Near-infrared Spectroscopy (NIRS) Measurements in Cold Water Immersion.

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Covid-19 Impact Statement

The disruption caused by Covid-19 led to an inability to conduct face to face research and a restriction of lab access. As a result, a new research question was developed in order to adjust to these constraints, and secondary analysis was conducted on an existing dataset obtained prior to the pandemic. The author was not present for the collection of data which features in the experimental study of this thesis. Communication was established with the individuals involved and an understanding of the methodology was ensured.

Thesis Abstract

One of the most popular recovery strategies utilised by elite and amateur athletes is a form of cryotherapy called cold-water immersion (CWI). CWI is suggested to augment recovery from exercise induced muscle damage (EIMD) through temperature induced reductions in microvascular blood flow and tissue metabolism. However, a review of CWI literature highlights a lack of physiologic data regarding muscle blood flow and muscle metabolic responses to its application (Chapter 1). In addition, an overview of the near-infrared spectroscopy (NIRS) technique and measurements is then discussed, highlighting its ability to measure muscle blood flow and tissue metabolism during CWI. Subsequently, a systematic review investigates the application of NIRS measurements during CWI (Chapter 2). Contrasting changes in local muscle blood volume (ΔtHb) were reported during and following post-exercise CWI, with an increase in tHb explained as a cold-induced vasodilatory response whilst a decrease in tHb suggested the occurrence of peripheral vasoconstriction. The measurement of local muscle oxygen saturation (ΔTSI %) was also common during and following post-exercise CWI but arguably was inadequate for providing useful insight into muscle metabolic activity as there tended to be no change in TSI (%), or it was reported as a standalone measure. An experimental study using male university level long distance runners (n = 11) investigated the utility of a NIRS occlusion procedure to quantify measures of muscle blood flow (mBF) and muscle oxygen consumption (mVO_2) (Chapter 3). Measures were obtained pre- and post-CWI at two different temperatures (10 ° and 15 °C). By performing arterial occlusion, a similar reduction in mVO_2 was shown in both CWI temperatures. This is consistent with the notion that CWI is capable of reducing

muscle metabolism. NIRS derived mBF and mVO_2 extends current findings and understanding of the muscle physiological response to CWI.

Key Words: Cold-water immersion, Near-infrared spectroscopy, Muscle Blood Flow, Muscle Oxygen Consumption, Recovery.

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Abbreviations

%SmO_2	Muscle oxygen saturation
AO	Arterial occlusion
AR	Active recovery
ASA's	Arteriovenous anastomoses
ATP	Adenosine triphosphate
ATT	Adipose tissue thickness
CBV	Central blood volume
CBF	Local cutaneous blood flow
CK	Creatine Kinase
CIVD	Cold-induced vasodilation
CMJ	Counter-movement jump
CNS	Central nervous system
CRP	C-reactive protein
CWI	Cold-water immersion
CW-NIRS	Continuous wave NIRS
CVC	Cutaneous vascular conductance
CVP	Central venous pressure

Cytox Cytochrome Oxidase

DOMS Delayed onset muscle soreness

E-C coupling Excitation-contraction coupling

EIMD Exercise-induced muscle damage

EMG Electromyography

NIRS Near-infrared spectroscopy

FABF Femoral artery blood flow

FD-NIRS Frequency domain NIRS

FOR Functional overreaching

Hb Haemoglobin

Hb_{diff} Haemoglobin difference

HHb Deoxyhaemoglobin

HMb Deoxymyoglobin

HR Heart rate

LDF Laser doppler flowmetry

MAP Mean arterial pressure

Mb Myoglobin

mBF NIRS derived muscle blood flow

MRI Magnetic resonance imaging

mVO_2 NIRS derived muscle oxygen consumption

MVC Maximal voluntary contraction

NFOR Non-functional overreaching

O_2 Oxygen

O_2Hb Oxyhaemoglobin

O_2Mb Oxymyoglobin

PBC Partial body cryotherapy

PET Positron emission topography

Q Cardiac output

RF Rectus Femoris

ROM Range of motion

ROS Reactive oxygen species

RPE Rating of perceived exertion

SRS Spatially resolved spectroscopy

SSC Stretch shortening cycle

ST Shivering thermogenesis

StO_2 Tissue oxygen saturation

SV Stroke Volume

T_{core} Core body temperature

T_{muscle} Intramuscular temperature

T_{rec} Rectal temperature

T_{skin} Skin temperature

TD-NIRS Time domain NIRS

TOI Tissue oxygenation index

TT Time trial

tHb Total haemoglobin concentration

TSI Tissue saturation index

VL Vastus Lateralis

VJP Vertical jump performance

VO Venous occlusion

VOP Venous occlusion plethysmography

VO_{2max} Maximal oxygen uptake

Thesis Overview

Thesis Justification

The increasing physical demands of athletic competition, particularly in sports with a high fixture frequency has further exacerbated the physical and mental loads placed upon athletes. Athletes routinely perform recovery strategies as part of their post-exercise regimen in order to restore homeostasis on a physiological and psychological level and maintain the equilibrium between training/ competition stress and recovery. One of the most popular recovery strategies utilised by elite and

amateur athletes is a form of cryotherapy called cold-water immersion (CWI). A primary application of CWI is following strenuous exercise that may invoke exercise-induced muscle damage (EIMD); the objective here is for athletes is to ameliorate the negative symptoms associated with EIMD in the days following exercise which include: a loss of muscle force production capacity and range of motion and increases in swelling and muscle soreness. CWI is suggested to achieve this primarily through temperature-induced reductions in microvascular blood flow and tissue metabolism, which may help minimise oedema manifestation, inflammation, and secondary muscle damage. Despite the proposed mechanistic benefits, there is conflicting evidence over the efficacy of CWI for improving the recovery of muscle damage indices, i.e., muscle function, muscle soreness and blood markers, post-exercise. Furthermore, in studies observing a beneficial effect of CWI on recovery, there remains a lack of physiologic data, thereby making it difficult to ascertain the underlying mechanisms involved. Research studies have aimed to assess the physiological response to CWI but are limited to assessment of whole limb blood flow or deep conduit artery measurement. Tissue metabolism in response to cooling has largely been based off animal models and medical/surgical studies involving amputated and stored limbs and organs; consequently, the extent of reduction in intramuscular temperature required to alter metabolic activity remains unclear. As such, there is a requirement to further investigate the muscle physiological response to CWI. Near-infrared spectroscopy (NIRS) is a light-based technique that informs upon the relationship between oxygen delivery and extraction at the site of gas exchange in the muscle. NIRS offers non-invasive and continuous monitoring, and advancements in technology including portable wearable devices with telemetric capability have enabled local muscle oxygenation and haemodynamics to be

assessed in numerous sport disciplines. Researchers have also successfully waterproofed portable NIRS devices, demonstrating reliability of the measurement in aquatic environments. Accordingly, NIRS has potential to provide insight into the muscle physiological response during CWI. A small number of studies have utilised NIRS measurement during and following post-exercise CWI, reporting on local muscle blood volume (tHb) and muscle oxygen saturation (O_2). Changes in these measures have been considered a proxy for muscle blood flow and muscle metabolic activity, thereby posing useful interpretation in regard to the proposed mechanisms by which CWI benefits post-exercise recovery. However, current findings have yet to be collectively reviewed and compared with consideration to the specific exercise and recovery contexts utilised, or contextualised with regard to athletic recovery. Evaluating current literature may help to establish the significance of NIRS measures in CWI exercise studies. Arguably, the information currently reported from NIRS during CWI can be further enhanced through incorporating existing methodology in the form of venous occlusion and arterial occlusion. Performing venous occlusion of a limb enables quantification of muscle blood flow (mBF) whereas arterial occlusion enables quantification of muscle oxygen consumption (mVO_2). Studies have reported comparable values between this non-invasive approach and other established methods including plethysmography or invasive blood gas sampling. Incorporating this simple procedure pre- and post-CWI will offer further insight into alterations in muscle haemodynamics and metabolic activity as a result of CWI, thereby improving the current physiological evidence underpinning the rationale for its implementation following EIMD. Moreover, a lack of literature has compared different CWI protocols, i.e., immersion temperature and duration, on physiological measures of blood flow and muscle metabolism. Such

investigations may improve CWI optimisation to achieve a desired physiological response and/ or meet a specific recovery objective. Despite this potential, the utility of NIRS-occlusion methodology has yet to be assessed in CWI research.

Thesis Aims

This thesis aims to understand the applicability and utility of Near-infrared Spectroscopy as a measurement tool pre-, during- and post-Cold Water Immersion. In order for this to be achieved, two studies were conducted:

Study 1: Review investigating the application or use of NIRS with CWI.

Specific study aims:

1. Identify the specific exercise and recovery contexts where NIRS has been utilised alongside CWI application.
2. Interpret the NIRS findings with regard to underlying physiological mechanisms within the muscle.
3. Highlight potential limitations regarding the information currently obtained from NIRS within CWI research.

Study 2: The effect of CWI temperature on lower limb muscle blood flow and muscle oxygen consumption.

Specific study aims:

1. Explore the utility of NIRS-occlusion methodology for obtaining measures of muscle blood flow and muscle oxygen consumption pre- and post-CWI.
2. Investigate how different immersion temperatures influence muscle blood flow and muscle oxygen consumption responses.
3. Discuss findings with regard to the proposed mechanisms by which CWI aids post-exercise recovery.

Thesis Outline

Chapter 1 introduces CWI, with a focus upon; its application post-exercise; the reported recovery benefits following its use; and the underlying physiological mechanisms which potentially aid recovery, including: changes in peripheral body fluid and blood flow, a reduction in skeletal muscle metabolism and alterations in cardiovascular and thermoregulatory control. This is followed by an overview of the NIRS technique and NIRS measurements, with a focus upon arterial and venous occlusion. Chapter 2 is a review of the studies which have utilised NIRS with CWI. Chapter 1 and 2 form the rationale for the experimental study (chapter 3). Chapter 3: (a) explores the utility of a NIRS-occlusion methodology for obtaining measures of muscle blood flow and muscle oxygen consumption pre- and post-CWI; (b) investigates how different immersion temperatures may influence these measures. The thesis is concluded in Chapter 4 where the experimental findings are discussed and contextualised in relation to the review and wider literature. Finally, recommendations for future research are provided.

1

Introduction

1.1 The rising importance of recovery in modern sport.

Recovery is regarded as a multifaceted (e.g., physiological, psychological) restorative process relative to time and modulated by external load, individual response to stress, and often dictated by external athletic competition and demand (1). The increasing physical demands of athletic competition, particularly team sports (2) involving high fixture frequency, has further exacerbated the physical and mental loads placed on athletes (3). Athletes are now routinely exposed to longitudinal demands where in some cases there is only 48 h of recovery time between competitions. As such, the recovery time between successive competitions or training may be insufficient to allow athletes to fully regenerate, leading to fatigue (4). Fatigue may be defined as “an inability to complete a task” (5,6), and is derived from central and/or peripheral origins (7). Peripheral fatigue refers to processes which originate in muscle cells and directly impair muscle contractile function (7). Mechanisms which are postulated to contribute to peripheral fatigue either directly or through interactive effects include metabolic factors (e.g., adenosine triphosphate, inorganic phosphate, phosphocreatine, lactate) (8), diminished glucose or glycogen availability (8), ionic factors (e.g., K^+, Na^+, Ca^{2+}, Cl^-) (8), acidosis (8), hypoxia (9), reactive oxygen species (ROS) (8), and/or ultrastructural damage (8). When a reduced muscle force occurs during volitional contractions, it may also arise through a lowered drive from the motor cortex in the brain, i.e., central fatigue (7). This inhibition of motor drive (reduced motoneuron firing frequency and/or de-recruitment of motor units) may be due to peripheral feedback from working muscles, heart or lungs and/or input from higher centres in the central nervous system (CNS). Direct evidence is available for reduced motor drive during hypoxia (lowered oxygen levels) (10), hyperthermia (11), hypoglycaemia (lowered plasma glucose) (12) or

consequent to greater firing of group III and IV muscle afferents (13).

A continuous or severe imbalance of inadequate recovery and excessive physical and mental demands could initiate a cascade of deleterious conditions such as under-performance, non-functional overreaching, injury, and illness (14–18). Demands are further increased in athletes competing in continental leagues, play-off phases, international tournaments, and in specific circumstances such as the English Premier League that does not include a winter break (3) or in recent times the effect of the COVID-19 pandemic upon returning to sport (19). Increased athlete training and competition availability as a result of a reduction in injuries substantially improves the likelihood of success for the individual or team (20). Changes in injury occurrence also have a significant impact, particularly, financial implications (team underachievement and player salaries) of sporting organisations due to injury-related decrements in performance (21). Collectively, the increased physical demands of athletic competition and the rising importance of recovery have prompted sports teams and individual athletes to invest in bespoke personal support in an attempt to accelerate recovery.

1.2 Recovery strategies

A certain degree of fatigue, resulting in functional overreaching (FOR), is required to mediate adaptations to training (22,23). FOR refers to the short-term decrement of performance without signs of maladaptation, which occurs as a consequence of intensive training (1,23). When in a functionally-overreached state, the individual undergoes an adaptive response to the training stimulus (23). This process, referred to as supercompensation, is suggested to produce long term performance enhancement over time by continually imposing a new homeostatic challenge to the physiological systems involved (23,24). From a physiological perspective, the target

system(s) require a frequent application of training stimulus in order to adapt (23,24). However, excessive fatigue through insufficient recovery may negatively affect the training stimulus for adaptation (e.g., insufficient recovery will result in a decreased performance) (24), and increase susceptibility to non-functional overreaching (NFOR) (1,23), injury (17,18) and illness of players (18,25). FOR and NFOR can be thought of as different stages of a fitness-fatigue adaptive continuum (23). Whereas FOR may be considered to be a sufficient and necessary component of a training program and is often deliberately induced, NFOR describes a state of extreme overreaching that results from the continuation of extended periods of overload training, leading to a stagnation or decrease in performance which may persist for several weeks or months (23). Fatigue can be compensated with recovery strategies which serve to restore homeostasis on a physiological and psychological level (26). In order to regain the equilibrium between training stress (e.g., the extent of disruption in physiological homeostasis) and recovery, athletes frequently implement recovery strategies as part of their post-training or competition regime (27,28). There are various recognised recovery strategies used by elite athletes (29–33). A recent investigation reviewed commonly used recovery strategies in professional soccer and found that all teams were utilising at least one recovery strategy following games; however, the range of interventions used between teams was substantially different with water immersion (cold and hot), massage and foam rolling representing 74, 70 and 57% respectively (33). Work featured on athlete perceptions has also shown that athletes may not be aware of the intended effects of a specific recovery strategy on their physical status despite around two-thirds performing some type of recovery after activity (30).

It is imperative that the origin of fatigue is understood in order to most effectively

return the body to homeostasis following exercise. Furthermore, an understanding of the origin of fatigue may help with tailoring an appropriate recovery strategy to enhance the accelerated return to homeostasis (34). The increased focus on athlete recovery within professional sport has naturally been followed by many scientific investigations attempting to understand the efficacy of a range of commonly performed strategies (28,35,36). However, few studies have been able to demonstrate the efficacy of such strategies in improving recovery in athletes following training or competition (37–40).

