglobin concentration and haematocrit were estimated from finger capillary blood ($\approx 10 \mu\text{L}$) using pre-calibrated device (EKF Hemo Control, EKF diagnostic, Germany).

b. Maximal assessment

Participant's stature, mass, body composition, thigh adipose tissue thickness, limb length, as well as ankle-to-brachial (ABI) ⁶⁶² were assessed in sessions 1 and 15 (Study 3 – Figure 1). Two capillary blood samples were taken at rest in a seated position.

Pre- and post-training, participants' cardiorespiratory fitness was evaluated with $\dot{V}O_{2max}$ test on a cycle ergometer (Lode Corival, Groningen, Netherlands). Prior to the first $\dot{V}O_{2max}$ test, participants completed a self-paced and self-timed warm-up, used to establish their preferred cadence (rpm). The participants were asked to keep this cadence (± 5 rpm) during all sessions of the study. Warm-up duration and cadence were kept the same between both pre- and post-training $\dot{V}O_{2max}$ tests. The $\dot{V}O_{2max}$ test started with one minute of baseline at 100 watts for males and 50 watts for females, with resistance increased by $0.42 \text{ W}\cdot\text{s}^{-1}$ (25 W per minute) until volitional exhaustion ⁶⁶³. Perceived exertion was recorded every minute during the test using the modified Borg Rating of Perceived Exertion (RPE) scale from 1 to 10 (CR-10) ^{284,664,665}. Continuous encouragement was provided by investigators throughout the test. The test stopped when the cadence decreased by more than 5 rpm from the

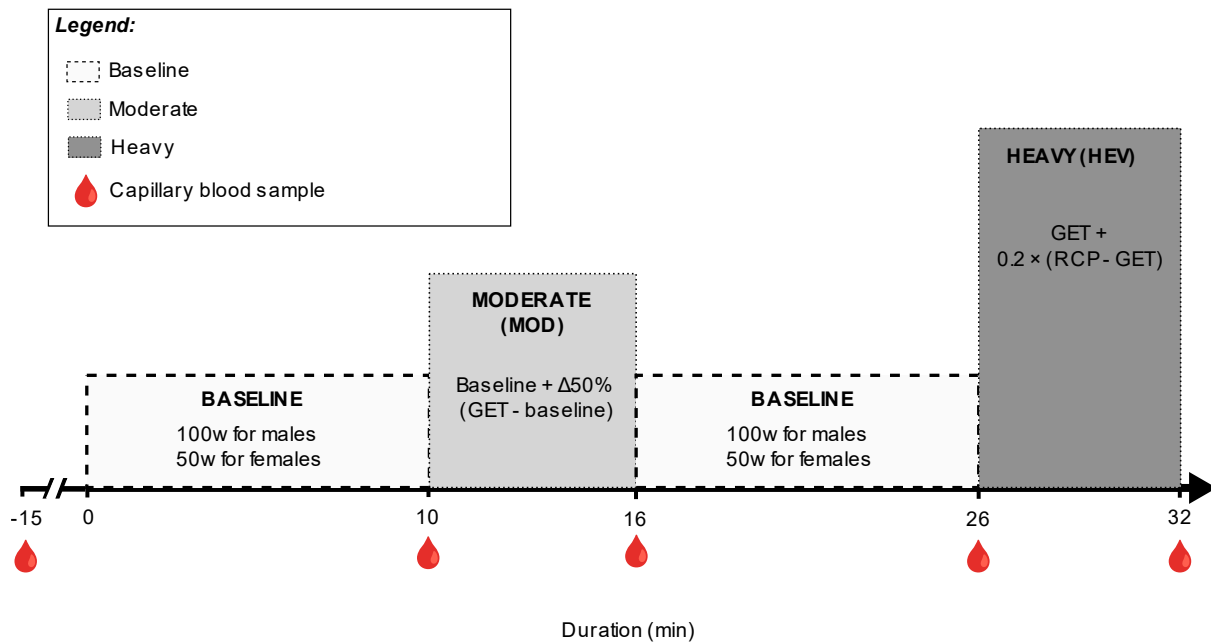
initial value, or when the participant reached volitional exhaustion. Straight after the end of the test, a capillary blood sample was taken at the finger for estimations of blood glucose and lactate concentrations.

Gases expired, cardiovascular and muscle oxygenation responses were recorded during the whole duration of pre- and post-training $\dot{V}O_{2max}$ test.

c. Submaximal assessment

Gas exchange threshold (GET) and respiratory compensation point (RCP) attained during the pre- or post-training maximal tests, were determined⁶⁶⁶ and used to calculate individuals moderate and heavy domains intensities³⁹³. Moderate intensity (MOD) was calculated as the sum of baseline intensity (100 W for males, 50 W for females) and 50 % of the difference between GET and baseline. Heavy intensity domain (HEV) was calculated as follows : $HEV = GET + 0.2 \times (RCP - GET)$. Pre- and post-training MOD and HEV were calculated based on the pre- and post-training GET and RCP, respectively. The submaximal test consisted of periods (6 minutes) of constant MOD or HEV intensities preceded by periods (10 minutes) of baseline intensity cycling (Study 3 – Figure 2). Pre- and post-training, participants' submaximal expired gases, cardiovascular and muscle oxygenation responses were recorded during the whole duration of the submaximal tests.

Capillary blood samples were taken at rest before the submaximal test, and at the end of each step (*i.e.* first baseline, MOD, second baseline, HEV) (Study 3 – Figure 2). RPE was collected at the end of each step.



Study 3 - Figure 2: Design of the submaximal test.

The protocol involves alternating between baseline intensities (100 W for males, 50 W for females) for 10 minutes, followed by square wave transitions to MOD and HEV intensities for 6 minutes each.

3. Data analysis

Datasets were time-aligned using the provided timecodes and manual markers placed at each event for $\dot{V}O_{2max}$ test (onset and end of the test) and submaximal test (onset of exercise, MOD trial, baseline, HEV trial, and end of the test).

For $\dot{V}O_{2max}$ tests, breath-by-breath and beats-by-beats data were smoothed using a 15-points rolling average⁶⁶⁷. $\dot{V}O_{2max}$, maximal heart rate (HR_{max}), maximal stroke volume (SV_{max}) and \dot{Q}_{max} were calculated as the highest 15 points average (non-rolling) in each dataset⁶⁶⁷. NIRS signal was smoothed using a 10th-order low-pass zero-phase Butterworth filter (cut-off frequency 0.1 Hz) using Pandas software library functions for Python (Python 3.8.8, Python Software Foundation, <https://www.python.org>)⁶⁶⁸. All data were normalized on the average of the 30-s preceding the beginning of each test.

For submaximal tests, breath-by-breath and beats-by-beats data were processed using a 9-point rolling average with points that were severely outlying (more than ± 2 SD from the mean of the rolling average) due to coughs or sighs, deleted^{663,669}. The data kinetics were then modelled using a bi-exponential equation (Study 3 – Equation 1) for the first baseline and MOD, and a tri-exponential equation for HEV trials (Study 3 – Equation 2)⁶⁷⁰. Due to a poor fitting for NIRS-derived parameters, muscle deoxygenation kinetics are not presented here.

Study 3 - Equation 1: bi-exponential model

$$x(t) = x_{baseline} + H(t-TD_{cd})A_{cd} \left(1 - e^{-\frac{t-TD_{cd}}{\tau_{cd}}} \right) + H(t-TD_p)A_p \left(1 - e^{-\frac{t-TD_p}{\tau_p}} \right)$$

Study 3 - Equation 2: tri-exponential model

$$x(t) = x_{baseline} + H(t-TD_{cd})A_{cd} \left(1 - e^{-\frac{t-TD_{cd}}{\tau_{cd}}} \right) + H(t-TD_p)A_p \left(1 - e^{-\frac{t-TD_p}{\tau_p}} \right) + H(t-TD_{sc})A_{sc} \left(1 - e^{-\frac{t-TD_{sc}}{\tau_{sc}}} \right)$$

Where, x is the data analysed, t is time in seconds, H is the heavyside step function (Study 3 – Equation 3)⁶⁷⁰, TD_p , TD_{cd} and TD_{sc} are the time delays in seconds of the primary phase, the cardiodynamic phase and slow component, respectively. A_p , A_{cd} and A_{sc} are the amplitudes of the primary phase, the cardiodynamic phase and slow component, respectively. And τ_p , τ_{cd} and τ_{sc} are the time constants in seconds of the primary phase, the cardiodynamic phase and slow component, respectively.

Study 3 - Equation 3: Heavyside step function

$$H(t) = \begin{cases} 0, & t < 0 \\ 1, & t \geq 0 \end{cases}$$

In addition, total amplitude (T_{Amp}) of each session were calculated as the sum of A_p and A_{sc} for bi-exponential model (Study 3 – Equation 1), and as the sum of A_{cd} , A_p and A_{sc} for the tri-exponential model (Study 3 – Equation 2).

The averaged last two minutes of each square-wave transition were compared pre- and post-training for breath-by-breath, beat-by-beat and muscle oxygenation data.

4. Statistical analysis

Data are presented as means \pm standard deviations (SD). After testing the normality (Shapiro-Wilk's test), the homogeneity of variance (Levene's test) and the sphericity (Mauchly's test), mixed analysis of variance (mixed ANOVA) with Bonferroni post hoc correction were used to determine the difference between conditions. One-way ANOVA with Bonferroni post hoc corrections were used to compare data between groups. When data deviated from normality, non-parametric tests were used: paired Wilcoxon signed-rank tests to compare sessions, and Kruskal-Wallis test to compare groups with post- and pre-training values difference (Δ) calculated. The magnitude of effects was calculated with eta-squared, Cohen's d and match ranked biserial correlation (r). Eta-squared was interpreted as small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$) and large ($\eta^2 \geq 0.14$). Cohen's d was interpreted as small ($0.2 \leq d < 0.5$), medium ($0.5 \leq d < 0.8$), and large ($d \geq 0.8$)¹⁴⁵. A r of 1 or -1 was interpreted as a perfect positive or negative correlation, respectively, whereas a r equals to 0 denotes the absence of correlation between the two variables⁶⁷¹. Statistical analysis was performed using JASP 0.17.1.0⁶⁷². Statistical significance was set at $p < 0.05$.

RESULTS

1. Anthropometric

During the training period, two participants voluntarily withdrew from the study for reasons unrelated to the study. No adverse effects were reported by the remaining participants ($n = 25$). Gender repartition in groups, age, stature and occlusion pressure used for training are provided in Study 3 – Table 1.

Study 3 - Table 1: Participants characteristics.

Variables	G1 N = 8	G2 N = 8	G3 N = 9
Females (n)	4	4	3
Males (n)	4	4	6
Age (years)	18 ± 2	18 ± 3	22 ± 10
Stature (cm)	175 ± 7	176 ± 7	175 ± 10
Occlusion pressure for training (mmHg)	20	72 ± 3	106 ± 2

Participants characteristics that were measured pre- and post-training are reported in (Study 3 -Table 2). The training resulted in a significant reduction in body mass ($p = .03$), fat mass ($p < .001$), and body fat percentage ($p < .001$) across all groups, with no significant differences observed between the groups. Additionally, there was a tendency towards increased muscle mass ($p = .06$) in all three groups. These changes in fat mass reduction and muscle mass increase were not accompanied by alterations in vastus lateralis circumference (Study 3 – Table 2).

Study 3 - Table 2: pre-and post-training anthropometric and blood assessments.

Variables	G1 N = 8		G2 N = 8		G3 N = 9		ANOVA			
	Pre	Post	Pre	Post	Pre	Post	T	G	INT	ES
Mass (kg)	65.76 ± 9.33	65.41 ± 9.23	67.21 ± 5.60	66.92 ± 5.40	67.24 ± 8.92	66.97 ± 8.80	0.03*	0.91	0.97	0.0004
Body fat (%)	17.44 ± 8.86	16.71 ± 8.51	18.91 ± 10.65	18.05 ± 10.41	15.76 ± 5.25	14.73 ± 5.31	< .001*	0.73	0.79	0.003
Muscle mass (kg)	51.54 ± 9.16	51.71 ± 9.06	51.77 ± 8.33	52.08 ± 7.82	53.94 ± 8.78	54.41 ± 8.97	0.06	0.80	0.75	0.0003
Fat mass (kg)	11.50 ± 6.37	10.96 ± 6.11	12.69 ± 7.41	12.10 ± 7.27	10.46 ± 3.09	9.69 ± 3.09	< .001*	0.71	0.73	0.003
Vastus lateralis circumference (cm)	48.42 ± 4.38	48.38 ± 3.94	47.52 ± 3.43	48.52 ± 4.88	47.97 ± 2.89	48.04 ± 2.33	0.33	0.97	0.42	0.002
Vastus lateralis skinfold (mm)	20.05 ± 11.91	19.30 ± 9.91	17.53 ± 11.21	17.35 ± 10.69	15.25 ± 6.84	15.02 ± 6.63	0.55	0.62	0.92	0.0004
SBP (mmHg)	113.50 ± 12.40	111.25 ± 7.48	121.88 ± 11.86	117.50 ± 11.89	118.11 ± 16.19	112.67 ± 1.75	0.03*	0.46	0.74	0.03
DBP (mmHg)	65.63 ± 5.63	61.13 ± 3.76	71.88 ± 6.31	63.25 ± 5.95	69.67 ± 5.68	66.89 ± 6.72	0.003*	0.06	0.32	0.16
ABI	1.18 ± 0.08	1.14 ± 0.06	1.11 ± 0.14	1.13 ± 0.08	1.24 ± 0.10	1.21 ± 0.10	0.40	0.04*	0.45	0.18
Haematocrit (%)	40.67 ± 3.83	39.00 ± 3.69	41.86 ± 3.34	40.43 ± 2.23	42.25 ± 2.12	38.63 ± 2.39	< .001*	0.67	0.22	0.14
Haemoglobin (g/dL)	13.87 ± 1.19	12.93 ± 1.38	13.90 ± 1.43	13.72 ± 0.71	14.00 ± 1.25	13.23 ± 0.79	0.04*	0.69	0.52	0.07

Note: * $p < .05$; ** $p < .01$; *** $p < .001$; T, G and INT are the p -values for the effect of time, group and interaction, respectively; ES: Effect size; SBP: systolic blood pressure; DBP: diastolic blood pressure; ABI: ankle-to-brachial index.

2. Blood pressure and blood composition

The training resulted in a significant decrease in both systolic ($p = .03$) and diastolic ($p = 0.003$) pressures measure at rest, with no differences between groups. Ankle-brachial index (ABI) remained unaffected in all groups (Study 3 – Table 2).

Analysis of blood composition showed a significant overall effect of training, with reductions observed in haematocrit ($p < .001$) and haemoglobin ($p = 0.04$) levels (Study 3 – Table 2). Prior to the $\dot{V}O_{2max}$ test, there was a significant increase in resting blood glucose levels following training ($p = 0.01$). However, blood glucose levels after the $\dot{V}O_{2max}$ test did not exhibit any significant changes between the pre- and post-training sessions. While resting lactate levels only tended to reduce after training ($p = 0.06$), there was a significant increase in lactate levels at the end of the test following the training period.