Arguably, much of the positive evidence for recovery strategies lies with an enhanced perceptual outcome of recovery, often attributed to an athlete's belief in the modality or the placebo effect (41,42). Indeed, evidence exists whereby recovery strategies have not improved fatigue levels post-exercise further than that of the placebo condition (41,43,44). To date, research has traditionally focused on administering one recovery strategy at a time, whereas in the applied setting athletes are more likely to administer multiple strategies in varying sequences due to the number of strategies available, many of which lack apparent efficacy (40,45,46). Although extensive, the existing literature investigating the efficacy of recovery strategies lacks clarity and directional progression for practitioners and athletes alike (34). A large proportion of the data involves study designs investigating changes in physical performance and perceptual or muscle damage markers following an exhaustive exercise protocol or athletic competition (36,40). Methodological variances in the exercise performed, laboratory protocols detached from contextual performance, investigation of only the acute recovery response (0 – 72 h) and including sub-elite subject cohorts are possible reasons why inconclusive data exists, indirectly creating confusion for practical application (28,34).

This thesis will focus on a recovery strategy known as cold water immersion (CWI). Recent reports and surveys suggest CWI is one of the most commonly utilised acute recovery strategies used by team sport athletes (29–33). Within scientific literature, a number of interventional research studies and reviews (47–49) on the efficacy of CWI for aiding post-exercise recovery have been published. Discussed within the text is an overview of studies investigating the influence of CWI on post-exercise recovery, including muscle damaging exercise. Additionally, there is a main focus on the proposed mechanisms by which CWI is suggested to improve recovery and the physiological evidence base for such mechanisms are presented.

1.3 Origin of Cryotherapy (cold exposure).

CWI is a form of hydrotherapy and cryotherapy as it utilises the combined recovery effects of water immersion (hydrotherapy) and cold exposure (cryotherapy) to the body. Cryotherapy refers to the application of ice to parts of the body and has been historically used in the management of soft-tissue injury in first aid and post-surgical settings via the common application of ice packs or other icing modalities (50–52). Cryotherapy has been promoted in the immediate and rehabilitative care of soft-tissue injury. The application of ice to an injured area causes a reduction in the temperature of the underlying tissue. Immediately post injury, this reduction in tissue temperature is proposed to reduce tissue metabolism, thereby minimising secondary hypoxic injury (i.e., damage that occurs to the tissue through a lack of O_2), and the degree of tissue damage, swelling and inflammation (53,54) . Furthermore, ice causes acute analgesia to the local area on which its applied likely due to a slowing of nerve conduction velocity (55), which can relieve pain during subsequent injury rehabilitation and facilitate an earlier and more aggressive return to daily activity and exercise (54,56).

The rationale for using CWI as a post-exercise recovery strategy originates from previous clinical applications of cryotherapy post-injury. Athletes primarily utilise CWI following strenuous activity which may provoke a degree of exercise-induced muscle damage (EIMD), in an attempt to ameliorate any negative influence on subsequent exercise performance. The symptoms of EIMD manifest as a temporary reduction in the force-generating capacity of the muscle and an increase in delayed onset muscle soreness (DOMS) (35,57). The intensity of soreness and discomfort associated with EIMD increases within the first 24 h, peaks around 24 – 72 h, before subsiding and eventually disappearing 5 – 7 days after exercise (35). Consequently, it is well established that EIMD negatively affects athletic performance and causes a less than optimal training intensity. Despite the rationale for cryotherapy's clinical application following soft-tissue trauma, the basis for its use in exercise recovery is not straightforward. The aetiology of EIMD and soft tissue trauma are different (58); upon incurring EIMD there is an inflammatory response which occurs subsequent to the initial muscle damage, however, if left unaltered there is an increase in inflammation and secondary cell damage which persists into the days following exercise which is the reason symptoms of EIMD peak during this time period (59,60). The initial cause of muscle damage may also be attributed to primarily mechanical or metabolic determinants, depending on the modality of prior exercise and the type of stress incurred (35,36,57), posing further considerations for CWI application.

1.4 How does Exercise-Induced Muscle Damage (EIMD) occur?

Depending on the modality of high-intensity exercise performed, varied perturbations may be observed in mechanical muscle damage, oxidative stress and subsequent inflammation (36). Mechanical stress is imparted on the active muscle(s) during eccentric exercise (e.g., resistance exercise, plyometric exercise) due to eccentric

contraction requiring muscle fibres to produce tension whilst lengthening (61–63). This repeated action can cause physical disruption to the excitation-coupling system (E-C coupling), thereby contributing to muscle soreness and a loss of force production capacity (62,64); furthermore, damage to the muscle cell membrane (sarcolemma) and sarcoplasmic reticula increases fibre permeability to Ca^{2+}. The accumulation of intracellular Ca^{2+} activates proteases (enzymes which catabolise proteins) and inflammatory signalling cell processes (61,63). Whilst the inflammatory response promotes muscle fibre restoration following damage (65,66), it includes the infiltration of neutrophils and macrophages to scavenge cellular debris, which can cause secondary enzymatic injury in the surrounding healthy cells through lysosomal mechanisms (58). Oxidative stress occurs when performing high-intensity endurance or interval training and is associated with an increase in reactive oxygen species (ROS) (67). ROS are thought to play an important role as mediators of EIMD and inflammation (68); they are a highly reactive and can denature proteins and lipids meaning muscle cell structures such as the E-C coupling system and sarcolemma are destabilised (63,69,70). Endurance exercise at or near maximal intensities also leads to the accumulation of intramuscular metabolites (71). Collectively, increased fibre permeability along with metabolite accumulation increases the muscle cells osmolality (63), thereby promoting an increase in interstitial fluid in localised regions, a condition called oedema.

Oedema manifestation causes swelling within the muscle(s) which increases extracellular pressure and mechanical compression of the capillaries, thereby impairing O_2 and fuel substrate delivery and the removal of waste substances from muscle cells (63,72). The term secondary ischaemic injury encompasses these three distinct inadequacies (58) and expands upon previous theory (53,54) regarding

secondary hypoxic injury, which specifically refers to cell damage/death through a lack of O_2 availability. It is likely a combination of the aforementioned mechanisms that contribute to secondary muscle damage, bringing about further oedema and inflammation. The increase in oedema and inflammation is likely responsible for DOMS: the sensation of pain or discomfort in the muscle, which may restrict range of motion (ROM) and last for several days following EIMD (35,57). The rationale for applying post-exercise cryotherapy is based upon its potential to lessen tissue ischemia and, therefore, secondary injury (73). Figure 1.4 summarises the aforementioned cascade of events which occur as a result of mechanical and metabolic muscle damage pathways.

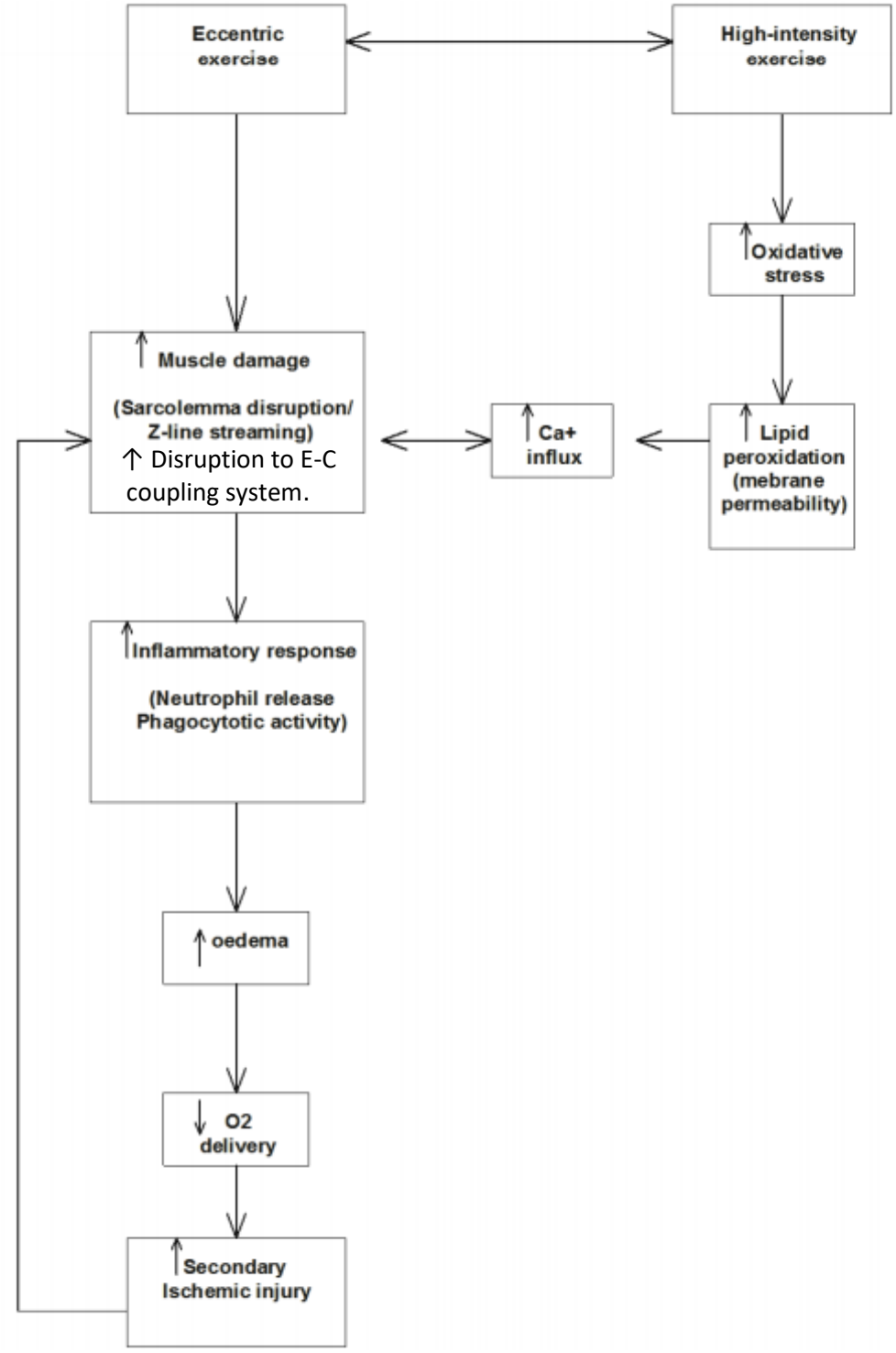

Figure 1.4. A simplified illustration of the mechanical and metabolic pathways contributing to EIMD. Ca^{+} Calcium ions; *E-C Coupling system* Excitation-contraction coupling system; O_2 Oxygen, ↑ increase in; ↓ decrease in.

1.5 Implementation of CWI post-exercise.

1.5.1 Acute utilisation of CWI for the recovery of muscle damage.

Studies have investigated the effectiveness of post-exercise CWI following exercise protocols consisting of isolated eccentric resistance exercise (74–77) (e.g., eccentric elbow contractions and eccentric leg press), whole-body plyometric movements (e.g., drop jumps and counter movement jumps) (78–80) and simulated team sport protocols and/or competitive matches (81–86). A common outcome associated with muscle damage is a reduction in muscle function, i.e., muscle strength and/or muscle power (35). The recovery of muscle function in the days following muscle damaging exercise is monitored using tests such as maximal voluntary contractions (MVC), vertical jump height, squat jumps, and sprint ability/performance (36). Generally, studies utilising eccentric resistance exercise and plyometric movements have shown negligible effects of CWI on the recovery of MVC (Table 1.5.1). For example, Eston & Peters (74) observed no difference in strength loss 0 – 72 h following eccentric maximal isokinetic contraction of the elbow, between the exercised arm undergoing CWI (15 min in 15 ˚C) and a control condition involving an unimmersed arm. Similarly, Paddon-Jones & Quigley (75) utilised a longer and colder CWI protocol (5 x 20 min in 5 ˚C, separated by 60 min) for the exercised arm. The authors reported no difference in isometric torque and isokinetic torque compared to the unimmersed arm 0 – 96 h following 64 eccentric elbow flexions. Additionally, Goodall & Howatson (78) and Howatson *et al*., (80) utilised an identical lower-body plyometric exercise protocol (5 x 20 drop jumps) and reported no difference in MVC for participants undergoing full lower-body CWI (12 min in 15 °C) compared to passive rest control when assessed 0 – 72 h following exercise (78), or following a repeated bout performed 14 – 21 days later (80).

Conversely, studies utilising intermittent sprint protocols or simulating team sport training and/or matches generally report an improved recovery of muscle strength (MVC), vertical jump performance (VJP), counter movement jump performance (CMJ) and/or sprint performance following post-exercise CWI (Table 1.5.1). For example, the recovery of MVC was improved at 24 h following a single competitive football match (90 min) when CWI (10 min in 10 °C) was performed post-match compared to thermoneutral immersion (10 min in 35 °C) (87). Bailey *et al*., (85) also reported improvements in the recovery of MVC at 24 and 48 h following the Loughborough-intermittent shuttle test when CWI (10 min in 10 °C) was performed post-exercise compared to a passive rest control. Following a basketball tournament involving four daily competitive matches, measures of muscle power (i.e., 20m sprint, VJP) showed the best recovery when intermittent CWI (5 x 1 min in 11 °C) was performed after each match compared to alternative recovery methods such as compression stockings and stretching (88). Furthermore, in a football tournament involving four daily competitive matches, there was an improvement in total running time and distance covered during each competitive match when CWI (5 x 1 min in 10 °C) was performed post-match compared to thermoneutral immersion (5 x 1 min in 34 °C) (84).

Overall, the recovery of jump performance (i.e., CMJ and VJP) following CWI has shown the most positive results from the aforementioned studies, indicating that CWI may be more effective for the recovery of the stretch-shortening cycle (SSC) movements rather than isolated concentric movements, such as those undertaken when assessing MVC (89). Indeed, reviews focusing on the use of post-exercise CWI within team sport suggest a beneficial effect on the recovery of sprint and jump performance (36,90,91) in the days following exercise (primarily 24 – 72 h). However,

the failure to observe an improved recovery of muscle function following eccentric resistance exercise or plyometric exercise in comparison to team sport exercise is likely attributed to differences in the exercise performed and the training status of the participants. For example, studies incorporating team sport training and/or competitive matches to monitor the recovery of muscle damage often do so with participants trained to a high standard (e.g., regional or national standard) and with numerous years training experience in their designated sports (82,84,87,88,92). On the other hand, studies utilising exercise protocols with a predominantly eccentric component (e.g., eccentric elbow contractions or plyometric exercise) have generally used physically active young adults (e.g., university students) not specifically trained in a sport or regularly competing at a high standard (76,78,80). It is well established that a single bout of eccentrically biased exercise can cause significant EIMD, particularly if the individual is unaccustomed to such activity (35). As such, it is likely that the degree of EIMD attained in participants performing eccentric exercise protocols is greater compared to those performing team sport activity, which may influence the effectiveness of CWI in facilitating recovery.

Alongside tests of muscle function, studies monitor indices of muscle damage, such as subjective ratings of muscle soreness (DOMS) and/or general fatigue, ROM measurements, muscle swelling and biomarkers of muscle damage and inflammation (e.g., serum CK, myoglobin, CRP concentration). Overall, the positive effects of CWI on the recovery of muscle function in the days following exercise tended to be independent to any observed changes in indices of muscle damage (Table 1.5.1). CWI produces a positive effect by reducing perceived ratings of DOMS in the days following muscle damaging exercise (most effective at 24 and 48 h) (36). Conversely, CWI may not have any significant effect on reductions in markers of

muscle damage and the associated inflammatory response post-exercise. However, results remain equivocal and direct comparison between studies is difficult due to variation in exercise protocols which could elicit differing extents of muscle damage, thereby influencing the ability to recover associated muscle damage markers. An overview of the studies assessing the influence of post-exercise CWI following muscle damaging exercise can be found in Table 1.5.1.

Table 1.5.1 A summary of studies investigating the application of CWI for recovery from muscle damaging exercise.

Study	Exercise Protocol	Subjects	Recovery intervention(s)	Main outcomes
Ascensão *et al*., (79)	Friendly football match, (full-length 90 min match comprising of two 45 min halves).	N = 20M, junior soccer players (national standard).	• CWI: 10 min in 10 °C to iliac crest. • Thermoneutral immersion (35 °C) for 10 min to iliac crest.	CWI vs. thermoneutral immersion: • ↓DOMS post-match (24 h). • ↑ MVC post-match (24 h). • ↓ in CK activity (48 h), serum myoglobin (30 min) and CRP conc. (24 h) post-match.
Bailey *et al*., (77)	Loughborough Intermittent-Shuttle Test, (90 min of varying intensities with average intensity ≈75% VO_{2max}).	N = 20M, physically active.	• CWI: 10 min in 10 °C to iliac crest. • CON: 10 min of seated rest.	CWI vs. CON: • ↓ DOMS post-test (48 h). • ↑ MVC post-test (24 and 48 h). • ↓ serum myoglobin conc. post-test (1 h)
Broodryk *et al*., (93)	15 min high-intensity fitness session: 10 x 15 s shuttle runs, followed by rugby simulating activities (tackling, sprinting, acceleration, deceleration phases) over a 50 m distance for 6 x 30 s.	N = 23M, university rugby players (mean playing experience of ≈10 y)	• CWI: 20 min in 8 °C to umbilicus. • CON: 20 min of seated rest.	CWI vs. CON: • [BLa], Na^+ and Hb returned to pre-exercise levels (0 h). • ↓ VJP, speed and power (0 h). Then ↑ in both groups (0 – 24 h). • ↑ grip strength (0 – 24 h).
Delextrat *et al*., (84)	For each gender, 8 competitive basketball matches (Wed) were performed within a standard week consisting of 3 training sessions (Mon, Tues, Fri). Recovery performed immediately post-match.	N = 8M, 8F, university basketball players (top 4 teams in university premier league)	• CWI: 5 x 2 min immersion in 11 °C to iliac crest, separated by 2 min. • Lower leg massage for 30 min • CON: Seated rest for 30 min.	CWI vs. CON: • ↑ CMJ performance post-match (24 h). • ↓ DOMS and general fatigue post-match (0 – 48 h).
De Nardi *et al*., (94)	4 x football training sessions (Tues-Fri, ≈140 min per day). • Warm up of soccer specific drills, dynamic stretching. • Performance tests such as CMJ, repeated sprint ability, 5 min shuttle run test. • 30 min of low intensity training involving technical/tactical skills. • Small-sided games (4 x 4 min).	N = 18M, junior soccer players (national standard)	• CWI: 8 min in 15 °C to iliac crest. • CON: 8 min seated rest.	CWI vs. CON: • ↓ perceptions of fatigue post-training (obtained daily).

	• Exercise performed at ≈32 °C.			
Eston & Peters, (66)	Eccentric and concentric maximal isokinetic contractions of the elbow flexors (8 sets x 5 reps). Recovery was performed 15 min post-exercise and every 12 h following for total 7 times.	N = 15F, university students.	• CWI: 15 min in 15 °C, exercise arm immersed. • CON: No treatment for unimmersed arm.	CWI vs. CON: • ↑ ROM post-exercise (48 and 72 h) • ↓ CK conc. post-exercise (48 and 72 h) • ↔ Strength loss (0 – 72 h)
Goodall & Howatson, (70)	Plyometric drop jumps (5 sets x 20 jumps).	N = 18M, physically active university students.	• CWI: 12 min in 15 °C to iliac crest. • CON: 12 min seated rest.	CWI vs. CON: • ↔ MVC, DOMS, CK conc., ROM, thigh swelling post-exercise (0 – 72 h)
Higgins *et al*., (95)	1 x rugby union game and 2 x training sessions per wk for 4 wks total. Recovery performed immediately post-match.	N = 26M, rugby union players.	• CWI: 5 min in 10 – 12 °C to above waistline level. • CON: 5 min seated rest.	CWI vs. CON: • ↔ repeated sprint ability post-match (0 – 72 h) • ↔ perceived muscle tightness post-match (0 – 72 h).
Howatson *et al*., (72)	Plyometric drop jumps (5 sets x 20 jumps). Repeated 14-21 days later.	N = 16M, physically active university students.	• CWI: 12 min in 15 °C to iliac crest. • CON: 12 min of seated rest.	CWI vs. CON: • ↔ MVC, DOMS, CK conc., ROM, thigh swelling following the 1st bout or repeated bout.
Ingram *et al*., (78)	Simulated team sport exercise: 4 x 20 min intermittent running, followed by 20m multistage shuttle run test.	N = 11M, team sport trained athletes.	• CWI: 2 x 5 min immersion in 10 °C to umbilicus, separated by 2.5 min. • CON: 15 min seated rest.	CWI vs. CON: • ↓ DOMS post-exercise (24 and 48 h). • ↑ best sprint and total sprint times post-exercise (48 h). • ↑ MVC post-exercise (48 h).
Jakeman *et al*., (71)	Counter movement jumps (10 sets x 10 jumps)	N = 18F, physically active.	• CWI: 10 min at 10 °C to iliac crest. • CON: 10 min seated rest.	CWI vs. CON: • ↔ MVC, DOMS, CK conc. post-exercise (0 – 72 h).
King & Duffield, (73)	Simulated netball intermittent sprint exercise circuit (4 x 15 min). Repeated 24 h later.	N = 10F, trained netball players	• CWI: 2 x 5 min in 9.3 ± 1.6 °C to iliac crest, separated by 2.5 min. • 15 min AR at 40% v-VO_{2max} • CON: 15 min seated rest.	CWI vs. CON: • ↑CMJ and 20-m sprint performance pre-Ex_2 (24 h post Ex_1). CWI vs. AR: • ↓ RPE and muscle soreness immediately post- Ex_1 (0 h)

				CWI vs. all other groups: • ↔ in performance measures during Ex_2.
L´ıllian Beatriz Fonseca *et al*., (96)	120 min Jiu-Jitsu training session: 40 min gymnastics, 40 min technical training, 40 min combat training.	N = 8M, trained jiu-jitsu athletes.	• CWI: 4 x 4 min in 6 °C to shoulder, separated by 1 min. • CON: 19 min seated rest.	CWI vs. CON: • ↓ serum LDH conc. post-training (24 h). • ↑ lower limb power post-training (24 h).
Montgomery *et al*., (80)	Basketball tournament: 1 x 48 min match per day for 3 consecutive days. Recovery performed immediately post-match.	N = 29M, basketball players (state regional level).	• CWI: 5 x 1 min in 11 °C to midsternal level, separated by 2 min. • Wearing compression garments post-match period. • CON: Carbohydrate intake + stretching.	Pre-tournament tests were repeated post-tournament. CWI vs. all other groups. • ↑ 20 m sprint, VJP, basketball line drill, sit and reach flexibility. • ↓ DOMS and general fatigue.
Paddon-Jones & Quigley, (67)	64 x eccentric elbow flexions per arm (8 sets x 8 reps at 110% of concentric 1RM).	N = 8M, resistance trained.	• CWI: 5 x 20 min immersion in 5 °C, separated by 60 min. Exercised arm immersed. • CON: No treatment for un-immersed arm.	CWI vs. CON: • ↔ isometric torque, isokinetic torque, DOMS, limb volume post-exercise (0 – 96 h)
Pooley *et al*., (97)	9 x 80 min competitive soccer matches, (2 x 40 min halves). 3 matches for each recovery intervention. Recovery performed immediately post-match.	N = 15M, elite youth soccer players.	• CWI: 10 min in 14 °C to iliac crest. • 10 mins of low intensity cycling (AR) equalling 80W power output. • Static stretching.	CWI vs. all other groups: • ↓ CK conc. post-match (48 h). CWI vs. AR: • ↔ DOMS, CMJ post-match (48 h).
Pournot *et al*., (75)	Exhaustive intermittent exercise protocol: 2 x 10 min of alternating 30 s CMJ and 30 s rowing at 80% mean power.	N = 41M, elite team sport athletes.	• CWI: 15 min at 10 °C up to iliac crest. • CON: 15 min seated rest.	CWI vs. CON: • ↑ MVC, CMJ post-exercise (1 h) • ↓ CK conc. post-exercise (24 h). • ↓ total leukocyte count post-exercise (1 h).

Rowsell *et al*., (76)	Football tournament: 1 x 90 min match per day for 4 consecutive days, ≈24 h between each match. Recovery performed immediately post-match.	N = 20M, junior soccer players (national standard).	• CWI: 5 x 1 min in 10 °C to midsternal level, separated by 1 min. • Thermoneutral immersion (34 °C) for same protocol.	CWI vs. thermoneutral immersion: • ↑ total running time and distance covered during matches. • ↓ leg soreness and general fatigue over course of tournament.
Rowsell *et al*., (74)	Football tournament: 1 x 90 min match per day for 4 consecutive days, ≈24 h between each match. Recovery performed immediately post-match.	N = 20M, junior soccer players (national standard).	• CWI: 5 x 1 min in 10 °C to midsternal level, separated by 1 min. • Thermoneutral immersion (34 °C) for same protocol.	CWI vs. thermoneutral immersion: • ↔ CMJ, repeated sprint ability, CK conc., LDH conc. • ↓ leg soreness and general fatigue over course of tournament.
Sellwood *et al*., (68)	Non-dominant leg extensions (5 sets x 10 reps at 120% concentric 1RM).	N = 11M, 29F, university students.	• CWI: 3 x 1 min in 5 °C to iliac crest, separated by 1 min. • CON: Same immersion protocol in 24 °C water.	CWI vs. CON: • ↔ DOMS, MVC, CK conc., thigh swelling post-exercise (0 – 72 h)
Vaile *et al*., (69)	Eccentric leg press protocol (5 sets x 10 reps at 120% of concentric 1RM. Followed by 2 sets x 10 reps at 100% 1RM).	N = 38M, resistance trained.	• CWI: 14 min in 15 °C to shoulder level. • CON: Seated rest for 14 min.	CWI vs. CON: • ↑ dynamic power (squat jump) immediately post-exercise (0 h). • ↓ thigh swelling, CK conc. post-exercise (24 and 72 h).

↑ increase, ↓ decrease, ↔ no change, *M* male, *F* female, *CWI* Cold water immersion, *CON* control, *AR* active recovery, Ex_1 first exercise bout, Ex_2 second exercise bout, *CK* creatine kinase, *CRP* C-reactive protein, *LDH* lactate dehydrogenase, *DOMS* delayed onset muscle soreness, *CMJ* counter movement jump performance, *MVC* maximal voluntary contraction, *VJP* vertical jump performance, *1RM* one repetition maximum, *ROM* range of motion, *RPE* rating perceived exertion, VO_{2max} maximal oxygen uptake, *v-*VO_{2max} velocity at VO_{2max}

1.6 Proposed mechanisms by which CWI improves recovery.

The proposed mechanisms by which CWI improves post-exercise recovery are associated primarily with the effects of hydrostatic pressure and cold exposure on the body (47,72). Hydrostatic pressure refers to the compressive force exerted on a body immersed in water. When immersed in water, hydrostatic pressure acts on the body in relation to the depth of immersion. The amount of pressure that acts on a body is equal to:

$$P = P_{atm} + g \cdot p \cdot h$$

Where P = water pressure; P_{atm} = atmospheric pressure (standard sea level 1013 hPa); g = gravity (9.81 m/sec^2); p = water density (1000 kg/m^3) and h = height of the water (m) (72).

Therefore, water pressure is a force per unit area and is equally transmitted throughout the water at a given level. If the walls of the container are solid then it exerts a pressure on the water equal to the pressure of the water at that depth. This means that during water immersion, the only factor which influences the hydrostatic pressure acting on the body is the depth of the water. An example would be a body part such as a foot immersed at a depth of 1m would have 981 Pa (7.34 mmHg) pressure acting on it, whereas at hip level (0.1m), only 98.1Pa (0.734 mmHg) of pressure would be applied (72). This hydrostatic effect of water immersion is suggested to facilitate metabolite efflux and blood and fluid redistribution from lower-body muscle regions, thereby improving metabolic and muscle damage recovery. The beneficial effects of hydrostatic pressure for post-exercise recovery feature in the following sections (1.6.2 and 1.6.3).

1.6.1 Reduction in cardiovascular strain

Cold exposure during CWI can influence physiological responses through reductions in core body (T_{core}) and tissue temperature (e.g., T_{skin}, T_{muscle}). Reductions in T_{core} and T_{skin} are commonly associated with a systemic vasoconstrictive response (98). Vasoconstriction leads to a reduction in blood flow to peripheral regions of the body such as the extremities, causing a redistribution of blood flow back toward the central vascular space to assist the maintenance of T_{core} (98). This redirection in peripheral blood flow results in an increased central blood volume (CBV) which is proposed to assist recovery and improve subsequent exercise performance through a reduction in cardiovascular strain (47). Cardiovascular strain is typified by reductions in cardiac output (*Q*), skin and muscle blood flow and systemic and muscle oxygen delivery during prolonged and intense exercise, thereby impairing exercise performance (99). Particularly during exercise in the heat, cardiovascular strain is elevated as blood flow is redirected from the active musculature to the cutaneous circulation for heat dissipation and temperature regulation (100). The redirection of blood to the peripheries results in reduced CBV, causing a decline in muscle blood flow and, consequently, impairment in O_2 delivery and performance (101,102). Therefore, vasoconstriction as a result of cooling counteracts this response by redirecting blood back to central circulation, leading to improved availability of O_2 and substrate for exercising muscle. Additionally, cold-induced reductions in T_{core} reduce the thermoregulatory demand for heat dissipation and therefore limits the need to redirect blood to the skin (47). Figure 1.6.1 illustrates the proposed mechanism by which CWI attenuates cardiovascular strain. Further insight into the effects of CWI on cardiovascular function is provided in section 1.7.2.