3. Maximal cardiovascular adaptations

Changes in cardiovascular, respiratory and performance responses are reported in Study 3 – Table 3. After 4 weeks of training, neither absolute $\dot{V}O_{2max}$ (L/min) nor relative $\dot{V}O_{2max}$ (mL/kg/min) exhibited changes in any of the groups. All participant groups demonstrated significant improvements in MAP ($p < .001$, $\eta^2 = 0.11$) and time to exhaustion (TTE) ($p < .001$, $\eta^2 = 0.28$), with no significant differences observed between the groups. Significant increases were found in GET (W) ($p < .001$, $\eta^2 = 0.09$) and RCP (W) ($p < .001$, $\eta^2 = 0.11$), without any significant differences between the groups. A main effect of training was found regarding the percentage of $\dot{V}O_{2max}$ used at GET ($p = 0.006$; ES : $r = -0.612$) and RCP ($p < 0.001$; ES : $d = -0.850$). These results indicate a rightward shift in the apparition of GET (pre: 67.54 ± 7.93 % vs. post: 75.02 ± 7.71 %, $p < 0.001$, $\eta^2 = 0.19$) and RCP (pre: 86.68 ± 5.62 % vs. post: 90.82 ± 4.17 %, $p = 0.028$, ES = 0.19) thresholds at higher percentage of $\dot{V}O_{2max}$. The linear relationship between $\dot{V}O_2$ and workload exhibited steeper slopes in the linear regressions before training compared to after training ($p < .001$, $\eta^2 = 0.23$).

A significant main effect of training was found in RER_{max} ($p < .001$, $\eta^2 = 0.16$) and blood lactate after the maximal test ($p = 0.002$, $\eta^2 = 0.07$), while no significant increase was observed in $\dot{V}CO_{2max}$ or $\dot{V}E_{max}$.

As stated in Study 3 – Table 4, no significant alterations were found in HR_{max} ($p = 0.56$, $d = -0.117$), SV_{max} ($p = 0.22$, $r = -0.239$), or \dot{Q}_{max} ($p = 0.13$, $r = -0.335$) following training. Additionally, the decrease in tissue saturation index (TSI) induced by exercise does not statistically differ between the two sessions ($p = 0.54$).

Study 3 - Table 3: Changes in gas exchanges, performance, and cardiovascular values between pre- and post-training maximal test

Variables	G1 N = 8		G2 N = 8		G3 N = 9		ANOVA		
	Pre	Post	Pre	Post	Pre	Post	Time	Group	Int
Expired air									
$\dot{V}O_{2max}$ (L/min)	2.93 ± 0.55	2.89 ± 0.56	2.98 ± 0.72	2.94 ± 0.54	3.04 ± 0.66	3.10 ± 0.57	0.93	0.84	0.76
$\dot{V}O_{2max}$ (mL/kg/min)	44.84 ± 6.59	44.40 ± 6.88	44.40 ± 9.84	44.07 ± 7.69	45.31 ± 8.49	46.50 ± 7.53	0.76	0.88	0.68
$\dot{V}CO_{2max}$ (L/min)	3.53 ± 0.66	3.53 ± 0.69	3.55 ± 0.84	3.62 ± 0.64	3.62 ± 0.84	3.84 ± 0.77	0.27	0.84	0.54
$\dot{V}E_{max}$ (L/min)	113.28 ± 24.31	115.41 ± 29.75	112.51 ± 34.38	110.86 ± 21.17	113.36 ± 27.89	119.84 ± 32.10	0.51	0.93	0.64
RER _{max}	1.20 ± 0.04	1.22 ± 0.03	1.19 ± 0.06	1.23 ± 0.03	1.18 ± 0.05	1.23 ± 0.04	< .001***	0.98	0.51
$\dot{V}O_2/WR$ slope	10.37 ± 1.21	9.07 ± 0.78	10.12 ± 1.51	8.99 ± 0.99	10.30 ± 1.69	9.00 ± 0.72	< .001***	0.95	0.95
End-exercise metrics									
RCP (W)	221.83 ± 46.34	257.67 ± 52.02	221 ± 44.39	258.29 ± 53.56	237.25 ± 52.81	268.50 ± 58.47	< .001***	0.85	0.84
GET (W)	174.50 ± 57.29	206 ± 47.15	173.07 ± 48.92	199.43 ± 42.26	183.94 ± 49.22	215.88 ± 60.12	< .001***	0.86	0.87
MAP (W)	262.38 ± 41.31	295.38 ± 55.39	264.88 ± 41.81	294.63 ± 41.65	268.75 ± 45.88	297.38 ± 47.92	< .001***	0.98	0.82
TTE (s)	516.38 ± 45.99	593.88 ± 74.79	521.75 ± 70.69	592.88 ± 56.88	514.5 ± 56.06	581.88 ± 58.20	< .001***	0.95	0.82
Lactate (mmol)	10.79 ± 1.43	11.32 ± 1.80	10.43 ± 2.99	12.67 ± 2.50	9.89 ± 2.14	10.54 ± 1.39	.002**	0.40	0.08

Note: * $p < .05$; ** $p < .01$; *** $p < .001$; $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide uptake; $\dot{V}E$: ventilation; RER: respiratory exchange ratio; WR: workload; RCP: respiratory compensation point; GET: gas exchange threshold; MAP: maximal aerobic power; TTE: time to exhaustion; T, G and INT are the p-values for the effect of time, group and interaction, respectively

Study 3 - Table 4: Haemodynamic changes between pre- and post-training maximal test

Variables	G1 N = 8		G2 N = 8		G3 N = 9	
	Pre	Post	Pre	Post	Pre	Post
Cardiovascular						
HR _{max} (bpm)	192.66 ± 4.77	192.31 ± 6.38	186.36 ± 9.69	187.81 ± 5.75	182.43 ± 15.60	184.57 ± 7.25
SV _{max} (mL/min)	118.23 ± 23.27	130.25 ± 12.95	126.90 ± 26.22	122.22 ± 12.74	126.69 ± 23.09	133.88 ± 25.69
\dot{Q}_{max} (L/min)	21.95 ± 4.01	24.41 ± 2.54	23.02 ± 4.89	22.65 ± 2.62	22.03 ± 4.99	23.56 ± 4.16
TSl _{max} (%)	-3.81 ± 10.61	0.16 ± 6.97	-0.30 ± 6.82	-0.38 ± 12.80	-1.34 ± 5.33	-2.31 ± 3.81

Note: due to a deviation from normality, ANOVA columns are not displayed in the present table, but statistical results are available in the text. HR: heart rate; SV: stroke volume; \dot{Q} : cardiac output; TSl: tissue saturation index.

4. Submaximal cardiovascular adaptations

For HR kinetics in the first baseline, post-training HR T_{Amp} (46.76 ± 51.18 bpm) and A_{cd} (30.15 ± 52.19 bpm) were found significantly lower ($p = 0.003$ and 0.011 , respectively) compared to pre-training values of T_{Amp} (90.94 ± 143.78 bpm) and A_{cd} (66.08 ± 137.01 bpm). In MOD, no statistical differences were found. But, in HEV, HR ΔA_{cd} was greater ($p = 0.048$) in G3 (10.70 ± 16.20) compared to G1 (-46.98 ± 50.76 bpm).

For $\dot{V}O_2$ kinetics, in the first baseline, a statistical difference ($p = 0.046$) was found in $\Delta\dot{V}O_{2baseline}$ between G2 (82.13 ± 188.48 mL/min) and G3 (-188.23 ± 256.66 mL/min). In MOD, τ_P was greater ($p < 0.001$) in post-training test (96.39 ± 239.46 mL/min) compared to pre-training test (25.84 ± 80.44 mL/min). ΔTD_p was found different ($p = 0.005$) between G2 (199.27 ± 257.84 mL/min) and G3 (-10.28 ± 13.44 mL/min). In HEV, post-training $\dot{V}O_{2baseline}$ (1355.40 ± 266.21 mL/min) was found lower ($p = 0.004$) than pre-training values (1457.62 ± 310.25 mL/min). Post-training τ_{sc} (497.75 ± 193.90 mL/min) and TD_{sc} (308.71 ± 83.31 mL/min) were found greater than pre-training (347.28 ± 303.73 and 254.07 ± 49.36 mL/min, respectively).

A reduced production of $\dot{V}CO_2$ was shown in the first baseline bout only for G3 ($p = .003$, ES = 1.067), and on the second baseline for G2 ($p = .032$, ES = 0.944) and G3 ($p = .015$; ES = 1.027) (Study 3 - Table 5). This reduction in $\dot{V}CO_2$ is also accompanied with a decrease in $\dot{V}O_2$ during the first baseline for G1 ($p = 0.048$, ES = 0.845) and G3 ($p = 0.001$, ES = 1.586) with a nonsignificant decrease for G3 compared to G1 ($p = .06$). The decrease in $\dot{V}O_2$ also occurred during the 2nd baseline for G2 ($p = 0.049$, ES = 0.843) and G3 ($p = 0.012$, ES = 1.370). A significant decrease in $\dot{V}E$ was found only for G3 in the first ($p < .001$, ES = 0.69) and second baseline ($p = 0.03$, ES = 1.11). A significantly larger decrease in $\dot{V}E$ was found between G3 and G1 ($p = 0.01$) for the first baseline. No significant changes were found in RER during the submaximal test.

A significant reduction in HR was found in the first ($p = 0.003$) and the second ($p = 0.003$) baseline with no difference between groups (Study 3 – Table 5). The changes in HR were not accompanied by any changes in SV during the submaximal test.

A significant reduction in the deoxygenation at first (pre: -2.64 ± 6.45 %, post: -0.91 ± 5.25 %, $p = 0.042$, $\eta^2 = 0.021$) and second (pre: -2.08 ± 7.53 %, post: 0.50 ± 6.08 %, $p = 0.013$, $\eta^2 = 0.036$) baseline bouts was only shown for TSI, with no differences between groups.

A main effect of time was found for blood lactate values collected at the end of first and second baselines ($p < 0.001$), and of HEV ($p = 0.001$) (Study 3 – Table 5), with no differences found between groups.

Study 3 - Table 5: Changes in submaximal gas exchanges, heart rate and lactate production between pre-and post-training submaximal test.

Variables and periods	G1 N = 8		G2 N = 8		G3 N = 9		Non-parametric tests			
	Pre	Post	Pre	Post	Pre	Post	Time	G1 – G2	G1 – G3	G2 – G3
<i>$\dot{V}O_2$</i>										
BL 1	18.36 ± 2.41	17.35 ± 2.60	18.19 ± 4.80	16.85 ± 4.11	20.29 ± 2.33	17.35 ± 2.85	< .001***	1.00	0.06	0.29
MOD	25.82 ± 4.72	26.94 ± 3.17	25.46 ± 6.52	26.00 ± 5.78	28.02 ± 4.27	26.54 ± 5.08	0.84	1.00	0.36	0.53
BL 2	18.61 ± 2.90	17.74 ± 2.88	18.57 ± 5.04	17.13 ± 4.23	20.21 ± 2.86	17.93 ± 2.91	0.01**	1.00	0.34	1.00
HEV	36.94 ± 9.05	40.02 ± 5.31	35.53 ± 8.36	38.54 ± 7.74	38.94 ± 8.14	40.01 ± 7.73	0.07	1.00	1.00	1.00
<i>$\dot{V}CO_2$</i>										
BL 1	1.12 ± 0.25	1.07 ± 0.28	1.17 ± 0.38	1.06 ± 0.31	1.27 ± 0.25	1.10 ± 0.21	0.003**	0.92	0.09	0.26
MOD	1.64 ± 0.44	1.72 ± 0.39	1.69 ± 0.51	1.72 ± 0.46	1.82 ± 0.37	1.72 ± 0.34	0.84	1.00	0.29	0.53
BL 2	1.12 ± 0.27	1.07 ± 0.28	1.17 ± 0.38	1.07 ± 0.32	1.24 ± 0.28	1.10 ± 0.20	0.01**	0.88	0.24	1.00
HEV	2.54 ± 0.85	2.86 ± 0.54	2.47 ± 0.66	2.82 ± 0.63	2.73 ± 0.64	2.91 ± 0.64	0.003**	1.00	1.00	1.00
<i>HR</i>										
BL 1	118.48 ± 12.05	110.65 ± 9.17	113.85 ± 12.02	110.01 ± 8.49	111.74 ± 9.80	106.03 ± 5.15	0.003**	0.74	1.00	0.98
MOD	144.75 ± 13.75	145.49 ± 8.22	136.84 ± 12.72	137.92 ± 10.11	137.08 ± 11.89	132.18 ± 10.61	0.55	1.00	1.00	1.00
BL 2	130.67 ± 11.53	122.74 ± 8.23	123.71 ± 14.26	119.13 ± 8.73	124.24 ± 14.62	114.60 ± 7.99	0.003**	1.00	1.00	0.81
HEV	170.19 ± 13.86	173.69 ± 4.01	159.02 ± 10.38	164.32 ± 9.69	161.86 ± 15.08	160.40 ± 11.03	0.84	1.00	0.84	0.30
<i>Lactate</i>										
BL 1	1.72 ± 0.59	1.16 ± 0.38	2.02 ± 1.33	1.13 ± 0.69	1.86 ± 0.76	1.08 ± 0.35	< .001***	1.00	1.00	1.00
MOD	2.29 ± 1.20	2.61 ± 1.14	2.61 ± 1.53	2.49 ± 1.89	2.59 ± 1.18	2.16 ± 0.83	0.63	0.75	0.16	1.00
BL 2	1.67 ± 0.50	1.43 ± 0.53	1.75 ± 1.33	1.46 ± 1.24	1.75 ± 0.97	1.03 ± 0.33	< .001***	1.00	0.79	0.95
HEV	6.15 ± 2.92	9.81 ± 2.20	6.44 ± 2.40	9.50 ± 3.64	6.92 ± 2.36	8.61 ± 2.87	< .001***	1.00	0.58	1.00

Note: Due to a deviation of the data from normality, only lactate START, MOD and HRV comparisons were analysed using a mixed ANOVA. The remaining comparisons were assessed using non-parametric tests. Time: p-value for the effect of time; * $p < .05$; ** $p < .01$; *** $p < .001$. $\dot{V}O_2$: oxygen uptake; $\dot{V}CO_2$: carbon dioxide uptake; HR: heart rate; MOD: moderate domain; HEV: heavy domain; BL1: first baseline; BL2: second baseline.

DISCUSSION

This study investigated the effects of four weeks of HIIT programme coupled with BFR intervention on cardiovascular and respiratory adaptations using three different restriction pressures.

This study showed submaximal physiological adaptations after training with a reduction in O_2 consumption per workload after 4 weeks of training, without differences between groups. This gain in efficiency is highlighted by the delayed appearance of both GET and RCP, without differences between groups. These results are consistent with the findings of De Oliveira et al. (2016), who found an improvement in the onset of blood lactate accumulation (OBLA), in both HIIT and HIIT + BFR groups after 4 weeks of training (12 sessions) without significant differences between groups. By increasing the power reached at GET and RCP in our study, the MOD and HEV domains were also increased in the submaximal test following training. Consequently, the same metabolic demand relative to GET and RCP was required to achieve both square-wave transitions. Our results show a slower τ_p after training in the MOD domain and a difference in ΔTDp between G2 and G3. These findings differ from recent studies that showed a faster τ_p in MOD domain after 4 weeks of endurance training with BFR or HIIT ^{341,344}. However, both studies used pre-training calculated intensities, implying that MOD intensity does not increase between the pre-and post-training submaximal test. As the intensity increased in both MOD and HEV in our study, it was expected not to find a reduction in τ_p . Our study also showed that HEV SC was delayed (slower TDSC and τ_{SC}) after training, but the end effort values highlight a greater metabolic demand showed by higher lactate and $\dot{V}CO_2$ and a tendency towards an increase in $\dot{V}O_2$ HEV, without differences between groups. In the literature, $\dot{V}O_2$ SC has been shown to be reduced after 24 sessions of continuous endurance training ⁶⁷³, or 6 to 8 weeks of HIIT ^{621,674}. However, $\dot{V}O_2$ SC remains unchanged after 8 weeks of interval training programme ⁶⁷⁵. Therefore, it is consistent with our findings since only 12 sessions of HIIT + BFR

were performed by the participants. A longer training programme would be required to induce a reduction in $\dot{V}O_2$ SC. Consequently, our results in $\dot{V}O_2$ or HR kinetics cannot show any significant improvement in the submaximal efficiency. However, the protocol implied two steps at 100 W in both pre- and post-training submaximal tests, completing the lack of information on submaximal adaptations provided by MOD and HEV domains. The training effectively increases cycling efficiency when comparing pre- and post-training first and second baseline values. The same effort (100 W for males or 50 W for females) required less $\dot{V}O_2$ after training, as shown by the lower $\dot{V}O_2$ for the first and second baseline bouts and the lower HEV $\dot{V}O_{2\text{baseline}}$. Submaximal adaptations are thought to be mainly driven by a greater O_2 delivery to the muscles induced by greater angiogenesis and capillary density^{317,332,333}, and greater O_2 utilisation by the muscles (oxidative enzyme activity)^{317,339,340}. In addition, BFR associated with aerobic exercise is known to stimulate the expression of key factors regulation the activation of the angiogenesis signalling pathway, such as vascular endothelium growth factor (VEGF), hypoxic-induced factor 1 α (HIF-1 α), peroxisome proliferator-activated receptor Gamma coactivator 1- α (PGC1- α)^{213,676}. BFR has also shown effectiveness in increasing capillary density, which has the potential to enhance microvascular perfusion capacity²⁴⁸.

Physiological adaptations to training depend highly on training intensity since contraction induced metabolic disturbances are involved in mitochondrial biogenesis^{647,677}. In addition to the increase in mitochondrial density after endurance training, mitochondrial activity is also enhanced up to 30 %^{291,678,679}. An increase in mitochondrial density and activity enhances the capacity for ATP production from both fat and carbohydrates²⁹¹. A greater reliance on fatty acid oxidation during submaximal intensity exercise might also delay the reliance on anaerobic glycolysis, as shown by lower lactate and $\dot{V}CO_2$ production at baseline intensity in the present study.

The reduction in $\dot{V}O_2$ during the first and second baseline bouts of the submaximal test in our study might mainly be linked to the reduction in $\dot{V}E$ and heart rate during the first and the second baseline bouts^{276,366} of the present study. Thus, the better performance in the maximal test and the improved efficiency during the submaximal test could be explained by a glycogen-sparing effect of increased fat oxidation^{290,680}, that is triggered by a training-induced increase in capillarization⁶⁸¹. This increase in muscular efficiency is consistent with the effects of endurance training on aerobic fitness³⁶⁶, and has also been shown at submaximal intensity (80 % $\dot{V}O_{2max}$) after 6 weeks of BFR and non-BFR training²⁷⁶, without significant differences between groups.

Our results also showed improvements in the maximal test, as shown by the greater TTE and MAP reached after the 4 weeks of training, without differences between groups. Indeed, G2 and G3 were unable to elicit a better increase than G1 in the maximal power reached at the end of the $\dot{V}O_{2max}$ test. The present results contrast early work on BFR, that showed significant greater improvements in TTE with the BFR-trained leg, compared to the control leg after 4 weeks of training⁶⁸². However, the protocol used in this study implied training sessions of 45-min bouts at low intensity. Recent studies showed significant improvements in maximal power after 4 weeks of HIIT on a cycle ergometer⁶⁵³ or on a treadmill⁶⁵⁷, to the same extent for both BFR and control group, which is consistent with our findings.

While submaximal efficiency and MAP were improved by training, $\dot{V}O_{2max}$, either absolute or relative, remained unaffected in all groups. This result is confirmed the absence of significant alterations found in all groups in HR_{max} , SV_{max} and \dot{Q}_{max} following training. These results are consistent with the findings of Keramidas et al. (2012), who showed no significant increases in $\dot{V}O_{2max}$ in both BFR and non-BFR groups after 6 weeks of training (3 sessions/week)²⁷⁶. The sessions consisted of repeated bouts of 2-min work at 90 % of $\dot{V}O_{2max}$ and 2-min of active recovery, with (90mmHg) or without BFR. While none of the experimental groups

shows a significant increase in $\dot{V}O_{2max}$, both have increased the peak power output reached at $\dot{V}O_{2max}$ (BFR \approx 20 %, non-BFR \approx 12 %). However, when BFR is coupled with running, either on a treadmill or during small-sided games, an increase of $\dot{V}O_{2max}$ is found after only 3 to 4 weeks^{310,658}. The differences between our results and those presented by Amani-Shalamzari may arise from the initial $\dot{V}O_{2max}$ of participants (13 % lower than in the present study), from the length of intervals (\geq 2-min vs. 90s in the present study), or from the pressure applied by the cuffs.

Investigating the role of occlusion pressure in physiological adaptations was one of the aims of this study. The literature found no significant difference in blood flow reduction between occlusions at 40 % and 80 % of LOP. The findings from Mouser et al. (2017) also suggest a similar reduction of blood flow between 50 % and 80 % of LOP, with the same response for three different sizes of cuffs⁸⁴. Therefore, the pressure recommendations for aerobic exercise lay between 40 % and 80 % of LOP²⁸. But recent findings of Salzmann et al. (2021) suggest an effect of pressure on the parameters of $\dot{V}O_2$ kinetics. The authors have found that the amplitude of $\dot{V}O_2$ SC increases as a function of BFR pressure since BFR at 40 % of AOP shows a lower SC amplitude compared to BFR at 50 % of AOP (6.7 ± 0.9 vs. 9.7 ± 1.0 mL/min/kg; $p < 0.05$) in a cycling task³⁴⁷. Thus, training with two different pressures as in the present study (40 % of AOP and 60 % of AOP) should result in two different $\dot{V}O_2$ kinetics responses. Slowing the $\dot{V}O_2$ kinetics response delays the stabilisation of $\dot{V}O_2$ and consequently increases the O_2 deficit, especially during short intervals, as in our study^{351,353}. It was then expected that differences in the physiological adaptations between the BFR and SHAM groups and between both BFR groups would be observed. However, no significant differences between groups were found in either submaximal or maximal parameters. This absence of differences between groups may come from the study design since training duration affects the $\dot{V}O_{2max}$ adaptations. With 4 weeks of training, longer intervals (\geq 2-min) or higher volume (\geq 15-min) may have been more efficient for increasing $\dot{V}O_{2max}$, and then observing differences between groups⁶⁸³.

This study has shown resting (lower systolic and diastolic blood pressures) and submaximal (lower HR) cardiovascular adaptations. In addition, BFR has been shown to affect the nervous system by soliciting affecting the group III/IV muscle afferents signal to the Central Nervous System (CNS) ⁵⁷. Consequently, assessing the effects of HIIT + BFR on the autonomic control of the cardiovascular system could provide useful information, especially for monitoring athletes' recovery and preventing overtraining. The effects of training on autonomic control of the cardiovascular system could be monitored by the analysis of the level of heart rate variability (HRV) ⁶⁸⁴⁻⁶⁸⁶ or the evaluation of baroreflex sensitivity (BRS) ^{685,687}.

LIMITATIONS

The main limitation of this study is linked to the protocol of the submaximal tests. Indeed, baseline bouts were set at 50 w for females and 100 w for males, which are much higher than the unloaded or 15 w pedalling observed in the literature ^{347,663,669}. Consequently, it is not possible to directly compare our results to the existing findings in the literature as all parameters of the bi- and tri-exponential models are affected.

Another limitation of this study lay in the results provided by NIRS devices. NIRS has been found reliable at rest ^{628,630} and during exercise ⁶²⁷⁻⁶²⁹, especially within occlusions of a single session. However, inter-sessions estimations of muscle oxygenation have shown poor reliability in a general population ⁵⁴⁹. In the present study, participants with greater thigh adipose tissue thickness than one-quarter the longest inter-optode distance were included in the study ⁶²⁴. Consequently, the relevance of muscle oxygenation estimations remains limited in participants over this recommended adipose tissue thickness.

CONCLUSION

In conclusion, this study demonstrated cardiorespiratory adaptations following the training intervention in both maximal and submaximal tests. The main finding of this study is that 4 weeks of HIIT or HIIT + BFR enhance maximal performance but not $\dot{V}O_{2max}$. Although no significant differences were observed between the three groups in cardiovascular adaptations during the maximal test, training has induced a greater cycling efficiency in the submaximal baseline bouts. Therefore, at the same absolute workload, post-training $\dot{V}O_2$ is lower than pre-training values, as for markers of exercise intensity such as blood lactate concentration and $\dot{V}CO_2$. While no differences between groups were found, G3 exhibited more significant improvements in the submaximal test. Then, a longer intervention (≥ 6 to 8 weeks) may have been more adequate to show the beneficial effects of training with BFR. These submaximal results highlight the utility of combining HIIT + BFR to optimise the effects of training⁴²³.

DISCUSSION AND PERSPECTIVES



The role of training with limited available O_2 in improving athletic performance is well-established⁶⁸⁸. Local hypoxia induced by cuff inflation (BFR or IPC) leads to the release of compounds such as NO, H^+ or ROS^{160,181}. These compounds affect haemodynamic parameters (e.g., vasodilation), haematological parameters (e.g., blood- O_2 affinity), and muscle oxygenation (e.g. O_2Hb , HHb, PORH)^{96,160}. These compounds accumulation activates metaboreceptors and mechanoreceptors, triggering a signalling cascade that releases angiogenesis-related factors such as VEGF, mTOR, IGF-1, PGC-1 α or HIF-1 α ^{125–127,211,213,216}. Consequently, acute oxidative stress induced by local hypoxia can activate signalling pathways that promotes cellular and vascular adaptations¹³⁰.

Since the 1990's, KAATSU or BFR interventions has shown effectiveness in improving muscle strength and establishing hypertrophy, through cellular adaptations⁶⁸⁹. However, the vascular adaptations induced by BFR also improve the perfusive and diffusive O_2 transport, driving aerobic adaptations. Indeed, BFR training seems also beneficial when combined with aerobic programme⁸², with increases in $\dot{V}O_{2max}$ shown after several weeks (> 3 weeks)^{28,80,420}. However, literature is lacking on the physiological processes involved and the methodological considerations leading to increases in $\dot{V}O_{2max}$. The pressure applied in the cuffs

during exercise may affect the haemodynamic response differently, and consequently produce different cardiovascular adaptations^{84,347}. Thus, the effects of cuff pressure on haemodynamic responses must be investigated to optimise pressure for maximum cardiovascular adaptations and safety.

Therefore, the primary objective of this present thesis was to examine the acute and chronic haemodynamic and respiratory responses to different levels (in mmHg) of blood flow restrictions. Consequently, this manuscript has been focused on the delivery and utilisation of O₂ in muscles, central elements of the Fick principle (Equation 1)⁸⁷. To do this, the following physiological parameters have been monitored: the changes in O₂ delivered and used in skeletal muscles using NIRS; the delivery of O₂ by the heart using cardiac impedance; and the ability of the body to intake, distribute and utilise this O₂ by assessing $\dot{V}O_2$.

OVERVIEW OF THE MAIN RESULTS

The promise of NIRS as non-invasive real-time monitoring of muscle oxygenation with a small and wireless device is interesting since it may bring a better understanding of the mechanisms involved in O₂ delivery and utilisation inside the muscles^{27,518,623}. Since restricting blood flow reduces the O₂ transport to the muscle, NIRS devices may allow a deeper understanding of the vascular responses to BFR or IPC. However, CW NIRS devices have several limitations for use in the general population, such as the influence of ATT on the results²⁶². Thus, it is necessary to assess the device's reliability within the same session (intra-day) to ensure consistency across multiple repetitions of the same stimulus (arterial occlusion). Additionally, it appears essential to investigate reliability between different sessions (inter-day) to ensure consistency of the results over time. Therefore, the first study of the present thesis aimed to test the intra- and inter-day reliability, at rest, of the NIRS Portamon devices and associated parameters (TSI, O₂Hb, HHb and tHb). Within-session results showed good to excellent reliability for maximums and minimums of TSI, O₂Hb, HHb and tHb, with low variability between occlusions of the

same session. The intra-day reliability of NIRS-derived parameters maximums and minimums at rest has rarely been investigated in the literature⁵⁴⁹. The TSI_{\min} ($36.6 \pm 7.9 \%$) and TSI_{\max} ($80.2 \pm 2.8 \%$) reached after arterial occlusion in McManus *et al.* (2018) are close to our results of TSI_{\min} (G2: $31.41 \pm 9.76 \%$; G3: $25.57 \pm 9.96 \%$) and TSI_{\max} (G2: $75.35 \pm 4.88 \%$; G3: $73.48 \pm 4.89 \%$)⁶⁹⁰. However, no results of reliability are provided for TSI after arterial occlusion in this study⁶⁹⁰. The good to excellent reliability found in our study is noteworthy since it demonstrates that only one occlusion – reperfusion cycle is necessary to provide a reliable minimum and maximum. Moreover, reliable minimums and maximums help to define a reliable maximal physiological range calculated as the difference between the minimum (0 % tissue oxygenation) and maximum (100 % tissue oxygenation). Thus, NIRS signal may be expressed as a percentage of this calibration^{629,690}. Contrary to within-session, between-session results did not show good reliability for most of the measurements. Our results are in agreement with the findings of Lacroix *et al.* (2012) for O_2Hb and tHb maximums⁶⁹¹. The authors found an ICC of 0.63 and 0.31, whereas we reported an ICC of 0.53 and 0.40 for O_2Hb and tHb maximums, respectively. Additionally, the authors also reported similar between-session CVs for O_2Hb (6.68 %) compared to 5.10 % in our study. As opposed to our findings, McLay *et al.* (2016) have reported excellent reliability for intra-day (ICC = 0.92) and inter-day (ICC = 0.94) reoxygenation rate⁶³⁰. A first explanation of the difference between these findings and our results is the reoxygenation rate calculation. Indeed, McLay *et al.* (2016) have calculated the reoxygenation rate as the upslope of a 10 sec window immediately following cuff release⁶³⁰. In our study, the reoxygenation rate has been calculated as the upslope between the start and the end of the reoxygenation curve, defined as the highest point during PORH. In addition, the authors have used FD-NIRS devices placed on the *tibialis anterior* muscle (lower leg), when CW-NIRS devices placed on the brachioradialis muscle (forearm) have been used in our study. The discrepancy between our findings and the literature is highlighted by the way ICCs are reported in reliability studies of NIRS-derived parameters. In our study, 50

% of within-session and none of the between-session measurements of TSI% reperfusion slope are reliable using the lower bound of the 95 % CI to indicate reliability⁶³¹. Without the 95 % CI, 89 % of within-session and 22 % of between-session measurements would be considered reliable. This noteworthy difference underscores the importance of reporting the 95 % CI in reliability studies.