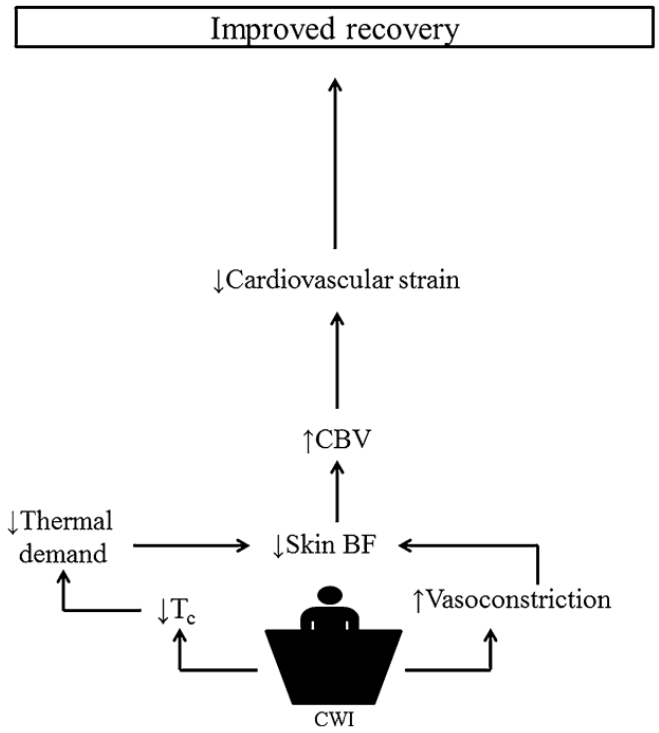

Figure 1.6.1. Illustrates the suggested mechanisms by which CWI attenuates cardiovascular strain and improves recovery. CWI reduces blood flow to the skin (skin BF) through cutaneous vasoconstriction and reduced thermoregulatory demand to dissipate heat. The reduction in skin BF results in an increased central blood volume (CBV), leading to the improved availability of oxygen and substrate for the exercising muscle. *CWI* cold water immersion, T_c core temperature, ↑ increase, ↓ decrease. (Adapted from Ihsan *et al*., (41)).

1.6.2 Removal of muscle metabolites.

Another mechanism by which CWI is proposed to aid post-exercise recovery is through an enhancement in muscle metabolite removal (47) . High-intensity exercise elicits the formation and accumulation of metabolites that are implicated in the development of muscle fatigue (71,103). Post-exercise CWI is suggested to accelerate the removal of these muscle metabolites, thereby improving metabolic recovery from intense exercise bouts (104–106). The combined effects of hydrostatic pressure and peripheral vasoconstriction facilitates haemodilution and blood displacement from the peripheral regions (107–109) (Figure 1.6.2), which in turn facilitates the transportation of metabolites from the muscle into central circulation. Haemodilution refers to fluid shifts from the interstitial (between cells) to intravascular

spaces (in the blood). Fluids leaving the interstitial space are then rapidly replaced by intracellular fluid, resulting in a higher extracellular (intravascular) to intracellular fluid content (107). This consequently results in an intracellular-intravascular osmotic gradient, facilitating the efflux of intracellular constituents and metabolic by-products from the intracellular and interstitial space into the peripheral circulation (47). Blood displacement through hydrostatic pressures, as well as peripheral vasoconstriction then further facilitates removal from peripheral circulation into central circulation (109,110). For example, increased hydrostatic pressure has been shown to displace blood from the splanchnic, abdominal regions and to a lesser extent the leg regions by increasing central venous pressures (109,110). Additionally, central circulation may be further augmented by decreases in limb arterial and cutaneous blood flow due to vasoconstriction in the limb and subcutaneous network (111,112). However, while acutely facilitating blood flow from peripheral regions into central circulation, CWI-induced peripheral vasoconstriction may also reduce muscle blood flow (47). Such a reduction in muscle blood flow may compromise O_2 and substrate delivery, enhance reliance on anaerobic metabolism and be detrimental rather than beneficial to recovery and subsequent exercise performance (47). This conflicting response to CWI may be responsible for the limited evidence demonstrating enhanced post-exercise metabolite removal following CWI (47). For example, a number of studies have observed no change in blood pH (104–106) or on the clearance of metabolites such as potassium and blood lactate (104–106,113,114) following post-exercise CWI. Some studies have even reported a tendency for an attenuated clearance of blood lactate when compared to passive resting (115) or light active recovery (30-40% peak cycling PO) (116,117). However, this attenuation of lactate clearance in the aforementioned studies was not found to impair performance during subsequent

exercise (115–117). Instead, CWI resulted in improved performance in these studies, indicating that the benefits of CWI may be associated with alternative mechanisms other than influencing the metabolite removal, such as alternating thermal and cardiovascular strain (115–117). Figure 1.6.2 illustrates the proposed mechanism by which CWI is suggested to improve clearance of post-exercise muscle metabolites, leading to improved recovery.

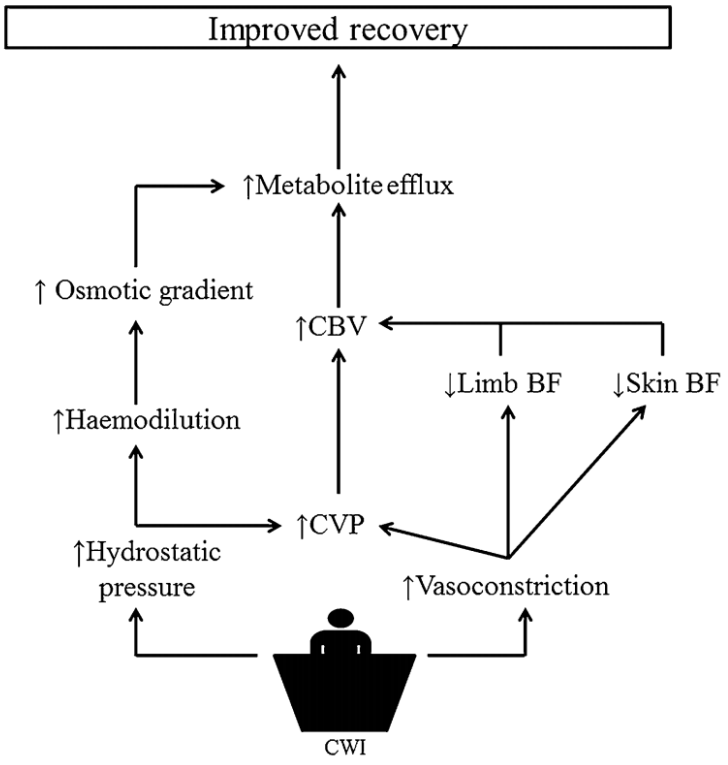

Figure 1.6.2. Illustrates the suggested mechanisms by which CWI is suggested to improve clearance of post-exercise muscle metabolites and improves recovery. The increase in osmotic gradient resulting from haemodilution promotes the efflux of intracellular constituents and metabolic by-products from the extravascular space into the peripheral circulation. These metabolites are subsequently displaced from the peripheries into the central circulation through the combined effects of hydrostatic pressure and peripheral vasoconstriction. *CWI* cold water immersion, *CVP* central venous pressure, *CBV* central blood volume, *BF* blood flow, ↑ increase, ↓ decrease. (Adapted from Ihsan *et al*., (41)).

1.6.3 Ameliorating exercise-induced muscle damage (EIMD)

For many coaches and athletes, a primary aim of post-exercise CWI is to ameliorate the symptoms of EIMD attained through training. CWI is suggested to ameliorate EIMD via several integrated mechanisms which are primarily associated with

localised cooling, increased hydrostatic pressures, and redistribution of blood flow (Figure 1.6.3) (36,50,72). For example, CWI is suggested to promote recovery by reducing muscle oedema (72,118). The presence of oedema impedes O_2 delivery to the muscles, as mechanical compression of the local capillaries is increased, resulting in an increased transit distance between capillaries and muscles fibres for O_2 exchange (118). CWI is proposed to reduce oedema by reducing incoming muscle blood flow and facilitating the removal of peripheral fluid (47). These effects are mediated through cold-induced peripheral vasoconstriction (111,112) and the increased hydrostatic pressure during water immersion (107–110), both of which lead to an increase in CBV. As detailed in section 1.6.2, peripheral vasoconstriction and hydrostatic pressure increase CBV by increasing the central venous pressure (CVP) and facilitating the movement of fluids from the intracellular and interstitial (extravascular) spaces to the intravascular compartments, respectively (107–110,119). Such fluid movements results in an intracellular-extravascular osmotic gradient, hence also encouraging the clearance of cellular debris and necrotic tissue from the damaged muscle into the central circulation (72). Furthermore, cold-induced reductions in T_{musle} may reduce intramuscular cellular metabolism and the overall muscle demand for O_2, thereby minimising the extent of secondary hypoxic cell damage and further inflammatory events (53,58). In combination with this, peripheral vasoconstriction limits the infiltration of neutrophils and macrophages during the inflammatory response (58,72). While the decrease in inflammation and/or oedema will likely contribute to reductions in DOMS in the days following muscle damaging exercise (35,57), CWI may also directly modulate sensations of muscle soreness through its acute analgesic effects, consequently improving perceptual recovery. Cold exposure has been shown to activate the transient receptor potential cation

channel M8 (TRPM8) receptors located in the Aδ and C fibres; the cutaneous equivalents of the muscle group III and IV nociceptive afferent neurons, respectively. Once activated, TRPM8 mediates analgesia through inhibitory inputs either through spinal inhibitory interneurons or directly to the muscle nociceptors (47).

Figure 1.6.3 illustrates the integrated mechanisms by CWI is suggested to improve recovery from EIMD.

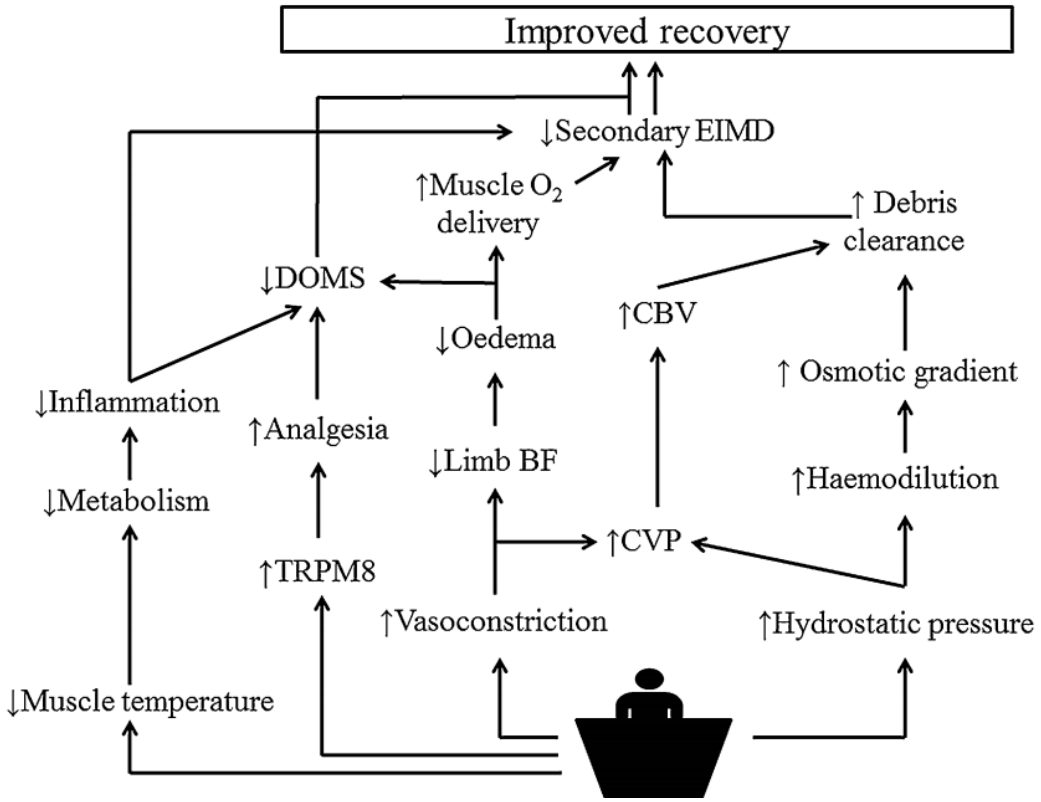

Figure 1.6.3. Illustrates the suggested mechanisms by which CWI improves recovered from EIMD. The increase in osmotic gradient resulting from haemodilution promotes the efflux of cell debris from the extravascular space into the peripheral circulation, where it is subsequently facilitated into central circulation through the effects of vasoconstriction and hydrostatic pressures. Vasoconstriction also reduces limb blood flow (Limb BF), leading to a decrease in oedema and a resultant improvement in muscle O_2 delivery. The decrease in muscle temperature following CWI reduces muscle metabolism and inflammation. Collectively, these aforementioned physiological responses reduce secondary EIMD, thus improving recovery. The decrease in inflammation and oedema may aid perceived perceptual recovery through ameliorating DOMS. Additionally, the analgesic effect of CWI may directly modulate the sensations of DOMS. *EIMD* exercise-induced muscle damage, *CWI* cold water immersion, *CVP* central venous pressure, *CBV* central blood volume, *DOMS* delayed onset muscle soreness. *TRPM8* transient receptor potential cation channel M8, ↑ increase, ↓ decrease. (Adapted from Ihsan *et al*., (41)).

1.7 Physiological response to CWI at rest and post-exercise.

The aforementioned literature highlights a number of potential mechanistic benefits regarding the application of CWI to improve recovery following different types of exercise (e.g., hyperthermic, metabolic or muscle damaging). However, it is worth noting that many of these studies focused on isolating the separated effects of water immersion *per se* (i.e., thermoneutral immersion) (108–110,119) or cold exposure via alternative cryotherapy methods (53,118). Furthermore, the observed physiological responses predominantly occurred following water immersion and/or cold exposure under resting conditions, therefore lacking efficacy for athletic context. Currently there is a lack of understanding regarding the physiological responses to post-exercise CWI. Since this is when CWI is primarily implemented during training, and the type of prior exercise is likely to directly influence the observed physiological response, further studies investigating the physiological response to post-exercise CWI are warranted. Nevertheless, there has been progression within this area, with studies monitoring the thermoregulatory, cardiovascular and blood flow responses both during and following immersion (111,117,120–122). Yet comparison between these studies is difficult due to experimental and methodological differences, including the CWI protocol utilised which will likely have a significant effect on the observed physiological response (49).