As the first study shows that within-session NIRS assessments are reliable, it is possible to investigate the effects of different occlusion pressures on muscle oxygenation. This is particularly interesting since a great variability in methodological aspects has been observed between studies, inducing heterogeneous results⁴¹. The pressure in the inflatable cuffs is one of the varying parameters between studies, and its effects on muscle oxygenation should be investigated. In IPC, both arbitrary and individualised pressures are used, with arbitrary pressures typically higher than individualised ones to ensure blood flow occlusion for all participants, regardless of their physiological characteristics^{41,426}. However, using higher pressures increases the risk of discomfort and complications (see Chapter 3 - Methodologies and recommendations to occlude blood flow, p. 103)^{446,489,692}. Therefore, employing individualised pressures, which also completely occlude blood flow, should produce similar effects on muscle oxygenation as arbitrary pressures, while reducing risks and discomfort. Thus, the second study aimed to test the peripheral haemodynamic responses to different occlusion pressures using NIRS. Since arterial inflow was not occluded, G1 (50 mmHg) showed a lower and slower deoxygenation and reoxygenation compared to G2 (SBP + 50 mmHg) and G3 (250 mmHg). These results are consistent with the findings of Cunniffe *et al.* (2017) who have reported greater deoxygenation for complete arterial occlusion (180 mmHg) compare to partial arterial occlusion (140 and 160 mmHg)⁶⁹³. No differences were found between G2 and G3 in the quantity of deoxygenation and reoxygenation, which is opposed to the hypothesis of a dose-response effect⁶⁹³. However, a significantly faster reoxygenation only for TSI was found in G3 compared to G2. The difference in reperfusion speed between G2 and G3 could also

result from greater vasodilation inducing a higher blood flow⁶⁹⁴. However, the speed of reoxygenation for TSI is the only significant difference between G2 and G3 when the results of the two sessions are averaged. Therefore, these findings need to be investigated in future studies to confirm or not our results. Since higher pressures could induce greater discomfort and higher risks of complications^{446,489,692}, the use of higher pressures should be justified (*see Chapter 3 - Methodologies and recommendations to occlude blood flow, p. 103*). This study showed that both suprasystolic (absolute and relative) pressures induce comparable deoxygenation and reoxygenation. In addition, using absolute pressure does not take into account individual characteristics⁴²⁵, potentially inducing unnecessary high pressure in the normotensive population and insufficient stimulus in hypertensive patients²⁷³. Therefore, the use of lower individualised pressure based on SBP should then be preferred during IPC procedure compared to using higher absolute pressure.

The use of individualised pressure is also recommended for BFR training, when associated with both resistance and aerobic training. Mouser *et al.* (2017) demonstrated that blood flow is similarly reduced between 40 % and 90 % of LOP at rest, which questions the benefit of using higher pressures during exercise⁸⁴. Conversely, Mattocks *et al.* (2017) showed a greater increase in AOP immediately after high pressure (90 % of AOP) elbow flexion exercises compared to lower pressures (10 % and 20 % of AOP)⁶⁹⁵. Consequently, greater pressure increased the cardiovascular response, which is consistent with the dose-response effect of BFR on $\dot{V}O_2$ kinetics response during HEV exercise at 40 % and 50 % of LOP³⁴⁷. In acute low-intensity cycling (40 % of $\dot{V}O_{2peak}$), a BFR at 80 % of LOP induced significantly greater increases in HR, $\dot{V}E$, tissue deoxygenation and blood $[La^-]$ responses compared to BFR at 60 % of LOP⁶⁹⁶. A higher pressure (80 % of LOP) is also associated with improvement of vascular function, as evidenced by greater vascular conductance after eight weeks of resistance training (elbow flexions), unlike the groups with low pressure (40 % of LOP) and without pressure⁶⁹⁷. Bell *et al.* (2018) found a reduced number of elbow flexions per set under high pressures (80 % of

AOP) versus lower pressures (40 % of AOP) ⁶⁹⁸. These results are also supported by Dankel et al. (2017), who reported greater fatigue but similar electromyographic activation during elbow flexions at 20 % of 1RM with a BFR at 80 % of AOP compared to 40 % of AOP ⁶⁹⁹. A recent meta-analysis showed that 60 % LOP induces higher myoelectric activity than 40 % LOP, resulting in greater type II fibre recruitment and earlier onset of fatigue ⁷⁰⁰. Thus, while higher BFR levels elicit more pronounced cardiovascular responses and adaptations, they also lead to earlier fatigue. Lower pressures, in contrast, are associated with reduced discomfort and pain ^{695,696}. Earlier onset of fatigue and greater discomfort might limit the participant's ability to perform high-intensity aerobic exercise with high pressure, reducing the overall exercise volume. Consequently, aerobic training with different degrees of BFR may not only be beneficial compared to non-BFR training but might also induce distinct haemodynamic and cardiorespiratory adaptations among different pressures.

The objective of the third study was to test the effectiveness of combining HIIT with BFR at 40 % or 60 % of LOP in eliciting cardiovascular and respiratory adaptations following 4 weeks of training. The results on anthropometric data showed a significant reduction in fat mass and an increase in muscle mass after training across all groups (G1: HIIT+SHAM; G2: HIIT+BFR at 40 % LOP; and G3: HIIT+BFR at 60 % LOP). Haematological analysis showed a reduction in HcT and Hb, and an increase in post-exercise $[La^-]$ with no difference between groups. Endurance performance in all groups during the maximal incremental test was enhanced, as shown by the increases in MAP and TTE. The absence of difference between groups contrasts with early work on BFR that showed greater ($P < .05$) improvements in TTE with the BFR-trained leg, compared to the control leg after 4 weeks of training ⁶⁸². However, the protocol used in this study implied training sessions of 45-min bouts at low intensity. More recent studies have been shown in agreement with our findings since significant improvements in MAP have been found after 4 weeks of HIIT on a cycle ergometer ⁶⁵³ or on a treadmill ⁶⁵⁷, to the same extent for both BFR and non-BFR groups.

Cardiorespiratory adaptations showed a rightward shift of GET and RCP in all groups, a sign of a successful training programme³⁶⁶. Indeed, a higher absolute exercise intensity was sustained after training before solicitation of anaerobic glycolysis and H⁺ accumulation in skeletal muscle. However, $\dot{V}O_{2max}$ has not been increased in any groups of our study, which is consistent with the findings of Keramidas *et al.* (2012), who have showed no significant increases in $\dot{V}O_{2max}$ in both BFR and non-BFR groups after 6 weeks of training (3 sessions/week)²⁷⁶. Contrary to our findings in $\dot{V}O_{2max}$, when BFR is coupled with running, either on a treadmill or during small-sided games, an increase of $\dot{V}O_{2max}$ is found after only 3 to 4 weeks^{310,658}. The differences between our results and those presented by Amani-Shalamzari may arise from the initial $\dot{V}O_{2max}$ of participants (13 % lower than in the present study), from the length of intervals (≥ 2 -min vs. 90-s in our study)⁶⁸³, or from the pressure applied by the cuffs. In addition, a significant increase in $\dot{V}O_{2max}$ has been found in both HIIT and HIIT+BFR groups following twelve weeks of training⁷⁴. These changes were accompanied by increases in \dot{Q}_{max} in both groups, which remained unaffected in all groups in our study. These results are consistent with the findings of a recent meta-analysis that found larger effects of moderate to long-term ($\geq 4 - 12$ weeks) intervention on $\dot{V}O_{2max}$ ^{147,683,701}. In addition, the training effectively increases cycling efficiency when comparing pre- and post-training baseline intensity values obtained during the submaximal test. The same effort (100 W for males or 50 W for females) required less $\dot{V}O_2$ after training, as shown by the lower $\dot{V}O_2$ for the first and second baseline bouts and the lower HEV $\dot{V}O_{2baseline}$.

Submaximal adaptations are thought to be mainly driven by a greater O₂ delivery to the muscles induced by greater angiogenesis and capillary density^{317,332,333}, and greater O₂ utilisation by the muscles (oxidative enzyme activity)^{317,339,340}. As explained in the present manuscript (*see Chapter 1 – Inducing cardiovascular and haematological adaptations with BFR, p. 58*), BFR associated with aerobic exercise is known to stimulate the expression of key factors regulating the activation of the angiogenesis signalling pathway, such VEGF, HIF-1 α , and PGC1- α

^{213,676}. BFR has also shown effectiveness in increasing capillary density, which has the potential to enhance microvascular perfusion capacity ²⁴⁸. However, this significantly greater efficiency following HIIT+BFR at 60 % of LOP is not found during MOD or HEV exercise. This finding could be explained by the fact that both MOD and HEV intensities have been increased between pre- and post-training submaximal tests. A beneficial effect of high pressure versus lower pressure of BFR seems consistent with the literature ^{347,695,696,698}. However, no evidence of significant differences between BFR at 60 % of LOP compared to BFR at 40 % of LOP in our study are shown in any parameter. This could be due to the fact that the relative reduction of blood flow decreases with exercise, as the blood pressure rises during exercise, especially associated with BFR ⁷⁰². In other words, a sufficient pressure to reduce blood flow at the beginning of exercise, may not be sufficient at the end of exercise ⁶⁹⁵. Consequently, increasing the occlusion pressure across the successive repetitions of high-intensity bouts may held a constant BFR. In addition, the use of handheld sphygmomanometer to inflate cuffs during training sessions present limitations such as a loss of pressure with time, a lack of precision due to muscle contractions and no automatic regulation of pressure during exercise. Therefore, the choice of an autoregulated BFR device may have prevent these limitations and amplified the difference between BFR at 40 % and 60 % of LOP ⁴³².

In conclusion, the three scientific contributions presented in this thesis have provided answers to the research questions outlined earlier (*see Aim of the thesis, p. 137*). Restricting the blood flow, whether at rest or during exercise, has induced physiological changes in the O₂ transport pathway.

Firstly, inducing ischaemia in the arm induces microvascular changes in the muscle oxygenation, which were evaluated with good to excellent reliability within a single session in a general population using the non-invasive Portamon NIRS device. The arterial occlusion induces a consequent deoxygenation and a PORH responses, which could be assessed using parameters such as maximums, minimums

or AUC. While the literature supports the reliability of reoxygenation and deoxygenation rates^{630,703}, our study did not confirm these findings⁵⁴⁹.

Secondly, at rest, while the pressure applied by the cuff was sufficient to occlude arterial blood flow, the magnitude of pressure (SBP + 50 mmHg or 250 mmHg) did not induce significant differences in muscle oxygenation responses. Similarly, applying either 40 % or 60 % of LOP during exercise did not result in significant differences in cardiovascular, respiratory or haemodynamic responses after 4 weeks of HIIT + BFR programme.

Finally, these findings suggest that within the range of pressures used across the three studies, variations in pressure appear to have an equivalent effect on the components of the O₂ transport pathway, in the specific contexts assessed in this thesis. Notably, participants were able to complete all sessions involving repeated strenuous exercise bouts (90 s at 90 % of $\dot{V}O_{2max}$, see *STUDY 2*, p. 166), while demonstrating improvements in muscular efficiency, as evidenced in the results of the third study (see *STUDY 3*, p. 183). Consequently, these results indicate that applying BFR during HIIT sessions is feasible without compromising the quality of the work performed during the session. Moreover, the present results suggest that the protocol could be further optimised to take advantage of the combined benefits of HIIT on cardiovascular adaptations and BFR on vascular function. It is noteworthy also that using HIIT + BFR at 60 % of LOP has shown promising effects on submaximal parameters, underscoring the need for further research on the impact of the magnitude of pressure during BFR.

LIMITATIONS OF THE STUDIES

The main limitation of the three studies included in the present thesis is the inclusion of participants from the general population, without excluding participants having a fat layer greater than one-quarter the distance between the NIRS receptor and the most distant emitter ²⁶². Consequently, participants with inconsistent responses were excluded. While the number of participants was sufficient in the first two studies to maintain statistical power, this was not the case in the third study. Indeed, NIRS results in study 3 are briefly presented as they did not all show consistent responses to exercise. In the first two studies, the muscle oxygenation was estimated on the arm, which may limit the fat layer thickness. But, in the third study, the muscle oxygenation was assessed on the *vastus lateralis*, which has been shown to have greater skinfold thickness than biceps or triceps, for instance ⁷⁰⁴. Therefore, parameters such as deoxygenation and reoxygenation kinetics cannot be calculated accurately.

Another limitation shared by the first two studies of the present thesis is the inability to control the room temperature, as microcirculation is sensitive to skin temperature ⁷⁰⁵. Thus, values assessed by NIRS may have been affected by changes in room temperatures during the session, but also between sessions and participants ⁷⁰⁵. Nonetheless, no significant differences in temperature were found between groups or sessions. This limitation is not shared by the third article since data collection was done in another lab, in which temperature was maintained constant throughout the experiment.

The protocol of the third study compares two groups with BFR and a group without BFR. The occlusion pressures used in these two groups were 40 % and 60 % of LOP. Despite it being recommended to assess the effectiveness of LOP by performing an arterial occlusion ^{28,706,707}, the LOP in the third study was determined using a predictive equation ²⁷³. This equation has been used because the BFR cuffs (The Occlusion Cuff Pro, The Occlusion Cuff, Sussex, London, UK) are too narrow to

occlude the blood flow in the thigh completely. Therefore, instead of overpassing cuff inflator capacities, which could provide inconsistent and biased pressure values, the decision to use an equation was made. Consequently, the pressure in the cuffs may have differ from the recommendations for aerobic exercise (40 – 80 % of LOP)²⁸, and potentially induce the same restriction between the groups with 40 % and 60 % of LOP. Thus, it may explain that no significant differences were found between groups.

The submaximal tests of the third study involve square-wave transitions, aiming to investigate effects of HIIT and HIIT+BFR on the O₂ kinetics response. Parameters of O₂ kinetics such as amplitude, TD or τ remained mostly unaffected by training, which differs from previous studies that found speeded kinetics after training^{317,333,621,708,709} and aerobic training with BFR³⁴¹. This divergence from literature could arise from the fact that MOD and HEV were increased between pre- and post-training submaximal tests. Indeed, MOD and HEV were based on GET and RCP calculated during pre- and post-training incremental maximal tests, which both increased due to training. Additionally, this discrepancy with literature findings may come from an excessive resistance applied during baseline bouts. Although baseline usually corresponds to unloaded cycling^{345,663,669}, baseline intensity in our study was set at 50 W for females and 100 W for males. Consequently, the “cardiodynamic phase” characterised by the rapid changes in HR, SV, and $\dot{V}E$ occurring in the first 15 – 30 s of exercise^{315,317} is altered, affecting the whole analysis of O₂ kinetics. The excessive baseline intensity is attributed to a misinterpretation of the experimental protocol’s methodological requirements. However, using higher absolute intensities enabled comparisons of pre- and post-training submaximal gas exchanges, independently of GET and RCP. This analysis showed greater cycling efficiency after training during baseline steps.

PERSPECTIVES

The second study of the present thesis has presented the muscle oxygenation responses to different levels of occlusion. Since within-session NIRS results are reliable, it is possible to compare different occlusion pressures within the same session with the BFR cuffs. While it could be done at rest^{84,131,509,510}, this assessment could also be done during exercise⁶⁹⁶. The effects of occlusion pressure during exercise on muscle oxygenation could be investigated through different exercise intensities, such as MOD and HEV domains. Because of muscular contractions, the results may differ from rest and low-intensity exercise and could provide insights to explain similarities or differences in the adaptations following a training protocol^{448,449}. In addition, comparing different degrees of BFR across increasing exercise intensity may help to select the most appropriate pressure to optimise training effects.

Mouser *et al.* (2017) have shown that the restriction of the blood flow depends on the cuff size and pressure, but the delay between the end of inflation and blood flow assessment is not provided⁸⁴. Indeed, the delay between the end of inflation and the beginning of exercise may affect muscle oxygenation and induce heterogeneous results. As shown by our second study, the deoxygenation lasted a few minutes before reaching a minimum. Then starting an exercise at the end of cuff inflation or at the end of deoxygenation results in a different quantity of O₂Hb (decrease of $\approx 17 - 19\%$). It is noteworthy that a reduction of blood flow or muscle oxygenation has been associated with fatigue^{700,710}. Therefore, whether the participant starts exercising right after cuff inflation, 10 s or 30 s after inflation should accelerate the onset of muscular fatigue. The delay between cuff inflation and maximal deoxygenation could be analysed by studying the deoxygenation kinetics. This method has already been used during repeated sprints by Buchheit and Ufland (2011)⁷¹¹ in normoxia and in different hypoxic training conditions including BFR²⁷¹. In their study, Solsona *et al.* (2022) compared the deoxygenation induced by

different hypoxic conditions. Similarly, this protocol could be adapted to explore the kinetics of deoxygenation and reoxygenation by varying occlusion pressures or by changing the time intervals between cuff inflation and the onset of exercise²⁷¹. Thus, a deeper understanding of haemodynamic responses during BFR aerobic exercise could be achieved, which would help in selecting more appropriate protocols. Furthermore, this analysis of muscle oxygenation kinetics, could be extended to cardiac and respiratory kinetics during exercise with BFR. Thus, $\dot{V}O_2$ and HR kinetics could be investigated during exercise with BFR or following a training period with BFR. The immediate response of TSI, $\dot{V}O_2$ and HR kinetics during exercise with BFR could provide insightful information on the aerobic energy provision. In addition, the speed of $\dot{V}O_2$ kinetics also influence the EPOC and the subsequent duration of recovery. Indeed, exercising with BFR at 40 %, 60 % or 80 % of LOP could influence $\dot{V}O_2$ kinetics as shown by Salzmann *et al.* (2021) who found a pressure effect between BFR at 40 % and 50 % on muscle and pulmonary $\dot{V}O_2$ kinetics³⁴⁷.

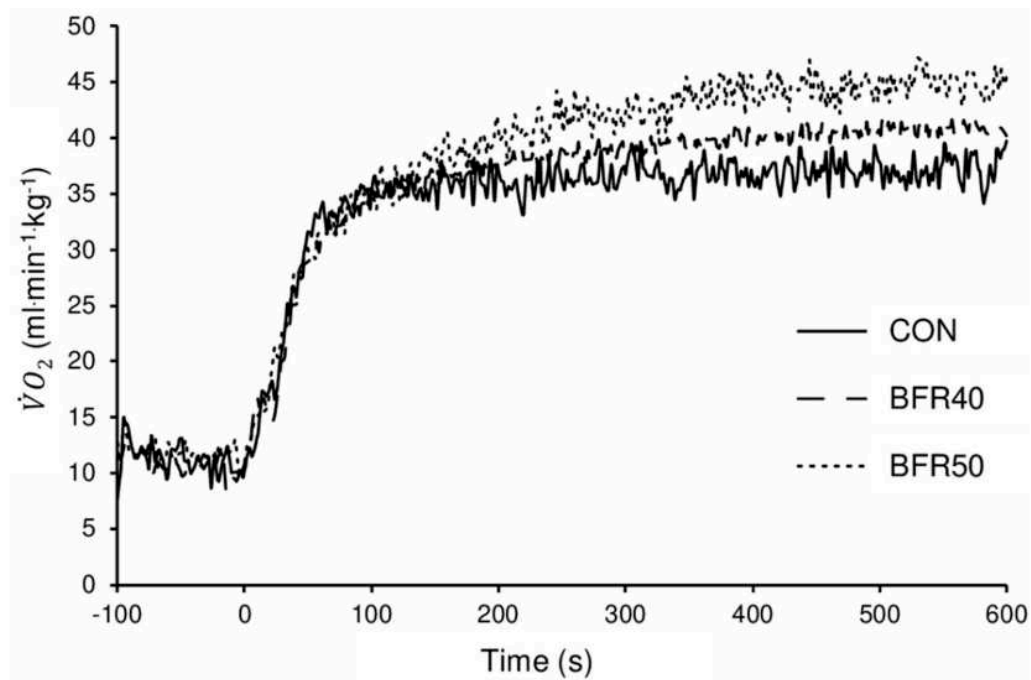


Figure 5-1: Representation of $\dot{V}O_2$ kinetics during a moderate intensity exercise with BFR at 40 % of LOP, BFR at 50 % of LOP and without BFR.

This figure shows an effect of pressure on the amplitude of $\dot{V}O_2$ at moderate intensity. BFR induces a $\dot{V}O_2$ SC, indicating an exercise intensity greater than GET. From Salzmann et al. (2021) ³⁴⁷.

As shown in Figure 5-1, a greater amplitude was found with BFR at 50 % of LOP than at 40 % of LOP than without BFR. In addition, the apparition of $\dot{V}O_2$ SC with BFR shifts the metabolic demand and could be impaired with the objectives of the trainer. In fact, if the aim of the training session is to work below the GET, it is necessary to find the appropriate BFR pressure and exercise intensity. Thus, implementing BFR in a training periodisation requires knowledge of both athlete's responses to BFR and methodological aspects of BFR.

BFR has first been demonstrated to be efficient in increasing force and hypertrophy when added to bodybuilding exercises^{57,58,450,712}. In addition, as shown by our third study and by the literature, BFR is effective in increasing MAP and aerobic capacity^{82,83}. The effects of BFR have mainly been investigated in tasks aiming to develop one physical quality at a time. However, most sports require the development of complementary physical qualities. Therefore, it could be interesting to investigate how BFR training could be integrated into a training periodisation, in which not only endurance or force should be developed. This approach has been investigated by Amani-Shalamzari (2019, 2020) with futsal training, where 3-a-side exercises performed with BFR induced significantly greater aerobic and anaerobic increases compared to the same training without BFR^{310,713}. In tennis, Brechbuhl *et al.* (2018, 2020) have demonstrated the efficiency of training in hypoxia to improve tennis-specific performance^{714–716}. However, training in hypoxia requires constraining and/or expensive equipment (hypoxic room or chamber) and limits its utilisation outside of the tennis federation or academy. Using BFR could be a more convenient approach to developing aerobic capacity and/or strength through tennis-specific exercises. While the cuff (depending on the cuff size and material used, (see *Chapter 3 - Methodologies and recommendations to occlude blood flow*, p. 97) could interfere with tennis gesture, BFR could be used during exercises in which precision is not the main outcome.

The perpetual quest of increasing one's performance in sports does not rely only on training but also on recovery⁷¹⁷. Before a competition, tapering consisting of a reduction of the training load, has been recommended to maximise performance gains. Training load could be reduced by either decreasing training volume, intensity, and frequency⁷¹⁸. Therefore, BFR could be an interesting approach to use during tapering when one of these parameters is reduced. Indeed, if the intensity is reduced, using low-intensity exercise with BFR could induce greater cardiovascular improvements compared to low-intensity exercise alone^{301,303,654}. In addition, it could induce cardiovascular adaptations close to high-intensity exercise

but exert lower fatigue than high-intensity⁷¹⁹. The same approach could be used with a reduced volume, as recommended in the literature for optimising tapering⁷¹⁸. Even with a reduced volume, BFR training could be more efficient than longer training without BFR³⁰¹. Thus, further investigations could be undertaken to provide a better comprehension of the usefulness of BFR in training periodisation and tapering in specific sports.

The effects of training loads on recovery could be monitored non-invasively by the analysis of the level of heart rate variability (HRV)⁶⁸⁶. HRV refers to the oscillation in the interval between consecutive heartbeats. HRV is influenced by the Autonomic Nervous System (ANS), which consists of the sympathetic and parasympathetic systems⁷²⁰. Analysing beat-to-beat fluctuations in HR provides a sensitive, quantitative, and non-invasive measure of cardiovascular control functioning⁷²¹. HRV is becoming one of the most used training and recovery monitoring tools in sport sciences⁷²², since it is a robust method to obtain valuable data concerning physiological responses to exercise⁷²². The possibility of applying HRV on a variety of sports is interesting since cardiovascular autonomic regulation is an important determinant of training adaptations⁷²³, thus it is meaningful in all types of exercise or populations.

The analysis of the level of HRV in association with BFR training could be particularly relevant since BFR has been shown to impact both cardiovascular (see *Chapter 1 – Inducing cardiovascular and haematological adaptations with BFR, p. 37*) and nervous system. Indeed, BFR has been shown to increase the metabolites accumulation and the acidification of the environment compared to the same task without BFR. This could contribute to increase the rate of discharge of group III and IV muscle afferents^{724–726} and may stimulate chemoreceptors, affecting the group III/IV muscle afferents signal to the Central Nervous System (CNS)⁵⁷. Several studies have investigated the effects of BFR on HRV, showing the effectiveness of BFR in improving the predominance of the parasympathetic branch of the autonomous

nervous system⁷²⁷⁻⁷²⁹. However, HRV has only been investigated in a few studies when coupled with aerobic training, evidence with daily follow-up is then lacking^{727,730}. In addition, the effects of cuff pressure on parameters of HRV have not been investigated, although this parameter could provide useful information on the choice of pressure.

Another way of assessing the autonomic control of cardiovascular system is the evaluation of baroreflex sensitivity (BRS)⁶⁸⁷. It has been shown that changes in the characteristics of baroreflex function reflect alterations in the autonomic control of the cardiovascular system⁷³¹. The arterial baroreceptor reflex system plays a dominant role in preventing short-term wide fluctuations of arterial blood pressure^{687,732}. Arterial baroreceptors provide the central nervous system with a continuous stream of information on changes in blood pressure (which are sensed by the stretch receptors in the wall of the carotid sinuses and aortic arch), on the basis of which efferent autonomic neural activity is dynamically modulated^{687,733,734}. Activation of arterial baroreceptors by increased systemic arterial pressure raises the discharge of vagal cardioinhibitory neurons and reduces the discharge of sympathetic neurons to the heart and peripheral blood vessels. This leads to bradycardia, decreased cardiac contractility, peripheral vascular resistance, and venous return. Conversely, decreased systemic arterial pressure deactivates baroreceptors, enhancing sympathetic activity and inhibiting vagal activity, resulting in tachycardia, increased cardiac contractility, vascular resistance, and venous return^{687,733,734}. BFR has been shown to increase the blood pressure during exercise compared to a non-BFR exercise, in both young and older adults^{735,736}. Therefore, BFR should influence the blood pressure regulation centre, that could be monitored through the evaluation of BRS. In addition, BFR has been shown to reduce significantly the systolic, diastolic and mean arterial pressures after 4 to 12 weeks, whereas no significant changes were found in the groups without BFR^{729,737-739}. These findings highlight the importance of monitoring the parameters of autonomic control of the cardiovascular system, such as BRS and HRV. These parameters could be assessed non-invasively at rest or

during exercise through a continuous blood pressure and HR monitoring, with a finger cuff (Finapres Medical Systems, Amsterdam, The Netherlands) and a simple 3-lead ECG (Electrocardiography; non-medical) or HR monitor belt (at least 1000 Hz)⁷⁴⁰.

Finally, another perspective of research that could be considered is the association between individuals' genetic profile and their responsiveness to BFR training. Indeed, genetic determinants have an essential role in individual responsiveness to diverse exercise modalities^{741,742}. Robust associations have been found between well-studied genes and exercise trainability or enhancement of exercise performance after training^{741,742}. Furthermore, an extensive number of genes exerts influence over a spectrum of physiological dimensions encompassing energy metabolism, cellular processes, protein synthesis, hormonal regulation, and enzymatic interactions⁷⁴³. Genes such as angiotensin-converting enzyme (ACE), Cytochrome c oxidase complex IV (COXIV) or PGC-1 α are associated with improvements in $\dot{V}O_{2max}$, whereas genes such as mTOR or VEGFA are mostly accounting in strength improvements⁷⁴³. A genetic profiling of participants may be considered to better understand responsiveness to BFR training and subsequent adaptations to training^{743,744}.

GENERAL CONCLUSION AND RECOMMENDATIONS



Whether at rest or during exercise, reducing the blood flow has shown effectiveness in inducing cardiovascular and/or respiratory adaptations. Indeed, reducing the blood flow disturbs the whole O_2 transport chain, which activates signalling pathways and haemodynamic responses. Endurance training with BFR has been shown effective enhancing parameters of the cardiovascular system, with a direct impact of maximal and submaximal O_2 uptake. However, studies are still lacking to prove the beneficial effect of applying BFR during HIIT. In addition, more research could be conducted about the effects of BFR pressure on physiological responses, using innovative and non-invasive technologies such as NIRS or cardiac impedance.

Given the evidence in the literature, the results of the studies presented in this thesis and the experience in the laboratory, several recommendations could be made: the pressure in the cuff should be individualised, and based on AOP when it is possible. The same cuff for estimating AOP and for training must be used. When it is not possible to precisely determine AOP, LOP should be estimated using a predictive equation, but the precision is reduced. The pressure in the cuff does have an impact on the perceived discomfort and high pressure may lead to participants

withdrawal. It is then necessary to select a pressure adapted to the studied population. Nevertheless, occlusion can be progressively increased during training, similar to training loads. BFR training, particularly when combined with HIIT, can be excessively strenuous for sedentary or less active individuals.

The field of BFR is an intriguing and fascinating journey across the cardiovascular, respiratory and nervous systems. It does disrupt with traditional exercise and force scientists and coaches to redesign their training programmes. Therefore, the field of BFR holds great promise for future research to fully unlock its potential.

SYNTHESE EN FRANÇAIS DU MANUSCRIT

Note explicative :

Cette synthèse, d'une quinzaine de pages, offre une traduction en français des principaux éléments abordés dans le manuscrit de thèse. Elle vise à refléter fidèlement le contenu original sans introduire de nouveaux éléments ni modifications. Les références bibliographiques citées dans cette synthèse sont identiques à celles utilisées dans le manuscrit.

INTRODUCTION

L'oxygène (ou dioxygène ; O₂) est capté lors de la respiration et circule dans les artères grâce au travail du cœur, afin d'atteindre tous les organes et tissus pour produire de l'énergie ². Ainsi, l' O₂ est indispensable au maintien de la vie et aux contractions musculaires. Par conséquent, l'idée de s'entraîner sous un flux sanguin réduit semble étonnante, voire complètement insensée. Cependant, depuis plus de trois décennies, la pratique du préconditionnement ischémique (IPC) au repos et de la restriction du flux sanguin (BFR) pendant l'exercice a démontré des améliorations notables de la performance, malgré leur caractère non conventionnel ^{3,4}. Ces méthodologies, bien que distinctes, convergent dans leur capacité à induire des adaptations physiologiques tant centrales que périphériques, en diminuant le flux sanguin. Ce processus entraîne des perturbations des paramètres cardiaques et vasculaires, ainsi qu'une altération de l'oxygénation des muscles squelettiques.

Lorsque l'apport de O₂ aux muscles squelettiques est altéré, soit par une diminution de la disponibilité en O₂ dans l'environnement (c'est-à-dire hypoxie), soit par une réduction du flux sanguin (c'est-à-dire ischémie), la perfusion en O₂ des

muscles est diminuée^{15,16}. Une disponibilité réduite de O₂ accélère l'apparition de la fatigue lors d'exercices en hypoxie¹⁷⁻¹⁹. Par conséquent, limiter la disponibilité en O₂, que ce soit par ischémie ou hypoxie, altère la performance, déplaçant la production d'ATP vers le métabolisme anaérobie et augmentant ainsi la fatigue musculaire²¹⁻²³.