1.7.1 Thermoregulatory effects during and immediately following CWI

The thermoregulatory effects during CWI have been well documented in literature to this date. CWI has the ability to rapidly cool whole limbs and large muscle groups due to the large surface area exposed to the water and the conductance heat

transfer, which is 25 times greater than air (106). Therefore, large decreases in tissue temperature are commonly associated with the use of CWI. T_{skin} is immediately reduced upon exposure to CWI by heat conduction (112,123), the temperature heat transfer gradient that exists between the skin and the cold water is responsible for its rapid reduction. T_{skin} is reduced during CWI independent of being preceded by rest (112,123,124) or exercise (106,120,121,125,126). The magnitude of temperature reduction is largely dependent on the water temperature, with greater decreases in T_{skin} associated with colder water temperatures (112). A pattern of gradual increase in T_{skin} towards baseline values occurs during the post-CWI recovery period (112,121,123–125). Under resting conditions, 8 °C CWI applied for 4 – 10 min, has been shown to reduce thigh T_{skin} from a pre-immersion value of ≈30 °C to a minimum of 15 – 21 °C during immersion (112,123). Similar changes in T_{skin} have been observed when CWI is applied after resistance (121) and endurance (125,126) exercise. For example, Roberts *et al*., (121) observed that 10 °C CWI applied to the lower body for 10 min after leg isokinetic exercise decreased thigh T_{skin} from ≈35 °C to a minimum of ≈24 °C at the end of immersion. A similar magnitude of decrease was reported when 20 min of 14 °C CWI, applied after 90 min of constant power cycling and a 16.1 km TT in the heat, reduced T_{skin} from ≈32 °C to 21 °C (126). Taken together, these findings suggest that a T_{skin} change of approximately 10 – 15 °C is achievable when 10 – 15 mins CWI (temperature range 8 – 14 °C) is applied after resting and exercise conditions, respectively.

T_{core} decreases during and following the application of CWI under resting (112,123) and exercise conditions (117,120,125,126). Previously, studies have reported that post-exercise CWI (14 °C for 5 – 20 min) significantly decreased (≈1.5 – 2 °C) pre-immersion rectal temperature (T_{rec}), which is commonly used to reflect T_{core}, by the

end of a post-cooling recovery period (117,120,125,126). This is in contrast to 8 °C CWI applied over durations of 4 min (123) and 10 min (112) under resting conditions, which resulted in a smaller decrease (≈0.2 – 0.3 °C) in T_{rec} during a post-cooling recovery period. The disparity in the magnitude of change in T_{rec} between resting and exercise conditions may be partly attributed to the initial and progressive fall in T_{core} after exercise *per se*, since T_{rec} has been documented to be similar to control (i.e., passive rest) trials during and after post-exercise CWI (120,126). Absolute T_{core} values remain above baseline values for a considerable period of time during the post-immersion period following exercise i.e., >20 min (120,126) compared with CWI applied after rest (112,123). However, T_{rec} is then commonly reported to fall below baseline values after >20 min into the period post-immersion (117,120,125,126). This is indicative of the delayed “after-drop” phenomenon in T_{core} (127) and is due to the return of cold blood from its redistribution to cooled peripheral tissue during rewarming (128). The after-drop in T_{core} has been shown to persist for up to 90 min post-CWI (125) with decrements ranging from 0.1 – 2.3 ˚C at 30 min post-immersion regardless of CWI duration (5 – 20 min). T_{core} has not been examined beyond 90 min post-CWI and the full time-course of its change during recovery remains unknown. Recent pooled data from multiple CWI protocols utilised from literature suggest a significant effect of water temperature and immersion duration on the resultant rate of reduction in T_{core} post-exercise. Specifically, colder water temperatures and greater immersion durations lead to a greater reduction in T_{core}, respectively (129). Therefore, there should be consideration for the magnitude of intended core cooling when selecting CWI protocol parameters.

Skin cooling also leads to reductions in T_{muscle} via conductive heat exchange between underlying tissue layers and convective cooling of the limb (112,130).

Colder water temperatures cause greater decreases in T_{muscle} with the magnitude of change dependent on the thermal gradient which exists between the muscle and the water (112,123). The change in T_{muscle} is also related to the duration of cold exposure (131), with very large decreases (18 °C) in T_{muscle} observed after 3 h of CWI (132). Under resting conditions, both 10 min of 8 °C (112) and 20 min of 10 °C CWI (133) was reported to decrease superficial (1 cm) T_{muscle} by ≈4 – 5 °C immediately after immersion. A continued slower rate of reduction (≈1 – 2 °C) in superficial T_{muscle} was noted during the post CWI recovery periods. Additionally, under resting conditions Rupp *et al*., (134) reported a significant 8 °C decrease in superficial (1 cm) T_{muscle} after approximately 40 min of 12 °C CWI which remained decreased to this extent up to 90 min post-immersion. In contrast to superficial muscle depths, Gregson *et al*., (112) reported deeper (3 cm) T_{muscle} to be similar to baseline values immediately upon exiting CWI, however a significant decrease (≈2 °C) in deep T_{muscle} was observed at the end of a 30 min post-immersion period. In support of these findings, Costello *et al*., (123) reported a similar pattern of change in deep T_{muscle} after 4 min CWI under resting conditions.

There are presently a limited number of studies which have investigated the effects of CWI on post-exercise T_{muscle}. After resistance exercise, superficial T_{muscle} (1 – 2 cm) has been shown to decrease to the greatest extent (≈6 – 7 °C) immediately after CWI (121). In contrast to resting conditions, where a continued post CWI decrease in superficial T_{muscle} occurs (112), an increase towards baseline values was documented over a 50 min post-immersion period. The observation of a slower rate of reduction and/or an increase of superficial T_{muscle} towards baseline values during the post-immersion period, along with a delayed afterdrop in deep T_{muscle} is consistent with the warmer deeper muscle tissue losing heat to more superficial

tissue via conductive and haemodynamic convective heat loss pathways (130). In other words, initially it is superficial muscle tissue that will lose temperature, however deeper muscle tissue will begin to lose heat to these superficial layers thereby causing deeper muscle temperatures to decrease and superficial muscle temperatures to increase over time following CWI. Unfortunately, deep (3 cm) T_{muscle} was not reported in the aforementioned resistance exercise study to witness this. However, Peiffer *et al*., (126) have reported deep (3 cm) T_{muscle} to follow a similar pattern of decrease to the aforementioned resting studies when 5 min of 14 ˚C CWI was applied after a 1 km cycling TT in the heat. Likewise, Choo *et al*., (122) reported deep (3cm) T_{muscle} to decrease by ≈2 – 3 ˚C from post-exercise to end of immersion, with a prolonged reduction (≈2 ˚C) up to 60 min post-immersion. The exercise-induced increase in limb temperature prior to immersion promotes a greater heat transfer gradient between the cooling medium, thereby hastening the rate of reduction in T_{muscle} during and following immersion compared to resting conditions. It is evident that adipose tissue (particularly subcutaneous) may affect T_{muscle} in the area where cooling is applied. Myrer *et al*., (135) found a significant inverse relationship between overlying adipose tissue and T_{muscle} changes during and after cryotherapy treatment of the calf (ice pack) at different skinfold thicknesses. They concluded that a greater amount of adipose tissue thickness resulted in a longer time for maximum cooling to be attained. Otte *et al*., (136) also demonstrated a significant effect of skinfold thickness on the cooling rates of the anterior thigh muscle. Unlike the previous two studies which were performed under resting conditions, Rech *et al*., (137) examined the effects of post-exercise CWI on superficial (2 cm) T_{muscle}. Their results showed that 5 out of 16 participants attained a reduction in T_{muscle} of 7 °C within 30 min of 10 °C CWI, with a lesser magnitude of reduction in T_{muscle} achieved

after 10 min and 15 min (2.5 °C and 4 °C, respectively). They also observed an after drop effect (i.e., continued gradual reduction) in T_{muscle} following CWI (average of 1.7 °C for average of 23 min) and found that adipose tissue thickness was significantly correlated with CWI cooling rate in their participants (137).

1.7.2 CWI effects on cardiovascular function.

The heart rate (HR) response to water immersion is well-documented, thermoneutral (35 ˚C) water immersion generally leads to a decreased HR via hydrostatic related central haemodynamic changes such as an increase in CBV expansion and associated atrial preload and SV (72). However, the HR response is inconsistent amongst different water temperatures. For example, Šrámek *et al*., (138) reported that both 20 °C and 32 °C head out water immersion decreased HR by ≈15%, whereas a colder 14 °C immersion caused a ≈5% increase in HR. Conversely, Blonde-Petersen *et al*., (139) observed 15 °C CWI applied to a similar depth to decrease HR by 15% compared with a control (non-immersion trial). This inconsistency in the magnitude of the HR response to water immersion *per se* has been related to competition between physiological feedback systems (72) and is also likely to provide an explanation for the disparity in documented HR response during CWI. For example, the sympathetic increase in mean arterial pressure (MAP), associated with CWI, activates arterial baroreceptors, which brings about a reflex slowing of the heart to prevent high blood pressure levels. In opposition, the increase in CBV, which is accentuated with immersion above hip level, stimulates atrial stretch receptors and activates a neural reflex called the Bainbridge reflex that increases HR (72). Despite the varied HR response during CWI, it is clear that colder water temperatures initially increase HR due to a cold shock response in the first few seconds of immersion (140). Therefore, reported increases in HR during CWI are

likely to be associated with an increased sympathetic activity and a decreased vagal outflow (141). Numerous studies have demonstrated that HR, which is elevated due to exercise prior to immersion, is decreased following CWI exposure (85,105,142). For example, Bailey *et al*., (85) reported that 10 min of 10 °C CWI applied up to level of iliac crest after a 90 min shuttle run decreased HR from 107 bpm to 94 bpm, however CWI had no effect on HR compared with the control group (non-immersion). Similarly, a consistent decrease in HR has been observed during a passive recovery period when CWI has been applied between two bouts of exercise (105,116). The decrease in exercise HR in the recovery period after CWI may be attributed to the competition between sympathetic and parasympathetic neural activity, with CWI thought to increase the latter during the post CWI period, leading to a reduction in HR (143,144).

Cardiac output (*Q*) is modulated via changes in both HR and stroke volume (SV) during water immersion. Increases in hydrostatic pressure due to water immersion cause venous and lymphatic compression (72). Venous return is sensitive to external pressure and when hydrostatic pressure exceeds venous pressure, blood flow is redirected from peripheral to central areas, indeed CVP has been shown to increase during thermoneutral water immersion (109,110). This redistribution of blood flow leads to an increased CBV which stimulates an increase in cardiac contractility and results in an increased SV (72). Under resting conditions, thermoneutral immersion (30 and 34 ˚C) to the neck has been shown to significantly increase *Q* by ≈50% compared with a non-immersion trial (119). In support of these findings, Lin *et al*., (145) used weighted averages to compare 8 studies, which all included immersion to the neck versus non-immersion trials, and found an ≈29% and 24% increase in SV and *Q*, respectively. Again, the *Q* and SV response also appears to be temperature

dependent as studies employing CWI observe contrasting results to the aforementioned studies. This may be a direct result of the contributing HR response during cold exposure. For example, Blonde-Peterson *et al*., (139) investigated the effects of CWI at rest (30 – 40 min CWI at 15 °C to the sternum) and reported that despite a 19% increase in SV, there was concomitantly relatively little change in *Q* due to a decreased HR response. Whereas Roberts *et al*., (121) reported that after resistance exercise, 10 min of 10 ˚C CWI to level of umbilicus led to a decrease in *Q* with a concomitant decrease in HR and SV. Overall, the abovementioned findings are difficult to compare since greater immersion depths are associated with a higher SV and *Q* during thermoneutral immersion (72). Consequently, the comparison of differences in SV and *Q* response between resting and exercise conditions is difficult to interpret due to the varied selection of water depths and the pre-immersion cardiovascular status. However, the failure to observe significant decreases in *Q* after CWI may be attributed to a peripheral vasoconstrictive response which facilitates blood redistribution from the periphery to the core, thereby increasing CBV (47,72), enhancing cardiovascular efficiency, and reducing cardiovascular strain (47,117).

1.7.3 Limb and muscle blood flow response to CWI.

As already discussed, the combined effects of hydrostatic pressure and cold exposure during CWI are suggested to cause a redistribution of blood flow and intracellular fluid from peripheral regions, which is associated with an increase in CBV, CVP and haemodilution after CWI (47,107–110,119). This may assist the transport of the abnormal increase in interstitial fluid from muscle fibre trauma, i.e.

oedema (118), and the removal of cellular debris back into central circulation (72), as well as enhance metabolite clearance (47) and limit secondary injury (58). Although the redistribution of peripheral blood flow may have benefits in the recovery following muscle damaging exercise, it may also negatively affect subsequent athletic performance due to a reduction in muscle blood flow, meaning O_2 and nutrient delivery to skeletal muscle is compromised if exercise is performed within a short timeframe (47). It therefore becomes important to investigate how CWI affects blood flow responses in order to better understand the implications for optimising recovery.

Previous studies have assessed the limb blood flow response to CWI at rest and immediately following the cessation of exercise utilising venous occlusion plethysmography (VOP). The VOP method entails keeping arterial inflow to a limb intact whilst venous outflow is temporarily arrested to allow strain gauge measures of limb circumference to be obtained for the calculation of limb blood flow (146). Without prior exercise, CWI was found to have no acute effect on leg blood flow (147). Whereas, Vaile *et al*., (117) found significant reductions in blood flow to the arm and leg from 5 – 40 min following post-exercise CWI. Large increases in the leg-to-arm blood flow ratio were also reported in this study, which were taken to reflect a greater reduction in skin blood flow than muscle blood flow, these changes were also correlated with the magnitude of the fall in T_{core} during CWI. Whilst reliable estimates of changes in whole limb blood flow can be calculated using VOP, the technique provides discontinuous blood flow measurements, i.e., one measure every 5-10 s, which only reflect an immediate post-exercise value and not exercise *per se* (146). Thus, due to these inherent limitations and the motion artifacts induced by exercise, VOP is limited to intermittent measurements at rest only, meaning it underestimates the true blood flow response to exercise (148). Furthermore, VOP cannot directly

differentiate between skin and muscle blood flow meaning the extent of change in these parameters can only be assumed. As previously shown, cooling induces significant changes in skin blood flow (112,149), therefore it is likely that VOP measures primarily reflect changes in this parameter, thereby making it difficult to determine the underlying muscle blood flow response (112).