Au cours de l'activité physique, on observe une réduction des niveaux de O₂ dans les muscles squelettiques, et l'étendue de cette diminution est influencée par l'intensité de l'exercice²⁴⁻²⁶. Cependant, lorsque l'exercice est effectué en hypoxie ou en ischémie, l'oxygénation des tissus montre une diminution plus prononcée que dans des conditions de normoxie¹⁶. L'oxygénation des tissus reflète alors l'équilibre entre l'utilisation de O₂ et son approvisionnement, traduisant la capacité oxydative des muscles squelettiques²⁷.

Présentation de l'IPC et du BFR

L'IPC se caractérise par de courtes périodes d'interruption du flux sanguin (ischémie), suivies de sa restauration (reperfusion), au repos. Cette méthode a d'abord été utilisée en clinique car elle permet d'améliorer la protection du cœur lors d'épisodes prolongés d'ischémie³⁰. Son utilisation s'est ensuite étendue au domaine du sport et de la performance. L'efficacité de l'IPC peut dépendre de divers facteurs tels que le type d'exercice spécifique, le protocole d'IPC utilisé, ainsi que les caractéristiques individuelles des athlètes^{42,46}. Par conséquent, davantage de recherches sont nécessaires pour comprendre les mécanismes sous-jacents contribuant aux effets de l'IPC sur la performance physique.

La méthode BFR consiste à diminuer le flux sanguin vers un muscle en appliquant un dispositif de constriction externe, tel qu'un brassard de pression sanguine ou un garrot, pour exercer une compression mécanique sur les vaisseaux sanguins sous-jacents⁵⁶. L'objectif de cette compression mécanique est de restreindre le retour veineux du sang vers le cœur, ce qui limite le processus de ré-

oxygénation et entraîne l'accumulation de métabolites ainsi que l'apparition précoce de la fatigue. Le BFR se distingue des autres techniques d'entraînement impliquant une occlusion vasculaire complète^{57,58}. Dans le BFR, le flux sanguin est partiellement restreint pendant l'exercice, plutôt que totalement occlus, maintenant ainsi la circulation, car la pression exercée par les brassards est inférieure à celle nécessaire pour induire une occlusion artérielle.

L'exercice aérobie de faible intensité réalisé avec BFR influence le transport de l' O_2 des poumons aux mitochondries en induisant des adaptations cardiaques et une croissance capillaire, améliorant ainsi les échanges d' O_2 dans les muscles, la consommation maximale d'oxygène ($\dot{V}O_{2max}$) et le temps jusqu'à l'épuisement (TTE)^{16,74,79-81}. Les entraînements à haute intensité (HIIT) réalisés avec BFR ont également montré des bénéfices sur les adaptations cardiovasculaires et la performance⁸³, et pourraient constituer une méthode d'entraînement efficace pour les athlètes de haut niveau⁴. Cependant, l'efficacité du HIIT avec BFR comparée à l'entraînement d'endurance seul reste à démontrer.

L'amélioration du $\dot{V}O_{2max}$ après des entraînements BFR pourrait dépendre de différences méthodologiques entre les études. En effet, plusieurs aspects méthodologiques visant à induire une ischémie peuvent affecter le niveau de réduction du flux sanguin. Parmi les différentes études sur l'entraînement BFR présentes dans la littérature, une large gamme de types de brassards est utilisée, avec différents matériaux, formes, tailles et technologies⁴³¹. Par conséquent, chaque type de brassard fournit un stimulus différent, même si la pression dans le brassard est la même. De plus, la sélection de la pression constitue également une source de biais, car des pressions arbitraires ou personnalisées peuvent être utilisées. Le rôle de la pression a été étudié à la fois sur les membres supérieurs et inférieurs⁸⁴, mais une large gamme de pressions a été recommandée²⁸. Ainsi, les conséquences de l'utilisation de faibles ou de hauts pourcentages de pression personnalisée sur les adaptations cardiovasculaires et respiratoires restent à étudier.

Tout comme l'entraînement en hypoxie, le BFR couplé à l'entraînement aérobie a montré des résultats prometteurs dans l'amélioration des adaptations cardiovasculaires et respiratoires⁸². Cependant, une grande partie de la littérature reste à explorer concernant les effets des variations méthodologiques sur les adaptations cardiovasculaires et respiratoires.

Structure de la thèse

L'objectif principal de cette thèse est d'explorer les réponses et adaptations cardiaques, vasculaires et respiratoires à différents niveaux de restriction du flux sanguin. Cette investigation met en lumière les perturbations de l'apport et de l'utilisation de l' O_2 dans les muscles, que ce soit au repos avec des occlusions complètes ou pendant l'exercice après un entraînement avec des occlusions partielles.

La thèse se concentrera sur les effets de l'entraînement BFR sur les composantes de la chaîne de transport du O_2 , à travers la relation entre la structure et l'efficacité cardiaque, le flux sanguin et l'oxygénation musculaire. Ensuite, les effets de l'entraînement BFR sur chacune des composantes de la chaîne de transport du O_2 , en mettant l'accent sur les améliorations du $\dot{V}O_{2max}$ et de l'efficacité du $\dot{V}O_2$ sous-maximal seront discutés. De même, les technologies utilisées pour estimer les adaptations cardiovasculaires et respiratoires à l'entraînement aérobie couplé au BFR seront examinées. Ainsi, les technologies disponibles telles que NIRS, l'impédance cardiaque ou les analyseurs de gaz pour évaluer l'oxygénation musculaire, les réponses cardiovasculaires et les échanges gazeux seront décrites. Les méthodologies d'induction de l'IPC et du BFR étant hétérogènes, les aspects méthodologiques relatifs à la réduction du flux sanguin seront abordés. Les effets du type de matériel choisi et de la pression utilisée pour gonfler les brassards seront présentés afin de mieux comprendre les divergences observées dans la littérature.

Ce manuscrit présente des contributions scientifiques originales centrées sur les réponses hémodynamiques et cardio-respiratoires, aiguës et chroniques, à différentes restrictions du flux sanguin au repos ou pendant l'exercice. La première contribution scientifique a examiné la fiabilité intra- et inter-séance des dispositifs NIRS au repos, après occlusion vasculaire. Dans une deuxième étude, les effets de différents degrés d'occlusion artérielle seront étudiés, en utilisant à la fois des pressions arbitraires et individualisées. Ensuite, une troisième étude se concentrera sur les adaptations hémodynamiques et cardio-respiratoires après quatre semaines d'entraînement aérobie avec deux pressions d'occlusion individualisées différentes, ou sans BFR. Enfin, les résultats des trois contributions scientifiques seront discutés dans une discussion générale, offrant une analyse critique des résultats dans le contexte de la littérature existante et esquissant des perspectives pour les recherches futures.

CADRE THEORIQUE

Chapitre 1 - Adaptations cardiovasculaires et hématologiques avec les entraînements BFR

Le $\dot{V}O_{2max}$ d'un individu est influencé par les capacités de perfusion et de diffusion de l'O₂. Par conséquent, augmenter le $\dot{V}O_{2max}$ nécessite d'améliorer les paramètres de transport de l'O₂ liés à la perfusion (flux sanguin et contenu artériel) ainsi qu'à la diffusion (DO₂).

Lors de l'exercice, le flux sanguin vers les muscles peut augmenter jusqu'à 100 fois par rapport à l'état de repos¹⁷⁴, facilité par les mécanismes de vasodilatation et l'augmentation du débit cardiaque (\dot{Q}). Le BFR s'est révélé efficace pour améliorer le \dot{Q}_{max} , grâce à une action combinée de stimulations mécaniques (surcharge volumique) et chimiques (voies de signalisation)²⁷⁵. Les résultats de l'étude de Tangchaisuriya et al. (2022) ont montré que l'augmentation du \dot{Q}_{max} lors d'entraînements traditionnels (CT), d'entraînements à haute intensité (HIIT) et de HIIT combiné au BFR est principalement due à une augmentation significative du volume d'éjection systolique maximal (SV_{max}) (+4 à 7 mL), tandis que la fréquence cardiaque maximale (HR_{max}) reste inchangée¹⁵⁷. L'entraînement avec BFR a démontré des améliorations de la fonction vasculaire, augmentant le flux sanguin post-occlusif et stimulant des marqueurs liés à l'angiogenèse tels que les protéines HIF-1 α , VEGF et PGC-1 α ^{163,193}. Cette angiogenèse, caractérisée par une croissance accrue des capillaires, présente un potentiel d'amélioration de la capacité de perfusion microvasculaire²⁴⁸. L'utilisation de mesures NIRS non invasives permet d'estimer indirectement l'angiogenèse après un entraînement avec BFR, évitant ainsi la nécessité de biopsies musculaires invasives^{266,267}. Les adaptations chroniques à l'entraînement avec BFR montrent des améliorations significatives de la fonction microvasculaire des muscles squelettiques, soulignant l'efficacité du BFR dans l'augmentation de l'apport en oxygène aux muscles, bien que la recherche sur les adaptations microvasculaires au BFR reste limitée^{74,274}.

Étant donné que le BFR améliore les fonctions cardiaques (HR, SV, \dot{Q}), vasculaires (angiogénèse) et diffusives (perfusion musculaire)^{69,193,248,277}, le transport et la diffusion de l' O₂ vers les mitochondries sont ainsi améliorées. Par conséquent, le BFR devrait être efficace pour augmenter le $\dot{V}O_{2max}$ et l'efficacité respiratoire submaximale.

Chapitre 2 – Amélioration de la capacité et de la puissance aérobie avec la restriction du flux sanguin

L'amélioration des conductances de perfusion et de diffusion de l' O₂ induites par les adaptations cardiovasculaires au BFR a des impacts substantiels sur le $\dot{V}O_2$ maximal and sous-maximal^{89,90}. Ces améliorations du système cardiovasculaire ont montré qu'elles retardent l'apparition des seuils de lactate et ventilatoire, qui sont des marqueurs sous-maximaux de la capacité aérobie. Ces seuils sont essentiels pour calibrer l'entraînement, car ils délimitent les domaines d'intensité de l'exercice⁴⁰⁵. Il a été démontré que le BFR influence ces domaines en ralentissant la cinétique du $\dot{V}O_2$ et en augmentant son amplitude^{352,421} pendant l'exercice. Cependant, après plusieurs semaines d'entraînement avec BFR, la cinétique de $\dot{V}O_2$ s'est accélérée, contrairement au groupe sans BFR³⁴¹. De plus, même avec un entraînement continu d'intensité modérée (MICT), le BFR s'est révélé efficace pour augmenter le $\dot{V}O_{2max}$, ce qui n'est pas le cas pour les groupes sans BFR^{302,422}. Cependant, lorsque le BFR est associé au HIIT, il semble efficace pour augmenter le $\dot{V}O_{2max}$ mais n'ajoute pas d'effet bénéfique supplémentaire par rapport au HIIT seul⁴²³. Compte tenu du nombre limité d'études sur les adaptations cardiovasculaires chroniques liées au HIIT+BFR ou au SIT+BFR, davantage de recherches sont nécessaires pour mieux comprendre si l'ajout de BFR au HIIT ou au SIT est réellement bénéfique. En outre, les divergences dans les résultats pourraient être dues à des différences méthodologiques entre les études, notamment concernant les types de brassards utilisés et les pressions sélectionnées.

Chapitre 3 – Méthodologies et recommandations pour occlure le flux sanguin

L'IPC et le BFR ont tous deux conduit à des réponses ou adaptations hétérogènes, pouvant découler de différences méthodologiques. La perturbation du flux sanguin est influencée par divers paramètres tels que la pression du brassard, le matériau et la largeur du brassard, ainsi que la pression artérielle et les caractéristiques des membres de l'individu ^{28,130,424,425}. Concernant les caractéristiques des brassards, une large gamme de largeurs de vessie (de 3,0 à 20,5 cm) a été rapportée dans la littérature associée au BFR. Ces différences de largeur influencent la pression transmise aux tissus plus profonds, les brassards plus larges nécessitant moins de pression que les brassards plus étroits pour induire la même restriction du flux sanguin ⁴³³. De plus, lorsque la largeur du brassard est trop petite (< 5 cm), la capacité du gonfleur peut être dépassée, entraînant une incapacité à restreindre de manière cohérente le flux sanguin ^{424,436,437}. Cette situation peut être exacerbée par le matériau de fabrication du brassard. En effet, les brassards peuvent être fabriqués en nylon ou en matière élastique, avec des propriétés physiques différentes en termes de transmission de la pression aux tissus. Il a été démontré que les brassards élastiques nécessitent une pression plus élevée pour occlure le flux sanguin par rapport aux brassards en nylon ^{439,440}. De plus, une étude a rapporté un inconfort accru avec les brassards élastiques, probablement dû à la pression plus élevée nécessaire ⁴³⁹. Enfin, le type de dispositif utilisé pour induire le BFR joue un rôle essentiel dans la pression appliquée aux membres, les dispositifs autorégulés fournissant un stimulus plus sûr et plus précis ⁴³².

En ce qui concerne la pression du brassard, elle peut être fixée de manière arbitraire (c'est-à-dire une même valeur absolue pour tous les participants) ou individualisée (c'est-à-dire relative aux caractéristiques individuelles, telles que la pression d'occlusion artérielle – AOP). Bien que l'utilisation d'une pression arbitraire soit plus simple, elle entraîne des réponses hémodynamiques différentes entre les participants et doit donc être évitée ²⁷³. L'utilisation de pressions absolues peut

également fournir un stimulus insuffisant ou, au contraire, induire une occlusion artérielle, ce qui doit être évité lors du BFR pour prévenir des effets indésirables ^{41,273,426}. Par conséquent, une pression individualisée basée sur des équations d'AOP ou de pression d'occlusion des membres (LOP) devrait être privilégiée pour fournir des réponses plus cohérentes et sûres ²⁷³. Puisqu'une réduction comparable du flux sanguin a été observée entre 40 % et 80 % de l'AOP ⁸⁴, il est recommandé d'utiliser entre 40 % et 80 % de l'AOP lors de l'entraînement avec BFR ²⁸. Cependant, les effets à long terme de l'entraînement avec 40 % ou 80 % de l'AOP n'ont pas encore été étudiés. De plus, il existe un manque de preuves sur l'impact des méthodologies BFR sur les adaptations cardiovasculaires, qui peuvent être surveillées à l'aide de diverses technologies tout au long de la chaîne de transport de l'O₂.

Chapitre 4 – Technologies utilisées pour surveiller les effets de l'occlusion sur les systèmes cardiovasculaire et respiratoire

Surveiller les réponses et adaptations cardiovasculaires et respiratoires à l'entraînement est essentiel pour mieux comprendre l'action du BFR. L'utilisation et l'importance de la spectroscopie proche infrarouge (NIRS) pour surveiller l'oxygénation musculaire squelettique sont abondamment documentées dans la littérature. Les dispositifs NIRS sont largement utilisés dans des contextes de repos et d'exercice en raison de leur coût modéré, de leur petite taille et de leur connectivité sans fil ^{27,518,623}. Cependant, il existe peu d'études sur leur fiabilité dans la population générale. La mesure de l'oxygénation musculaire à l'aide de la NIRS pourrait être associée à une évaluation du système cardiovasculaire. Plusieurs techniques ont été développées au fil des ans pour surveiller la fonction cardiovasculaire, mais la plupart sont invasives. Le développement de la technologie d'impédance cardiaque (PhysioFlow) offre une alternative non invasive et fiable à d'autres techniques, tout en étant particulièrement adaptée à l'exercice ⁵⁸⁹.

Enfin, l'évaluation non invasive des différentes étapes du transport de l'O₂ pourrait être complétée par l'analyse des échanges gazeux. Les effets de l'entraînement BFR sur les réponses et adaptations respiratoires peuvent être surveillés via un analyseur de gaz, permettant une analyse en temps réel, contrairement à l'ancienne technique des sacs Douglas. En combinant ces trois méthodes, un large spectre du système de transport de l'O₂ pourrait être évalué, offrant ainsi une meilleure compréhension des réponses physiologiques au BFR.

PROBLEMATIQUE GENERALE DE LA THESE

Le cadre théorique de la présente thèse montre que l'ischémie induite, que ce soit au repos via l'IPC ou pendant l'exercice avec le BFR, entraîne des effets aigus ou chroniques sur les composantes de la chaîne de transport de l'O₂. De plus, les variations méthodologiques entre les études, notamment concernant la pression sélectionnée, tendent à accentuer les résultats hétérogènes. Par conséquent, cette thèse vise à répondre aux questions suivantes :

1. Comment évaluer les effets d'une ischémie induite sur la réponse hémodynamique ?
2. Comment les composantes de la chaîne de transport de l'O₂ sont-elles affectées par une ischémie induite, au repos ou pendant l'exercice ?
3. Quels sont les effets aigus et chroniques des différentes pressions utilisées sur les réponses cardiovasculaires et respiratoires ?

Deux protocoles complémentaires, l'IPC et le BFR, ont été utilisés pour répondre à ces questions. Avant d'examiner les effets spécifiques de l'ischémie, il était essentiel d'établir la fiabilité de l'appareil utilisé pour les évaluations. Ainsi, la première étude a évalué la fiabilité des réponses en oxygénation musculaire à l'aide de la NIRS, sous différentes pressions. Le domaine de l'IPC présente des résultats hétérogènes quant aux effets bénéfiques sur la performance, l'une des principales variations entre les études étant la pression du brassard utilisée pour induire une occlusion artérielle. Pour répondre à cela, une deuxième étude a examiné la réponse aiguë de l'oxygénation musculaire à une ischémie induite par des pressions absolues et personnalisées. Enfin, le dernier objectif de cette thèse était d'étudier les effets de l'incorporation du BFR dans un programme d'entraînement aérobic à haute intensité. Ainsi, une troisième étude a examiné les effets d'un programme de HIIT réalisé avec différents niveaux de BFR sur les paramètres cardiovasculaires, hémodynamiques et respiratoires.

Globalement, cette thèse vise à étudier le rôle de la pression du brassard sur les réponses aiguës et chroniques des systèmes cardiovasculaire, hémodynamique et respiratoire à l'ischémie, que ce soit au repos ou pendant l'exercice.

CONTRIBUTIONS SCIENTIFIQUES

Etude 1: Fiabilité intra- et inter-sessions des appareils NIRS Portamon

Desanlis, J., Gordon, D., Calveyrac, C., Cottin, F., Gernigon, M. (2022). Intra- and inter-day reliability of the NIRS Portamon device after three induced muscle ischemias. *Sensors*, 22(14), 5165. **DOI:** [10.3390/s22145165](https://doi.org/10.3390/s22145165) | **IF WoS 2021:** 3,847 | **Quartile Scimago 2021:** 1

Introduction : La NIRS est une technologie innovante et non invasive utilisée pour explorer l'oxygénation musculaire. Son utilité et son importance dans la mesure des niveaux d'O₂ musculaire squelettique ont été largement détaillées dans diverses revues de littérature, parallèlement aux avancées en matière de technologie et d'oxymètres^{27,535,623,624}. Les études utilisant la NIRS ont démontré une forte fiabilité lors d'exercices statiques et dynamiques, ainsi qu'au repos après des occlusions artérielles au-dessus de la pression artérielle systolique (SBP)⁶²⁵⁻⁶³⁰. La fiabilité a été examinée pour des mesures intra-journalières et inter-journalières, mais dans la plupart des études, l'intervalle de confiance à 95 % (CI-95 %) du coefficient de corrélation intraclass (ICC) a été omis, ce qui peut potentiellement modifier l'interprétation du niveau de fiabilité⁶³¹. La fiabilité des valeurs maximales et minimales de la NIRS après occlusion, indiquant l'amplitude de désoxygénation pendant l'occlusion et la réoxygénation subséquente, est rarement rapportée. Par conséquent, cette étude vise à examiner la fiabilité intra-journalière et inter-journalière des paramètres dérivés de la NIRS, ainsi que les réponses maximales et minimales associées, après trois occlusions du flux sanguin à différents niveaux de pression, en état de repos.

Méthodes : Cinquante-trois jeunes hommes (moyenne ± écart-type : 23 ± 3 ans, 75 ± 12 kg, 178 ± 7 cm, 13,8 ± 4,1 % de masse grasse, 7,8 ± 5,7 heures d'activité physique par semaine) se sont portés volontaires pour participer et ont été attribués

aléatoirement à un groupe expérimental (G1, G2, ou G3). Selon le groupe expérimental, un brassard a été gonflé trois fois au niveau du bras supérieur gauche à 50 mmHg (G1), SBP + 50 mmHg (G2) ou 250 mmHg (G3). Les participants se sont rendus au laboratoire à deux reprises, dans un intervalle de 24 à 72 heures. Lors de la première visite, des mesures anthropométriques ont été enregistrées (taille, masse corporelle, et épaisseur du tissu adipeux), tandis que la longueur des membres a été mesurée pour déterminer l'emplacement du dispositif NIRS. Lors des deux séances, le même protocole a été suivi, consistant en une période de référence suivie de trois phases d'occlusion de 7 minutes entrecoupées de trois phases de reperfusion, où le brassard était dégonflé. Les valeurs maximales, minimales et le taux de réoxygénation ont été évalués après chaque phase d'occlusion, à l'aide d'un dispositif NIRS Portamon (Artinis, Elst, Pays-Bas) placé sur le muscle brachioradial gauche. La fiabilité a été évaluée avec les valeurs d'ICC et de CI-95 %, le coefficient de variation (CV) et l'erreur standard de mesure (SEM).

Résultats : Les résultats intra-séance ont montré une bonne à excellente fiabilité pour les valeurs maximales et minimales de TSI, O₂Hb, HHb et tHb, avec un coefficient de variation moyen de $2,58 \pm 1,82$ % et $5,14 \pm 3,81$ % pour les valeurs maximales et minimales, respectivement. Cependant, les résultats inter-séances n'ont pas montré une bonne fiabilité pour la plupart des mesures. De plus, le calcul du taux de réoxygénation a produit des valeurs non fiables pour les évaluations intra- et inter-séances.

Conclusion : Les mesures multiples des valeurs maximales et minimales au cours d'une seule séance semblent être fiables, ce qui montre qu'une seule mesure est nécessaire pour évaluer ces paramètres. Cependant, il est nécessaire d'être prudent lors de la comparaison des valeurs maximales, minimales et du taux de réoxygénation entre les séances. Ce manque de fiabilité pourrait être dû au fait que l'épaisseur du tissu adipeux (ATT) n'a pas été considérée comme un critère d'exclusion. Cependant, les participants étaient jeunes et pratiquaient généralement

une activité physique régulière et importante. Par conséquent, ces résultats remettent en question l'utilité des dispositifs NIRS pour la mesure de l'oxygénation musculaire à plusieurs reprises (par exemple, avant et après un entraînement) dans la population générale.

Etude 2 : Effets de la pression d'occlusion sur les réponses hémodynamiques mesurées par l'appareil NIRS Portamon

Desanlis J, Gordon D, French C, Calveyrac C, Cottin F and Gernigon M (2024)

Effects of occlusion pressure on hemodynamic responses recorded by near-infrared spectroscopy across two visits. *Front. Physiol.* DOI: [10.3389/fphys.2024.1441239](https://doi.org/10.3389/fphys.2024.1441239) | **IF**

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Introduction : Il a été démontré que l'IPC est bénéfique à des fins médicales et qu'elle a des effets positifs sur l'amélioration des performances. Cependant, une exposition prolongée à une occlusion du flux sanguin peut entraîner des effets indésirables délétères^{632,633}. Ces effets négatifs découlent de la compression des tissus sous le brassard gonflé ou de l'ischémie des tissus⁶³³. La revue d'O'Brien et Jacobs (2021) montre une hétérogénéité considérable dans les niveaux de pression utilisés pour induire une ischémie⁴¹. Deux méthodes principales pour déterminer la pression du brassard sont observées dans la littérature : une pression absolue ou une pression individualisée basée sur la SBP⁴¹. Les pressions absolues varient généralement de 180 mmHg à 250 mmHg (180, 185, 200, 220, 225, 240, 250 et >300 mmHg)⁴¹. En revanche, les pressions individualisées vont de la valeur de la SBP à SBP + 50 mmHg (SBP, 120 % de la SBP, et SBP + 10, 15, 20, 30, 40 ou 50 mmHg)⁴¹. Cependant, l'utilisation d'une pression absolue peut induire des changements hémodynamiques hétérogènes entre les participants en raison des caractéristiques individuelles (telles que la SBP)²⁷³. En outre, des études récentes ont suggéré que la SBP + 50 mmHg devrait être privilégiée pour atteindre une occlusion complète du flux sanguin lors de l'utilisation de pressions individualisées^{489,634}. Par conséquent,

le choix entre une pression absolue ou individualisée pour l'occlusion du flux sanguin peut entraîner des variations dans les perturbations hémodynamiques. La NIRS est une méthode viable pour évaluer les réponses hémodynamiques périphériques, tant au repos que pendant l'exercice, et pour quantifier l'amplitude de la désaturation induite par des pressions absolues (250 mmHg) ou individualisées (SBP + 50 mmHg) ²⁶¹. Ainsi, cette étude avait pour objectif d'évaluer les réponses hémodynamiques périphériques mesurées par NIRS lors d'occlusions partielles ou complètes du flux sanguin, avec des pressions absolues ou individualisées.

Méthodes : Quarante-six jeunes hommes (moyenne \pm écart-type : 23 \pm 3 ans, 75 \pm 12 kg, 178 \pm 7 cm, 13,8 \pm 4,1 % de masse grasse, 7,8 \pm 5,7 heures d'activité physique par semaine) se sont portés volontaires pour participer et ont été répartis aléatoirement en trois groupes expérimentaux (G1, G2 ou G3). Selon le groupe expérimental, un brassard a été gonflé trois fois au niveau du bras supérieur gauche à 50 mmHg (G1), SBP + 50 mmHg (G2) ou 250 mmHg (G3). Les participants ont visité le laboratoire à deux reprises, dans une période de 24 à 72 heures. Lors de la première visite, des mesures anthropométriques ont été enregistrées (taille, masse corporelle et épaisseur du tissu adipeux), tandis que la longueur des membres a été mesurée pour déterminer le placement du dispositif NIRS. Lors des deux séances, le même protocole a été suivi : une période de référence, suivie de trois phases d'occlusion de 7 minutes, entrecoupées de trois phases de reperfusion (10 minutes) où le brassard était dégonflé. Les valeurs maximales, minimales et le taux de réoxygénation ont été évalués après chaque phase d'occlusion, à l'aide d'un dispositif NIRS Portamon placé sur le muscle brachioradial gauche. L'aire sous la courbe (AUC) a été calculée pour chaque occlusion à partir des valeurs absolues (TSI_{AUC} , $[O_2Hb]_{AUC}$, $[HHb]_{AUC}$, ΔHb_{AUC}), afin d'évaluer l'amplitude des variations d'oxygénation pendant la désaturation et la réoxygénation ^{627,635,636}. Une analyse de variance mixte (ANOVA mixte) avec correction post hoc de Bonferroni a été utilisée pour déterminer les différences de désaturation et de reperfusion tissulaire entre les groupes.

Résultats: Les résultats ont montré une amplitude significativement plus faible de désaturation (TSI_{AUC}) pour le G1 comparé aux groupes G2 ($P < .001$) et G3 ($P < .001$), sans différence entre G2 et G3. La désaturation était plus rapide pour les groupes G2 et G3 par rapport au G1 ($P < .001$), mais aucune différence n'a été observée entre G2 et G3. Cependant, le groupe G3 a montré une réoxygénation significativement plus rapide, uniquement pour l'indice de saturation tissulaire (TSI_{slope}), par rapport au groupe G2 ($P = 0,010$), mais sans différence dans la vitesse de récupération de la désoxyhémoglobine ($[HHb]_{slope}$) ou dans l'amplitude de l'hyperémie réactive post-occlusive (PORH). En dehors de la vitesse de réoxygénation du TSI, les groupes G2 et G3 ont produit des réponses hémodynamiques au repos comparables mesurées par NIRS.

Conclusion: Les données ont montré une désaturation plus importante et plus rapide induite par des pressions suprasystoliques comparées au groupe G1, mais n'ont pas révélé de différences significatives entre les deux groupes ayant des pressions d'occlusion au-dessus de la SBP (G2 et G3). La réoxygénation était plus rapide pour les pressions suprasystoliques par rapport à G1, les résultats indiquant qu'une pression plus élevée (250 mmHg contre SBP + 50 mmHg) utilisée pour occlure le flux sanguin pourrait induire une réponse de réoxygénation plus rapide dans les 30 secondes suivant le dégonflage du brassard. Cependant, cette différence entre G2 et G3 n'a pas été retrouvée dans le TSI_{AUC} , indiquant que les deux pressions suprasystoliques induisent une désaturation comparable. Par conséquent, la plupart des résultats ne montrent pas de différences significatives entre G2 et G3, ce qui remet en question l'utilité de recourir à une pression absolue plus élevée. Il est donc recommandé d'utiliser une pression individualisée basée sur la SBP pour induire une ischémie pendant l'IPC.

Étude 3 : Adaptations cardiovasculaires et respiratoires après quatre semaines d'entraînement HIIT avec BFR

Introduction : La revue systématique récente de Chua et al. (2022) a inclus seulement sept études portant sur les adaptations chroniques du HIIT + BFR sur la performance aérobie ⁸³. La plupart des études ont trouvé des augmentations significatives du $\dot{V}O_{2max}$ dans le groupe HIIT + BFR, mais seulement trois études ont démontré une supériorité du HIIT + BFR par rapport au HIIT seul pour augmenter le $\dot{V}O_{2max}$ ^{454,637,638}. Des augmentations $\dot{V}O_{2max}$ ont été observées après aussi peu que 8 séances d'entraînement sur 4 semaines ^{637,638}. La divergence des résultats peut provenir de méthodologies hétérogènes pour induire le BFR. En effet, les aspects méthodologiques sont cruciaux avec le BFR puisque la perturbation du flux sanguin dépend non seulement de la quantité de pression appliquée au brassard, mais aussi des caractéristiques du brassard et de l'individu ²⁸. Il est recommandé que la pression d'occlusion soit individualisée en utilisant la pression d'occlusion des membres (LOP) plutôt qu'absolue pour améliorer la cohérence des résultats et la sécurité des participants ²⁷³. Des équations prédictives de la LOP ont été établies en fonction de la circonférence des membres et de la largeur du brassard ^{273,446,508}. Parmi les sept études de la revue de Chua et al. (2022) ⁸³, deux ont utilisé le BFR post-exercice, ce qui ne restreint pas le flux sanguin pendant l'exercice, impliquant ainsi des mécanismes physiologiques différents ^{637,638}. Parmi les cinq études restantes, aucune n'a utilisé la LOP pour sélectionner la pression d'occlusion, comme recommandé par McEwen et al. (2019) ²⁷³. Une revue récente recommande d'utiliser une pression comprise entre 40 % et 80 % de la LOP lorsqu'elle est combinée à un exercice aérobie ²⁸. Cependant, les résultats dans la littérature tendent à suggérer qu'une pression supérieure à 40 % de la LOP peut ne pas être nécessaire pour induire des adaptations physiologiques lors de l'entraînement en résistance avec BFR. Néanmoins, les effets de la pression d'occlusion pendant le HIIT + BFR restent encore flous ⁴²³. Par conséquent, cette étude visait à évaluer l'efficacité du HIIT + BFR à 40 % ou 60 % de la LOP pour induire des adaptations cardiovasculaires et respiratoires après 4

semaines d'entraînement.