Limb blood flow responses to lower body CWI at rest (112) and post-exercise (111,150) have also been investigated using Doppler Ultrasound to insonate the superficial femoral artery. This method uses measures of blood velocity and arterial diameter to determine arterial blood flow and offers the advantage over VOP of high temporal resolution, i.e., absolute blood flow measurements can be taken continuously during exercise and rest (146). Under resting conditions, Gregson *et al*., (112) compared cold (8 ˚C) and cool (22 ˚C) CWI temperatures for two lots of 5 min (10 min total immersion) and demonstrated that femoral artery blood flow (FABF) was reduced by a similar magnitude of ≈30% in both temperatures immediately after immersion and for up to 30 min following (112). Furthermore, following a prior submaximal cycling protocol (performed 10 min before CWI), a 10 min continuous immersion in either 8 ˚C or 22 ˚C led to a similar reduction in FABF of ≈55% in both temperatures by the end of a 30 min post-immersion period (111). One might conclude that a reduction in FABF, which takes into account vessel diameter, indicates the occurrence of peripheral vasoconstriction following CWI. However, interpreting FABF measures with the intention of relating these to changes in muscle blood flow requires careful consideration. FABF represents the sum of flow to the skeletal muscle, skin, subcutaneous tissue, and bone. As such, making accurate estimations regarding changes in muscle blood flow from the FABF measurement alone is difficult, especially during exercise conditions where the relative contribution

of flow to the cutaneous region increases. Accordingly, the aforementioned studies which obtained FABF measures also monitored changes in local cutaneous blood flow (CBF), which was indirectly estimated using Laser doppler flowmetry (LDF). Utilising this combined assessment approach, Gregson *et al*., (112) observed that despite FABF decreasing to a similar extent, CBF was observed to be higher in the colder (8 ˚C) water temperature. This finding was interpreted as a reduction in the underlying muscle blood flow in the 8 ˚C temperature due to a “stealing” from the cutaneous region (112). In contrast, Mawhinney *et al*., (111) observed a similar reduction in both FABF and CBF for both water temperatures following exercise. Since there was no opposing direction of change in FABF and CBF, interpretation of muscle blood flow becomes more problematic as the reduction in FABF could be more reflective of a reduction in CBF during CWI rather than any change in muscle blood flow (111). Nevertheless, the combined assessment of FABF and CBF still only allows for indirect estimates to be inferred for muscle blood flow based off changes in these independent measures. It should also be noted, other techniques such as contrast-enhanced ultrasound and magnetic resonance imagining (MRI) via intravascular tracer injection have observed blood flow kinetics through the local muscle microvasculature (i.e., smaller vessels and capillaries) to act in a different way compared with bulk flow through large conduit vessels (151). Such potential differences between local and global blood flow kinetics means the use of global blood flow measures (e.g., VOP and Doppler measurements) for determining changes muscle blood flow may not provide an accurate estimation (152).

In contrast to global measures of blood flow, a number of techniques have previously been utilised to measure local tissue blood flow and been adapted to provide indirect measures of regional muscle blood flow (146,148). For example, Blonde-peterson *et*

al., (139) used a local isotope clearance technique and observed no change in forearm muscle blood flow after 30 – 40 min of 15 °C CWI to the neck. This technique allows measurement of muscle perfusion by observing the clearance rate of an injective, i.e. Xe^{133}, however it has been shown to underestimate muscle blood flow compared to alternative invasive methods such as assessing direct venous outflow and microsphere trapping flow determinations (146). Additionally, the collection of repeated measurements during CWI is difficult due to the initial injection artifact delaying measurements by approximately 10 min, along with the potential inaccuracy in injecting the tracer into the same spot (146). Arguably the gold standard approach to measuring changes in muscle blood flow is via positron-emission tomography (PET). This method uses intravenous radiowater $[^{15}O]H_2O$ to measure blood flow in tissues where there is an exchange of water molecules, i.e. where exchange of nutrients and O_2 occur (146,153). The PET technique can directly distinguish between skin and muscle blood flow and can provide insight into capillary level blood flow (153). Using this technique, Mawhinney *et al*., (154) recently examined the influence of 10 min of CWI at either 8 °, 15 ° or 22 °C on global and individual muscle perfusion of the quadriceps. Their results showed that CWI (8 – 22 °C) does not reduce global quadriceps perfusion to a clinically relevant extent (assessed against a clinically important difference of 0.75 mL·100 g·min^{-1}, value based on comparable reduction of resting muscle perfusion with nitric oxide inhibition), however colder water increases (8 °C) deep muscle perfusion (i.e., *vastus intermedius*) and reduces (15 °C) superficial muscle perfusion (i.e., *vastus lateralis, rectus femoris*) in the quadriceps muscle (154). This study is the first to suggest muscle perfusion heterogeneity in the quadriceps in response to different water temperatures used for CWI. Unfortunately, PET scanners are few in number, very

expensive and require expertise to operate, consequently current research incorporating this technique is limited (146,148).

1.7.4 Skeletal muscle metabolism

The initial management of muscoskeletal trauma has commonly been based on the premise that cooling reduces the metabolic rate of underlying tissues, thereby reducing the extent of hypoxia and ensuing damage (54,73). This hypothesis appears reasonable considering the fact that the rate of chemical reaction *in vitro* or *in vivo* is temperature dependent via the Q_{10} effect. Specifically, Van't Hoffs law states that for every 10 °C reduction in tissue temperature, the rate of chemical reaction will decrease between 2 to 3-fold (155). As such, reductions in tissue temperature would lower the rate of chemical reactions and, therefore, the demand for ATP. Decreased cellular ATP demand would translate into less demand for O_2 at the terminal step of the oxidative phase of oxidative phosphorylation (58), potentially leading to prolonged cell survival during hypoxia. Indeed, previous work suggests mitochondrial function is temperature-dependent and the rate of O_2 consumption to be lower at reduced temperatures (156). Therefore, if cryotherapy modalities (e.g., CWI) are indeed effective in reducing muscle metabolism via a cooling effect on tissue temperature, they may potentially alter the sequelae of injury (e.g., EIMD) through a reduction in secondary muscle damage (58).

Most of the current evidence is limited to animal models (58,155) and medical/surgical studies that performed cryotherapy on amputated and stored limbs and organs (131,157). These advocate that metabolism is optimally reduced after injury when tissue temperatures are decreased to between 5 ˚C and 15 ˚C (58,155,158,159). Furthermore, to maximise this effect, the relevant temperature reductions must occur within the injured tissue (e.g., the muscle layer at and around

the point of injury) and not simply the overlying skin (155). It is less clear what reductions intramuscular temperature are required to achieve the desired physiological response *in vivo* (160). Meeusen & Lievens (131) recommend ice pack application for 10 – 15 min is sufficient to reduce T_{muscle} by 7 – 10 °C at a depth of 1 cm. This critical 10 ˚C reduction in T_{muscle} may be required for cellular metabolism to slow by approximately 50% based on the Q_{10} effect (155). Previous studies (section 1.7.1) suggest such a reduction in T_{muscle} is rarely achieved when utilising CWI at rest or post-exercise, primarily attributed to subcutaneous adiposity which has a low thermal conductivity and diffusivity (the ability of thermal energy to disperse through a substance) thereby creating an insulating effect. For example, Rech *et al*., (137) showed that only 31% of participants reached the desired 7 °C reduction in T_{muscle} of the *rectus femoris* (RF) (measurement obtained at 2 cm sub-adipose) within 30 min of immersion (10 °C up to iliac crest). This was similar to another study which showed approximately 75% of participants did not achieve an 8 °C reduction in T_{muscle} of the *gastrocnemius* (1 cm sub-adipose) within 30 min of immersion (12 ˚C single leg immersion to knee level) (134). Additionally, Rech *et al*., (137) reported that when the RF muscle was cooled by CWI, the amount of muscle cooled (2 cm sub-adipose depth) was estimated to be 35.5% (calculated using a series of limb and muscle volume equations). Collectively, these studies indicate that cooling the majority of a damaged muscle until recommended temperature reductions (7 – 10 ˚C) is difficult to achieve with CWI, and the potential for such reductions in T_{muscle} may be limited to extremely lean populations or body parts with less adipose tissue thickness (ATT) (155). However, this does not necessarily make the objective of reducing tissue metabolism a redundant benefit of CWI. As already mentioned, the current theoretical guidelines for reducing tissue temperature are largely based on

animal models and cryotherapy performed on amputated and stored limbs and organs, meaning further human studies are needed in this area. Furthermore, a pragmatic approach may argue that decreasing T_{muscle} by 1 ˚C is preferable to inducing no change. In this regard, achieving a minor or moderate reduction in T_{muscle} may still be of clinical benefit (155). To date, no studies have directly obtained measures to estimate skeletal muscle metabolism (e.g., measures of O_2 consumption) during CWI, this has only been inferred from monitoring changes in tissue temperature. However, advancements and development of new technologies such as near-infrared spectroscopy (NIRS) have allowed for more informative data regarding muscle oxidative metabolism to be attained.

1.8 Near-infrared spectroscopy (NIRS) technique and measurement

The NIRS technique utilises the fact that, unlike light in the visible spectrum (400 – 650 nm), light in the near-infrared range (700 – 900 nm) is capable of penetrating human tissue (i.e., skin, adipose tissue and muscle tissue) (161). This provides access to the light absorbing chromophores: Hb, myoglobin (Mb) and cytochrome oxidase (cytox) (162,163). Hb is the main component of the erythrocytes and the O_2 carrier of the blood. Mb is present within the muscle cell and facilitates intracellular O_2 transport. Due to identical spectral absorbance, it is not possible for NIRS to distinguish between Hb and Mb (161–163). Cytox is the terminal enzyme of the mitochondrial respiratory chain reaction which transfers electrons from cytochrome c to molecular oxygen (163). The amount of cytox in muscle is relatively low (≈5%) (164) compared with Hb and Mb meaning changes are lost within the noise of the NIRS signal (163). As such, the contribution of cytox is often neglected within *in vivo* muscle studies utilising NIRS.

When near-infrared light penetrates the muscle layer, the NIRS signals are the result

of the weighted average O_2 saturations of the heme groups of Hb in the vascular bed (arterioles, venules, capillaries) and of the Mb heme groups in the muscle fibres (162). In terms of Hb, most of the NIRS signal is from small blood vessels (arterioles, venules, capillaries), since larger vessels (greater than 1 mm) possess higher concentrations of Hb which completely absorb all the light (162). However, it is generally assumed that the majority of the Hb-related NIRS signal comes from capillaries since these micro-vessels compose the largest proportion (>90%) of vascular volume in skeletal muscle (165). When O_2 binds to the heme groups in either of these molecules, their absorbance properties of near-infrared light changes, meaning oxyhaemoglobin/myoglobin (O_2Hb/O_2Mb) and deoxyhaemoglobin/myoglobin (HHb/HMb) will absorb different wavelengths of near-infrared light (161,163,164). Figure 1.8.1 illustrates the basic process of NIRS, including the offload of O_2 from Hb which occurs within the mitochondria of the muscle cells. The near-infrared light (NIR-light) emitted from the device penetrates into the muscle layer to a pre-defined depth and depending on the relative concentrations of O_2Hb and HHb present, the NIR-light will be absorbed to a differing degree, which is detected via the devices receiver. Since it is not currently possible to differentiate between Hb and Mb chromophores, for the remainder of this thesis, when discussing the relative concentrations of O_2Hb and HHb, these will be inclusive of the Mb contribution.

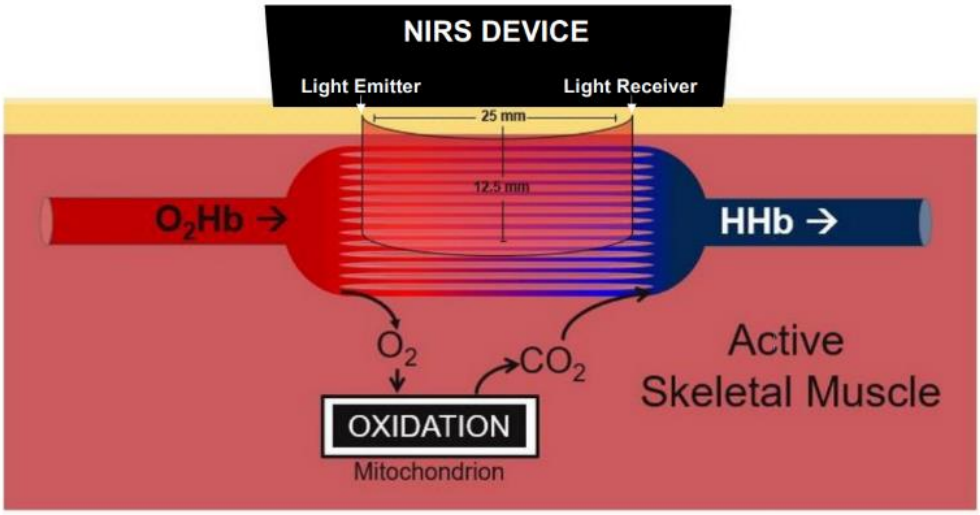

Figure 1.8.1 Diagram to illustrate the basic process of near-infrared spectroscopy (NIRS) (Adapted from Luck (158)).
(166)

Currently three types of oximeters have been used in NIRS research, namely: continuous wave NIRS (CW-NIRS); time domain NIRS (TD-NIRS) and frequency domain NIRS (FD-NIRS) (164). These techniques differ in the intensity and pattern of near-infrared light emitted (Figure 1.8.2). The most commonly used is CW-NIRS which utilises a single, consistent beam of emitted light, with the attenuation in light intensity recorded by the receiver (164).

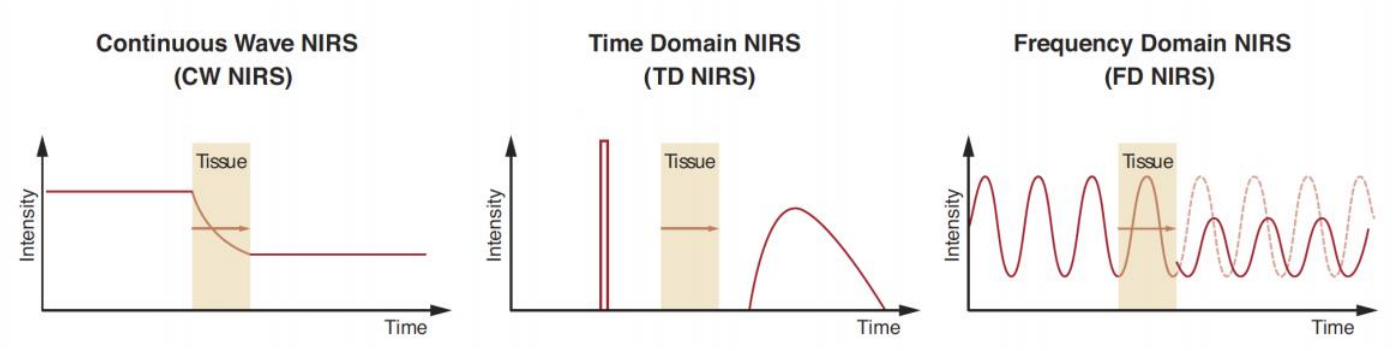

Figure 1.8.2 Diagram to illustrate how light intensity is monitored through tissue with the three types of muscle oximeter used in near-infrared spectroscopy (NIRS) research (adapted from Barstow (156))

Advancements in CW-NIRS technology include spatially resolved spectrometers (SRS) (167,168), such as the PortaMon (Artinis Medical Systems, Netherlands) portable CW-NIRS device used in chapter 3, where multiple source detector pairings are used, each utilising different wavelengths of near-infrared light to differentiate between O_2Hb and HHb. This is possible due to the specific extinction coefficients and the oxygen-dependent absorption spectra of these chromophore molecules (163). Peak absorbency for HHb occurs at shorter wavelengths (750 nm), whereas this occurs at longer wavelengths (850 nm) in O_2Hb. For most NIRS devices, the light penetration depth into tissue is approximately equal to half the distance between the light source and the receiver (164,167,168). It is accepted that the NIRS signal is significantly influenced by superficial tissue layers such as the subcutaneous adipose tissue layer (163). Therefore, for the purpose of measuring the NIRS signal within the underlying muscle layer, a larger source-detector distance is necessary (typically 30-50 mm). Biological tissue (i.e., muscle) is highly scattering and therefore the near-infrared light emitted into the tissue does not follow a uniform path. Light is either scattered or absorbed, with the light that reaches the detector described to have travelled in a “banana shaped” path through the tissue from the emitting source (Figure 1.8.3) (169). NIRS devices incorporate the modified Beer-Lambert Law (170–172) to account for light that: is lost due to absorption, takes a non-scattering path, scatters between emitter and receiver, and is lost due to scattering (Figure 1.8.4). As the specific path length of light is unknown, CW-NIRS devices provide relative values instead of absolute concentrations of O_2Hb and HHb, where changes in relative concentrations are typically tracked from a baseline value (i.e., obtained at rest) (164).

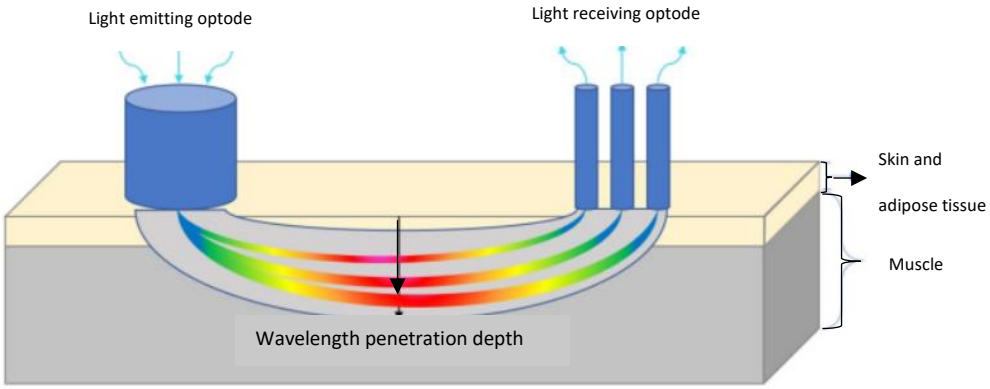

Figure 1.8.3 Diagram to illustrate the light path taken through the muscle tissue at different penetration depths, which is then detected by the device's light receiver (adapted from Beckett-Brown (165)). (173)

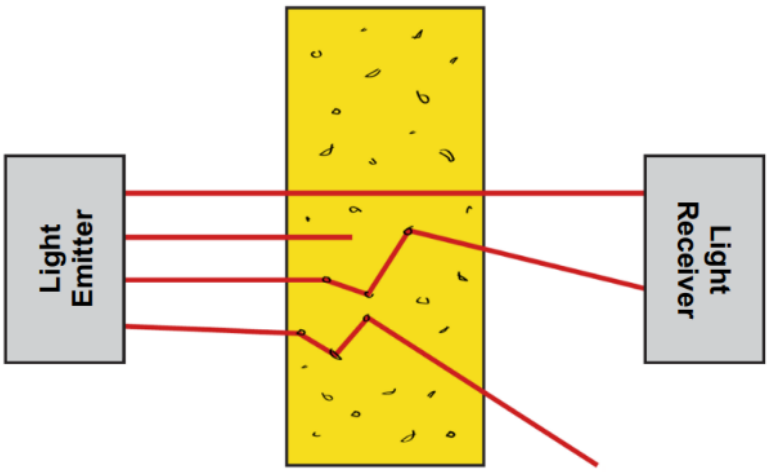

Figure 1.8.4 Diagram to illustrate the light scattering process accounted for by the modified Beer-Lambert Law (adapted from Barstow (156)).

The sum of relative O_2Hb + HHb concentrations gives an estimation for total haemoglobin concentration (tHb), therefore providing a measure of total blood volume within the local area of muscle interrogated (162,163). Changes in tHb (ΔtHb)

have previously been attributed to blood flow within the muscle microvasculature (121,174,175). SRS NIRS devices also utilise a multi-distance algorithm whereby the multiple source-detectors pairs allow for measurement at two or more tissue depths (161,164,176) (Figure 1.8.3). The ratio between O_2Hb and tHb (O_2Hb + HHb) provides the balance between delivery and removal of O_2 and hence estimates overall skeletal muscle saturation as a percentage (162,177). This is calculated by:

$$\text{Tissue saturation index} = \frac{\text{Oxyhaemoglobin}}{\text{Deoxyhaemoglobin + Oxyhaemoglobin}} \times 100\ (\%)$$

Equation 1. Tissue saturation index (TSI %) calculation.

The SRS derived TSI (%) signal is thought to account for the influence of superficial tissue layers and is therefore directly comparable between subjects (161,162). It is worth noting that different NIRS manufacturers derive their own set of calculations and formulas to approximate similar readings. As a result, the literature has referred to tissue saturation index (TSI), tissue oxygen saturation (StO_2), tissue oxygenation index (TOI) and muscle oxygen saturation (%SmO_2); these are all synonymous terms that inform upon the same measurement.

Depending on the NIRS device used, the muscle it is placed upon and the exercise context employed there is likely to be variation in the measurements obtained. Within literature there is a range of coefficients of variation reported. The NIRS measurement can be obtained with good reproducibility at rest (163,178,179) and across various intensities of exercise (163,180) with an accepted threshold of 10% (179). The coefficient of variation when using a PortaMon device has reported values of 4.7% in the *Vastus Lateralis* (VL) of clinical patients (181), and 1.8% - 2.5% in the VL during supine rest in male participants (179). It is also common in NIRS literature to report change or “delta (Δ)” values, rather than absolute values, to eliminate any

variation in measurement caused by differences in baseline absolute values. Previous research has reported no significant differences in NIRS output across multiple inter and intra-day analyses (182); specifically this study monitored deoxygenation kinetics during several moderate-intensity exercise transitions (MOD's) during a single visit and across separate visits. The study showed that deoxygenation kinetics were largely unaffected by data collection sequence, and the day to day reproducibility estimates for deoxygenation mean response time, as determined by CI_{95}, were improved by averaging at least three MOD's. The aforementioned research indicates the NIRS measurement is reliable, highly reproducible, and therefore holds potential as a measurement tool for use in training, recovery, and competition.

In summary, NIRS is capable of estimating changes in O_2Hb, HHb, tHb and TSI (%) at the superficial level, thereby informing upon muscle blood volume and O_2 saturation within the local muscle region on which the NIRS device is placed upon (167,168,177).

1.9 The potential of NIRS to monitor the physiological response to CWI.

The application of NIRS in sport physiology literature is now extensively documented (177). The non-invasive nature of the technique and the potential to provide insight into the peripheral changes occurring during dynamic exercise are its leading strengths. The development of portable NIRS technology with telemetric capability has allowed for the monitoring of local muscle haemodynamics and oxygenation in the applied sporting environment (167,168,177). The wearable devices can be securely attached onto the skin above the muscle site of interest using non-restrictive tape or strapping, allowing free movement and application in most sports

(177). Recently, investigators successfully waterproofed and attached portable NIRS devices (PortaMon, Artinis Medical Systems, Netherlands) to participants performing dynamic swim exercise (183,184). Significant changes in the TSI (%) and tHb signals were observed pre to post-training, representing phases of muscle deoxygenation and reoxygenation during specific elements of the stroke pattern (i.e., tumble turns). These studies demonstrate the reliability and robustness of NIRS measurement in underwater exercise, which was a previously unexplored application.

The capability of NIRS to provide measurement underwater means it also has utility during CWI where the participants muscle(s) are submerged underwater. To date, a small number of studies have utilised NIRS during CWI and in the acute period (i.e., up to 60 min) post-immersion in order to monitor the local muscle physiological response (121,122,174,185–189), (*for a review of the studies utilising NIRS with post-exercise CWI, the reader is directed to Chapter 2 of this thesis*).

1.10 Vascular occlusion technique.

Since NIRS only measures relative changes in muscle blood volume and muscle oxygenation, a direct measurement of muscle blood flow (mBF) and muscle oxygen consumption (mVO_2) is not possible. In order to obtain a quantitative value for mBF and mVO_2, a physiologic intervention (e.g., arterial occlusion (AO) or venous occlusion (VO)) must be used to control the inflow and outflow of blood to a limb (163,190). VO can be applied to a limb by inflating a cuff to a pressure of ≈50 mmHg, thereby sufficient to block venous outflow, but does not impede arterial inflow. As a result, venous blood volume and venous pressure increases. The increase in volume

is monitored by NIRS as an increase in O_2Hb, HHb and tHb signals (163) (Figure 1.10.1)

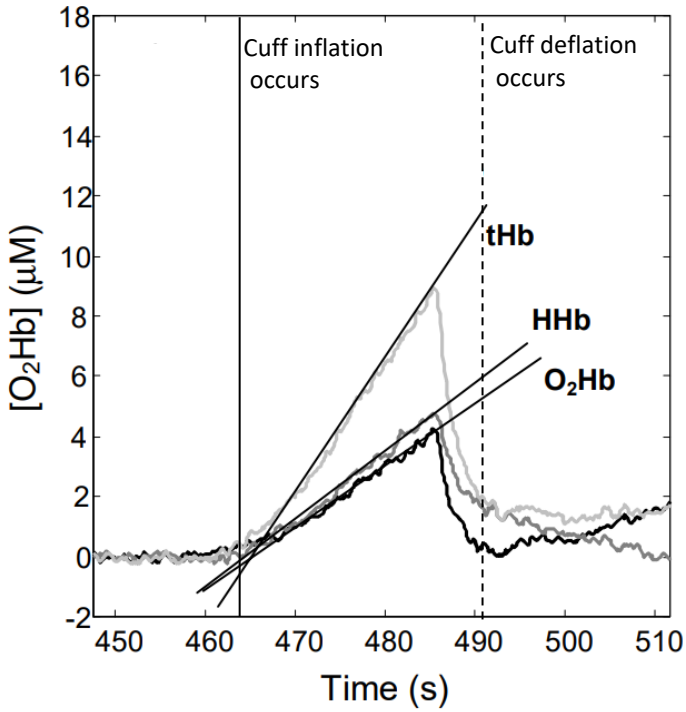

Figure 1.10.1 Illustrates the representative NIRS *tHb* total haemoglobin, *O_2Hb* oxy-haemoglobin, and *HHb* deoxyhaemoglobin traces during venous occlusion. The vertical solid black line indicates when cuff inflation occurred, dashed black line indicates when cuff deflation occured (adapted from Van Beekvelt (155))

NIRS blood flow measurements are similar to the well-established method of strain-gauge plethysmography. VO is used to provoke a blood volume increase in the part of the limb distal from the pneumatic cuff. Within the initial period of occlusion, the increase in blood volume per time is a measure for the blood flow (163). Strain-gauge plethysmography measures blood volume changes by changes in limb circumference but is unable to distinguish between the various tissues of the limb. NIRS measures blood volume changes directly in the muscle of interest by monitoring changes in total haemoglobin (tHb) content. As such, mBF in arm or leg can be measured during VO by evaluating the linear rate of increase in tHb (ΔtHb)

within the first seconds of VO (163,178,191–194) (Figure 1.10.1). Since venous outflow is blocked, the increase in tHb is directly related to arterial inflow (163,178). Concentration changes of tHb are expressed in micromolars per second ($\mu M \cdot s^{-1}$) and converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using the individual Hb-concentration ([Hb] in $mmol.L^{-1}$) obtained from blood samples or male and female values derived from literature. The molecular mass of haemoglobin (64.458 $g \cdot mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) must also be considered (163). mBF can then be calculated using the following equation (Equation 2) which has been previously used to calculate mBF at rest and post-exercise (163,192,195,196).

Equation 2.

$$mBF = \frac{(\left((\Delta \mathrm{tHb} \times 60) \div \left(([HB] \; x \; 1000) \div 4\right)\right) x \; 1000)}{10}$$

AO is applied by inflating the cuff to a pressure of at least 60-80 mmHg above systolic blood pressure. This ensures both venous outflow and arterial inflow are blocked, and systemic circulatory changes are sufficiently eliminated within the limb (163). Lacking the supply of well oxygenated blood, muscle metabolism fully depends on the available O_2 in local capillaries and muscle cells (163). Depletion of the local O_2 stores during AO is monitored by NIRS as a decrease in O_2Hb and a concurrent increase in HHb, while tHb remains constant (163) (Figure 1.10.2). After release of the AO there is a hyperaemic response, blood volume rises rapidly, resulting in a fresh pool of O_2Hb and a quick washout of HHb (163).

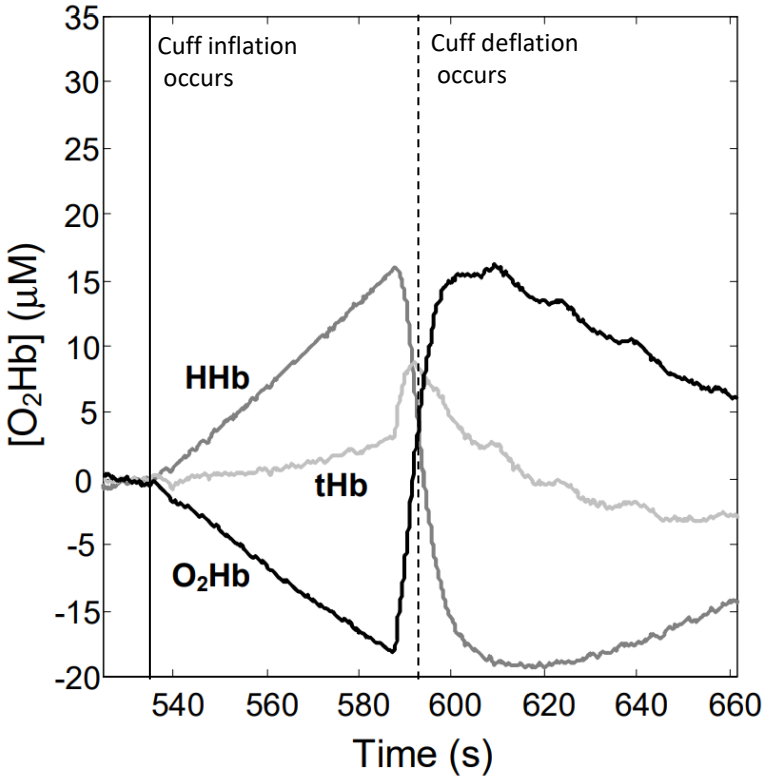

Figure 1.10.2 Illustrates the representative NIRS *tHb* total haemoglobin, *O_2Hb* oxy-haemoglobin, and *HHb* deoxy-haemoglobin traces during arterial occlusion. The vertical solid black line indicates when cuff inflation occurred, dashed black line indicates when cuff deflation occured (adapted from Van Beekvelt (155))

Measurement of mVO_2 is of great importance in the investigation of *in vivo* muscle metabolism (162–164,167,168). More conventional techniques like strain-gauge plethysmography combined with blood gas analysis are invasive and provide global values for the entire limb, thereby including tissues other than skeletal muscle. In contrast, NIRS is non-invasive and measures local oxygenation directly in the muscle. NIRS mVO_2 can be calculated using AO by evaluating the rate of decrease in O_2Hb (ΔO_2Hb) (Figure 1.10.2) or from the rate of decrease in Hb_{diff} (ΔHb_{diff}) divided by two (163). Hb_{diff} is simply calculated as $[O_2Hb] - [HHb]$, it provides a better noise to signal ratio and is also preferable for calculating mVO_2 compared to O_2Hb

as there have been reports where tHb does not remain constant throughout AO (163,195). When this occurs Hb_{diff} should be used to calculate mVO_2, since it is currently unknown whether the change in tHb during AO originates from the arterial or venous site and choosing the wrong variable could increase measurement error (163). The obstruction of inflow and outflow results in a static compartment of blood in the limb, where the decrease of O_2 from O_2Hb is directly related to consumption (163). Concentration changes of O_2Hb and Hb_{diff} are expressed in $\mu M \cdot s^{-1}$ and converted to millilitres O_2 per minute per 100 gram tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$), taking into account the molecular ratio between Hb and O_2 (1:4) and that the molar volume of gas is 22.4L assuming STPD conditions. A muscle density value of 1.04 $kg \cdot L^{-1}$ is also applied, resulting in Equation 3 for calculating mVO_2 (163):

Equation 3.

$$mVO_2 = \frac{\left(\left(\left(\frac{\Delta Hbdiff}{2} \, x \, 60\right) \div (10 \, x \, 1.04)\right) x \, 4\right) x \, 22.4}{1000}$$

The NIRS-occlusion method for measuring mBF and mVO_2 has been used in the forearm and leg at rest and during exercise (163,191–194,196), and when compared to existing methods such as strain gauge plethysmography and the invasive yet well-established Fick method, NIRS-occlusion produced similar values (163). Utilising the NIRS-occlusion methodology with CWI application enables assessment of its potential influence on mBF and mVO_2 (the latter being taken as a direct measure for muscle metabolism). These measures may provide further insight into the physiological changes occurring within the muscle and extend upon previous findings using global blood flow measures. Furthermore, applying occlusions

arguably allows for more theoretically valid estimations of mBF and muscle metabolic activity than studies which have simply monitored ΔtHb and ΔTSI (%) values during and following CWI.