Méthodes : Vingt-sept participants sains et actifs (11 femmes, 16 hommes ; 19 ± 7 ans ; 175 ± 8 cm ; 67 ± 8 kg ; 17 ± 8 % de masse grasse ; 9 ± 5 h d'activité physique par semaine) se sont portés volontaires pour participer et ont été répartis aléatoirement dans l'un des trois groupes d'entraînement : G1 (HIIT + 20 mmHg ; SHAM), G2 (HIIT + BFR à 40 % de la LOP), et G3 (HIIT + BFR à 60 % de la LOP). Les participants ont effectué 12 séances d'entraînement sur 4 semaines en utilisant un cyclo-ergomètre. Chaque groupe suivait le même protocole d'entraînement consistant en 4 à 7 séries de 90 secondes à 90 % de la puissance atteinte au $\dot{V}O_{2max}$, entrecoupées de 120 secondes de récupération. Les brassards ont été placés sur les deux cuisses et dégonflés pendant les phases de récupération. Les paramètres cardiovasculaires et respiratoires (\dot{Q} , oxygénation musculaire et échanges gazeux), les données anthropométriques et la composition sanguine (Hct, Hb, concentrations de lactate sanguin et de glucose) ont été enregistrés avant et après l'intervention d'entraînement. Ces paramètres ont été évalués au repos, lors d'un test d'exercice progressif, ou à travers un test sous-maximal composé de deux périodes d'exercice à charge constante de 6 minutes dans les domaines d'exercice modéré et intense.

Résultats : L'entraînement a entraîné une réduction significative de la masse corporelle ($P = .03$), de la masse grasse ($P < .001$) et du pourcentage de masse grasse ($P < .001$) dans tous les groupes, sans différences significatives entre les groupes. Des réductions significatives du Hct ($P < .001$) et de l'hémoglobine (Hb) ($P = .04$) ainsi qu'une augmentation du lactate sanguin post-exercice ont été observées après l'entraînement, sans différences entre les groupes. La pression artérielle moyenne (MAP) ($P < .001$) et le temps jusqu'à l'épuisement (TTE) ($P < .001$) ont augmenté sans différences entre les groupes, mais le $\dot{V}O_{2max}$ n'a pas changé dans aucun des groupes ($P > .05$). L'entraînement a induit un déplacement significatif vers la droite du seuil ventilatoire d'exercice (GET) ($P < .001$) et du seuil de compensation respiratoire (RCP) ($P < .001$), sans différences entre les groupes. Le \dot{Q}_{max} et le TSI

sont restés inchangés après l'entraînement ($P > .05$). Dans le test sous-maximal, une diminution de la fréquence cardiaque (HR) ($P < .001$) a été observée après l'entraînement, sans différences entre les groupes. De plus, des réductions du $\dot{V}O_2$ ($P < .001$) et du $\dot{V}CO_2$ ($P < .001$) ont été observées dans les deux phases de base du test sous-maximal uniquement pour le groupe G3.

Conclusion: Dans la présente étude, les trois groupes ont augmenté leur MAP et leur TTE, mais pas leurs paramètres cardiovasculaires et respiratoires maximaux (\dot{Q} , TSI, ou $\dot{V}O_{2max}$). Associé au protocole HIIT actuel, l'ajout du BFR ne semble pas constituer un stimulus puissant pour des adaptations cardiovasculaires et respiratoires maximales. Cependant, les résultats du test sous-maximal suggèrent que le HIIT + BFR (en particulier à 60 % de la LOP) peut conduire à une plus grande efficacité de l'entraînement. Des investigations supplémentaires sont nécessaires pour optimiser l'intégration des protocoles BFR et HIIT afin d'obtenir des adaptations d'entraînement optimales.

DISCUSSION ET PERSPECTIVES

Le rôle de l'entraînement avec une disponibilité limitée en oxygène dans l'amélioration des performances sportives est bien établi ⁶⁸⁸. Le stress oxydatif aigu induit par l'hypoxie locale peut activer des voies de signalisation qui favorisent des adaptations cellulaires et vasculaires ¹³⁰.

L'entraînement avec BFR semble également bénéfique lorsqu'il est combiné à un programme aérobie ⁸², avec une augmentation du $\dot{V}O_{2max}$ observée après plusieurs semaines (> 3 semaines) ^{28,80,420}. Cependant, la littérature manque d'informations sur les processus physiologiques impliqués et les considérations méthodologiques qui conduisent à ces augmentations de $\dot{V}O_{2max}$. La pression appliquée par les brassards pendant l'exercice peut affecter la réponse hémodynamique de manière différente, et par conséquent produire des adaptations cardiovasculaires variées ^{84,347}. Ainsi, les effets de la pression des brassards sur les réponses hémodynamiques doivent être étudiés afin d'optimiser la pression pour maximiser les adaptations cardiovasculaires tout en garantissant la sécurité.

Le principal objectif de cette thèse était d'examiner les réponses hémodynamiques et respiratoires aiguës et chroniques à différents niveaux de restriction du flux sanguin (en mmHg). Ce travail s'est donc concentré sur l'apport et l'utilisation de l'O₂ dans les muscles, éléments centraux du principe de Fick ⁸⁷. Pour ce faire, les paramètres physiologiques suivants ont été surveillés : les changements dans l'apport et l'utilisation de l'O₂ dans les muscles squelettiques à l'aide de la NIRS ; l'apport d'O₂ par le cœur à l'aide de l'impédance cardiaque ; et la capacité du corps à absorber, distribuer et utiliser cet O₂ en évaluant le $\dot{V}O_2$.

Synthèse des principaux résultats

Le potentiel de la NIRS en tant que technologie non invasive et en temps réel pour surveiller l'oxygénation musculaire est intéressant, car il permet de mieux comprendre les mécanismes impliqués dans l'apport et l'utilisation de l'oxygène dans les muscles^{27,518,623}. La première étude de cette thèse visait à tester la fiabilité intra- et inter-journalière, au repos, des dispositifs Portamon NIRS et des paramètres associés (TSI, O₂Hb, HHb et tHb). Les résultats intra-séance ont montré une bonne à excellente fiabilité pour les valeurs maximales et minimales de TSI, O₂Hb, HHb et tHb, avec une faible variabilité entre les occlusions d'une même session. Contrairement aux résultats intra-séance, les résultats inter-séances n'ont pas montré une bonne fiabilité pour la plupart des mesures. Nos résultats concordent avec ceux de Lacroix et al. (2012) pour les valeurs maximales d'O₂Hb et tHb⁶⁹¹. Les auteurs ont trouvé un ICC de 0,63 et 0,31, tandis que nous avons rapporté un ICC de 0,53 et 0,40 pour les maximums d'O₂Hb et tHb, respectivement.

La deuxième étude visait à tester les réponses hémodynamiques périphériques à différentes pressions d'occlusion à l'aide de la NIRS. Étant donné que le flux sanguin artériel n'était pas occlus, le groupe G1 (50 mmHg) a montré une désaturation et une réoxygénation plus faibles et plus lentes comparativement à G2 (SBP + 50 mmHg) et G3 (250 mmHg). Ces résultats sont cohérents avec ceux de Cunniffe et al. (2017), qui ont rapporté une désoxygénation plus importante pour une occlusion artérielle complète (180 mmHg) par rapport à une occlusion partielle (140 et 160 mmHg)⁶⁹³. Aucune différence n'a été observée entre G2 et G3 quant à la quantité de désoxygénation et de réoxygénation, ce qui contredit l'hypothèse d'un effet dose-réponse⁶⁹³.

Cette étude a montré que les pressions suprasystoliques (absolue et relative) induisent des désaturations et réoxygénations comparables. De plus, l'utilisation d'une pression absolue ne prend pas en compte les caractéristiques individuelles⁴²⁵, ce qui pourrait induire une pression inutilement élevée chez les populations

normotendus et un stimulus insuffisant chez les patients hypertendus ²⁷³. Par conséquent, l'utilisation de pressions individualisées basées sur la SBP devrait être préférée pendant la procédure IPC par rapport à l'utilisation de pressions absolues plus élevées.

L'objectif de la troisième étude était de tester l'efficacité de combiner le HIIT avec le BFR à 40 % ou 60 % de la LOP pour induire des adaptations cardiovasculaires et respiratoires après 4 semaines d'entraînement. Les performances d'endurance dans tous les groupes, lors du test incrémental maximal, se sont améliorées, comme l'ont montré les augmentations de la MAP et du TTE. Des études récentes ont montré des résultats cohérents avec les nôtres, car des améliorations significatives de la MAP ont été observées après 4 semaines de HIIT sur un cyclo-ergomètre ⁶⁵³ ou sur un tapis roulant ⁶⁵⁷, à la même échelle pour les groupes avec et sans BFR. Les adaptations cardiorespiratoires ont montré un déplacement vers la droite du GET et du RCP dans tous les groupes, signe d'un programme d'entraînement réussi ³⁶⁶. Cependant, le $\dot{V}O_{2max}$ n'a augmenté dans aucun des groupes de notre étude, ce qui est cohérent avec les résultats de Keramidas et al. (2012), qui n'ont pas montré d'augmentation significative du $\dot{V}O_{2max}$ dans les groupes BFR et non-BFR après 6 semaines d'entraînement (3 sessions/semaine) ²⁷⁶. Contrairement à nos résultats sur le $\dot{V}O_{2max}$, lorsque le BFR est associé à la course à pied, que ce soit sur un tapis roulant ou pendant des activités physiques en petits groupes, une augmentation du $\dot{V}O_{2max}$ est observée après seulement 3 à 4 semaines ^{310,658}. Les différences entre nos résultats et ceux présentés par Amani-Shalamzari pourraient provenir du $\dot{V}O_{2max}$ des participants (13 % inférieur à celui de notre étude), de la durée des intervalles (≥ 2 minutes contre 90 secondes dans notre étude) ⁶⁸³, ou de la pression appliquée par les brassards. Ces changements se sont accompagnés d'augmentations du \dot{Q}_{max} dans les deux groupes, ce qui n'a pas été affecté dans notre étude. En outre, l'entraînement a efficacement amélioré l'efficacité du cyclisme, comme en témoignent les valeurs d'intensité de base avant et après l'entraînement lors du test sous-maximal. Le même effort (100 W pour les hommes

ou 50 W pour les femmes) nécessitait moins de $\dot{V}O_2$ après l'entraînement, comme le montre la réduction du $\dot{V}O_2$ pour les deux premières phases de base et la diminution du $\dot{V}O_{2\text{baseline}}$ dans le domaine sous-maximal.

Principales limites des études

La principale limite des trois études présentées dans cette thèse est l'inclusion de participants issus de la population générale, sans exclure ceux ayant une couche de tissu adipeux supérieure à un quart de la distance entre le récepteur NIRS et l'émetteur le plus distant ²⁶².

Une autre limitation, partagée par les deux premières études de cette thèse, est l'incapacité de contrôler la température de la pièce, car la microcirculation est sensible à la température de la peau ⁷⁰⁵. Ainsi, les valeurs évaluées par NIRS pourraient avoir été affectées par des variations de température ambiante au cours de la séance, ainsi qu'entre les séances et les participants ⁷⁰⁵.

Le protocole de la troisième étude compare deux groupes avec BFR et un groupe sans BFR. Les pressions d'occlusion utilisées dans ces deux groupes étaient de 40 % et 60 % de la LOP. Bien qu'il soit recommandé d'évaluer l'efficacité de la LOP en effectuant une occlusion artérielle ^{28,706,707}, la LOP dans la troisième étude a été déterminée à l'aide d'une équation prédictive ²⁷³. Cette équation a été utilisée car les brassards BFR (The Occlusion Cuff Pro, The Occlusion Cuff, Sussex, London, UK) étaient trop étroits pour occlure complètement le flux sanguin dans la cuisse.

Perspectives de recherche

L'analyse du niveau de la variabilité de la fréquence cardiaque (HRV) en lien avec l'entraînement en BFR pourrait être particulièrement pertinente, car il a été démontré que le BFR impacte à la fois le système cardiovasculaire et le système nerveux. En effet, le BFR a montré qu'il augmentait l'accumulation de métabolites et l'acidification de l'environnement, comparé à la même tâche sans BFR. Cela pourrait contribuer à augmenter le taux de décharge des afférences musculaires des groupes

III et IV ⁷²⁴⁻⁷²⁶ et pourrait stimuler les chémorécepteurs, affectant le signal des afférences musculaires des groupes III/IV vers le système nerveux central (CNS) ⁵⁷. Plusieurs études ont examiné les effets du BFR sur la HRV, démontrant l'efficacité du BFR pour améliorer la prédominance de la branche parasympathique du système nerveux autonome ⁷²⁷⁻⁷²⁹. Cependant, la HRV n'a été étudiée que dans quelques études en association avec un entraînement aérobie, et les preuves d'un suivi quotidien manquent ^{727,730}. En outre, les effets de la pression des brassards sur les paramètres de la HRV n'ont pas été investigués, bien que ce paramètre puisse fournir des informations utiles pour le choix de la pression.

CONCLUSION GENERALE

Que ce soit au repos ou pendant l'exercice, la réduction du flux sanguin a montré son efficacité pour induire des adaptations cardiovasculaires et/ou respiratoires. En effet, la réduction du flux sanguin perturbe l'ensemble de la chaîne de transport de l'O₂, ce qui active des voies de signalisation et des réponses hémodynamiques. L'entraînement d'endurance avec BFR s'est révélé efficace pour améliorer les paramètres du système cardiovasculaire, avec un impact direct sur la consommation maximale et sous-maximale d'O₂. Cependant, il manque encore des études pour prouver l'effet bénéfique de l'application du BFR pendant le HIIT. De plus, davantage de recherches pourraient être menées sur les effets de la pression du BFR sur les réponses physiologiques, en utilisant des technologies innovantes et non invasives telles que la NIRS ou l'impédance cardiaque.

Compte tenu des preuves dans la littérature, des résultats des études présentées dans cette thèse et de l'expérience en laboratoire, plusieurs recommandations peuvent être faites : la pression doit être individualisée et basée sur l'AOP lorsque cela est possible ; utiliser le même brassard pour estimer l'AOP et pour l'entraînement ; lorsque l'AOP ne peut être précisément déterminée, la LOP peut être estimée à l'aide d'une équation prédictive ; la pression dans le brassard a un impact sur l'inconfort perçu, et une pression trop élevée peut entraîner l'abandon des participants. La pression doit donc être adaptée à la population étudiée et peut être augmentée progressivement au cours de l'entraînement, de manière similaire à la charge de travail. L'entraînement avec BFR, lorsqu'il est combiné au HIIT, est excessivement exigeant, notamment pour les individus sédentaires ou moins actifs.

Le domaine du BFR explore les systèmes cardiovasculaire, respiratoire et nerveux. Il perturbe les exercices traditionnels et oblige les scientifiques et les entraîneurs à repenser leurs programmes d'entraînement. Par conséquent, le BFR détient un grand potentiel pour les recherches futures afin de dévoiler pleinement ses avantages.

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