1.11 Justification for chapter 2.

The reviewed literature of this chapter highlights a lack of physiologic data regarding the muscle physiological response to CWI. Applied research has shown conflicting findings regarding the effectiveness of CWI in recovering muscle damage indices; however, in the absence of physiologic data, the underlying mechanisms associated with a beneficial effect are yet to be fully elucidated. Temperature-induced alterations in microvascular blood flow and muscle metabolism are two ways CWI is suggested to promote recovery from EIMD. Studies have inferred changes in these parameters using a variety of measurement techniques. Doppler and VOP techniques have been used to assess changes in global flow measures such as conduit artery (FABF) and whole limb blood flow but offer limited interpretation regarding muscle blood flow. Alterations in muscle metabolic activity are indirectly inferred from changes in intramuscular temperature as a result of CWI, with current guidelines largely based off cryotherapy performed on animal models and medical/surgical studies that performed cryotherapy on amputated and stored limbs. Therefore, a technique which enables accurate estimation of muscle blood flow and muscle metabolism in response to CWI is required. NIRS informs upon local muscle haemodynamics and muscle oxygenation, with studies demonstrating capability for underwater measurement. As such, the technique offers insight into the muscle physiological response to CWI, (including during immersion), which may aid understanding of the underlying mechanisms, and provide evidence-based rationale for CWI implementation during recovery. A number of studies have incorporated NIRS

measurement with CWI, but their findings have not been collectively discussed or framed within the wider context of post-exercise recovery. It is important to establish the NIRS measurements obtained within the literature, and what these can inform upon with respect to muscle blood flow and muscle metabolism. The following chapter follows a literature search procedure to obtain these studies and review their findings.

2

The use of Near-infrared spectroscopy (NIRS) with Cold water immersion (CWI).

2.1 Introduction

The application of cold-water immersion (CWI) as a post-exercise recovery strategy is now widespread in a range of sports and activities (30,32,33). CWI is often applied following strenuous exercise/training which invokes exercise-induced muscle damage (EIMD) (36,47,48,197). For athletes, the objective here is to ameliorate the negative symptoms associated with EIMD such as reductions in muscle force-producing capacity and range of motion (ROM), and increases in swelling and delayed onset muscle soreness (DOMS), thereby hastening recovery and maintaining subsequent training intensity (35,36). Performing CWI provides the combined effects of cooling, i.e., causing significant reductions in core body (T_{core}), and tissue temperature (T_{skin}, T_{muscle}), and increased hydrostatic pressure which occurs when immersing the entire body or parts of the body in water (72). As such, CWI is proposed to facilitate a number of physiological changes such as peripheral vasoconstriction (98,198,199), haemodilution (107–110,119), a reduction in cardiovascular strain (47), a reduction in cellular metabolism (47,50,73,131) and acute analgesia (47), for a more detailed overview of the proposed mechanisms by which CWI aids recovery please refer to *section 1.6*. Collectively, these physiological changes are suggested to promote an acute reduction in swelling (i.e., oedema manifestation) and inflammation, thereby limiting the extent of secondary injury and possibly aiding the recovery of muscle function and DOMS in the days following EIMD.

Despite the proposed mechanistic benefits, the efficacy of CWI in facilitating recovery from EIMD has shown conflicting results, with studies reporting improved (77,87), unchanged (78,79) or impaired recovery (105,126) of muscle function and/or indirect muscle damage markers (i.e., DOMS and CK concentration). To greater

understand the underlying recovery mechanisms associated with an improvement or lack thereof in recovery parameters following EIMD, a greater number of studies monitoring the physiological responses to post-exercise CWI is warranted. Previously, studies have utilised venous-occlusion plethysmography (VOP) (117) and Doppler Ultrasound (111,112,150) to estimate whole limb blood flow and limb arterial blood flow responses, respectively; demonstrating a reduction in flow following post-exercise CWI. However, distinguishing the contribution of muscle blood flow from bulk flow measures can be problematic, since a reduction in flow may predominantly originate from the cutaneous region, rather than representing any significant change in muscle blood flow *per se* (111). A reduction in muscle blood flow following EIMD is proposed to reduce oedema manifestation and the infiltration of pro-inflammatory cells, thereby potentially reducing acute inflammation and secondary muscle damage (47,72). However, to date there is currently limited evidence regarding the muscle blood flow response during post-exercise CWI, and any associated recovery benefits. Furthermore, with regard to investigating tissue metabolic responses to cooling, current best evidence is largely inferred from animal models (73,155) and medical/surgical studies that performed cryotherapy on amputated and stored limbs and organs (131,157). From these studies it is suggested that tissue temperatures of 5 – 15 ˚C are required for metabolic reductions to be facilitated (200), however such reductions in T_{muscle} are difficult to achieve during CWI due the insulating effect of the sub-adipose layer (135,137,155). Therefore, the efficacy of post-exercise CWI for reducing muscle metabolic activity remains largely unestablished.

Another practical consideration is the CWI protocol utilised, and how this may alter the associated physiological response. Currently optimal guidelines regarding the

temperature of water, duration of immersion, depth of immersion, and number/ frequency of immersions are yet to be established (49), resulting in large protocol variation within literature, and limited rationale for the protocol selection. For targeting reductions in muscle metabolism, it is likely that a large reduction in tissue temperature is required (200). However, previous studies have reported only ≈25 – 30% of participants achieved a 7 – 8 °C reduction in T_{muscle} (depth 1 – 2 cm sub-adipose) after a 30 min CWI at 10 – 12 °C (134,137). It remains to be elucidated if this leads to a significant, if any reduction in muscle metabolic activity. Similar uncertainty exists for the measurement of muscle blood flow. Previous studies investigating the blood flow response to CWI have utilised a 10 min CWI at either 8 °C or 22 °C and observed a similar reduction in femoral artery blood flow (FABF) between the two temperatures when CWI was performed at rest (112) and following sub-maximal exercise (111). Only recently has the investigation of muscle blood flow following CWI been observed using positron-emission topography (PET) (154). This study suggested colder (8 °C) temperatures increased deep muscle blood flow (i.e., *vastus intermedius*), whereas more modest (15 °C) temperatures decreased superficial muscle blood flow (*i.e., vastus lateralis, rectus femoris*). However, limitations exist with the PET technique such as its invasiveness, low accessibility, high costs and expertise required by the user. Collectively, the aforementioned research highlights how certain CWI protocols may be better optimised than others depending on the desired physiological response (e.g., reduction in flow vs. reduction in metabolism).

One such technique capable of non-invasively measuring tissue oxygenation and haemodynamics *in vivo* is near-infrared spectroscopy (NIRS) (162,163,167,168,177); for a detailed overview of the NIRS technique and measurement, please refer to

section 1.8. Advancements in technology such as the development of compact portable devices with telemetric capabilities (167,177) have enabled application during exercise in a number of applied sport environments (201–204). More recently, successful device waterproofing has also been demonstrated, with significant changes in muscle oxygenation patterns observed during dynamic swim exercise (183,184). NIRS devices, once waterproofed, allow for continuous physiological monitoring of a muscle including pre-, during- and post-CWI. As such, NIRS offers the methodological advantage of directly observing when physiological changes occur whilst performing CWI, which may aid with protocol optimisation. Changes in the NIRS-derived tHb and TSI (%) measurements represent changes in local muscle blood volume and O_2 saturation, respectively. In other words, NIRS can provide insight into the dynamic balance between O_2 supply and demand within the muscle, which poses useful interpretation with regard to the proposed mechanisms by which CWI improves recovery following EIMD (i.e., reductions in muscle blood flow, reductions in muscle oxidative metabolism).

The aims of the present review were as follows (1) to review the available literature concerning near-infrared spectroscopy and post-exercise cold-water immersion, (2) identify the acute effects of post-exercise CWI on the local muscle physiological response, including NIRS measures of blood volume and O_2 saturation and (3) to identify evidence-based implication for athletic recovery and (4) to develop recommendations for future research in this area.

2.2 Methods

2.2.1 Search strategy and selection of studies

Relevant studies were identified using a computer-based literature search on a total of 3 databases: Google scholar, SPORTDiscus and Web of Science. Separate searches were carried out on each database with no date range for articles specified. To maximise the yield of relevant articles, the search strategy only incorporated two main phrases combined using Boolean logic (AND). The search terms were (“cold water immersion” AND “near infrared spectroscopy”). Study titles and abstracts were searched in all databases. To be included within the review, studies had to fulfil the following inclusion criteria: (1) published in English in a peer-reviewed academic journal, (2) report at least one empirical study, (3) conducted with healthy participants, (4) incorporated CWI as a recovery treatment, which was used in isolation to other treatments (5) comparisons should have been made to a no treatment control, a different mode of cryotherapy or other specified recovery treatment (5) CWI treatment should have been applied post-exercise within 30 min or between successive bouts of exercise (6) outcome measures must have included either/or both tHb and TSI (%) responses during and following CWI, as obtained from NIRS.

In the first stage of selection, the titles and abstracts of all studies were assessed for the above eligibility criteria. If it was absolutely clear from the information provided in the title and/or abstract that the study was not relevant, it was excluded. If it was unclear from the available abstract and/or title, the full-text article was retrieved. Full-

text articles were also retrieved with a relevant title but no available online abstract. The primary researcher assessed the content of all full-text articles, making the final inclusion/exclusion decisions.

2.2.2 Data extraction

The primary researcher used a standardised spreadsheet form to extract the data and other key methodological details, e.g., exercise and CWI protocols. The focus was primarily on data showing the NIRS response to CWI both during and following immersion, this also included the effects of chronic exposure to CWI during a training period.

2.2.3 Data synthesis

Data showing the NIRS response to CWI was synthesised into three timepoints at which data was recorded during the experimental protocol. These were data obtained during immersion, data obtained following immersion (e.g., up to 1 hr) and data obtained during exercise being performed. This method of data synthesis was chosen to enable an extent of comparison between studies, since the NIRS response likely depends on the timepoint at which data was obtained.

2.2.4 Assessment of Methodological Quality.

All eligible articles were rated for methodological quality, using the PEDro scale (Table 2.2.2). The full PEDro scale, derived from the Delphi list (205), consists of an 11-item checklist, configured by expert consensus to rate the quality of randomised-controlled trials. Reviewed studies were awarded one point for each criterion that

was clearly satisfied. As criterion 1 is a measure of the study's external validity, it is not included in the final PEDro score, giving each study a possible maximum score of 10 on the PEDro scale. The scoring thresholds for the PEDro scale are as follows: high quality = PEDro score 6-10, fair quality = PEDro score 4-5, poor quality = PEDro score ≤ 3. The primary researcher assessed the quality of eligible studies. Where needed, discussion between the primary researcher and principal supervisors for the review would take place.

Table 2.2.4 Physiotherapy Evidence Database (PEDro) Scoring Scale.

		Yes/No
1.	Eligibility criteria were specified	-
2.	Subjects were randomly allocated in groups	1
3.	Allocation was concealed	1
4.	The groups were similar at baseline regarding the most important prognostic indicators	1
5.	There was blinding of all subjects	1
6.	There was blinding of all therapists who ad ministered the therapy	1
7.	There was blinding of all assessors who measured at least one key outcome	1
8.	Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	1
9.	All subjects from whom outcome measures were available received the treatment or control condition as allocated or, when this was not the case, data for at least one key outcome were analysed by "intention to treat"	1
10.	The results of between-group statistical comparisons are reported for at least one key outcome measure	1
11.	The study provides both point measures and measures of variability for at least one key outcome	1
Total points		10

2.3 Results Summary of studies included

2.3.1 Search Results

The initial literature search (conducted in March 2021) yielded 305 results, which became 297 after duplicates were removed. After reviewing titles, 67 records remained. After reading abstracts, 12 records remained. After reviewing full-texts, 9

articles reporting 9 studies with a total of 152 participants were identified and included in the review. There was a large degree of heterogeneity between studies, in terms of study design, experimental protocol and outcome measures, and so results were analysed and discussed qualitatively. Figure 2.3.1 shows the PRISMA flow chart (206) for this review, which illustrates the method of study selection and the number of studies excluded at each stage, with reasons.

2.3.2 Study Quality

The 10 criteria and the final scores assigned to each study are presented in Table 2.3.2. Overall, the source of participants and their eligibility were well reported. Randomisation was stringently performed, with all studies reporting random allocation of participants to treatment or control groups. Likewise, all studies provided adequate information on participant's baseline data. Blinded application of treatment intervention was not performed in any study, due to the nature of CWI as the treatment intervention it is impossible to blind the participant or investigator administering the treatment, however it would've been possible to blind the assessor responsible for carrying out statistical analysis separately, though no studies reported this. All but one study (121) did supply adequate information on the number of participants from whom key outcomes were obtained, and this was at least 85% of the original number who were allocated to a group. Between-group statistical comparisons were reported in all studies, along with measures of group variability. Final values were considered "fair quality", with eight studies (122,174,185–189,207) achieving a PEDro score of 5, and one study (121) a PEDro score of 4.

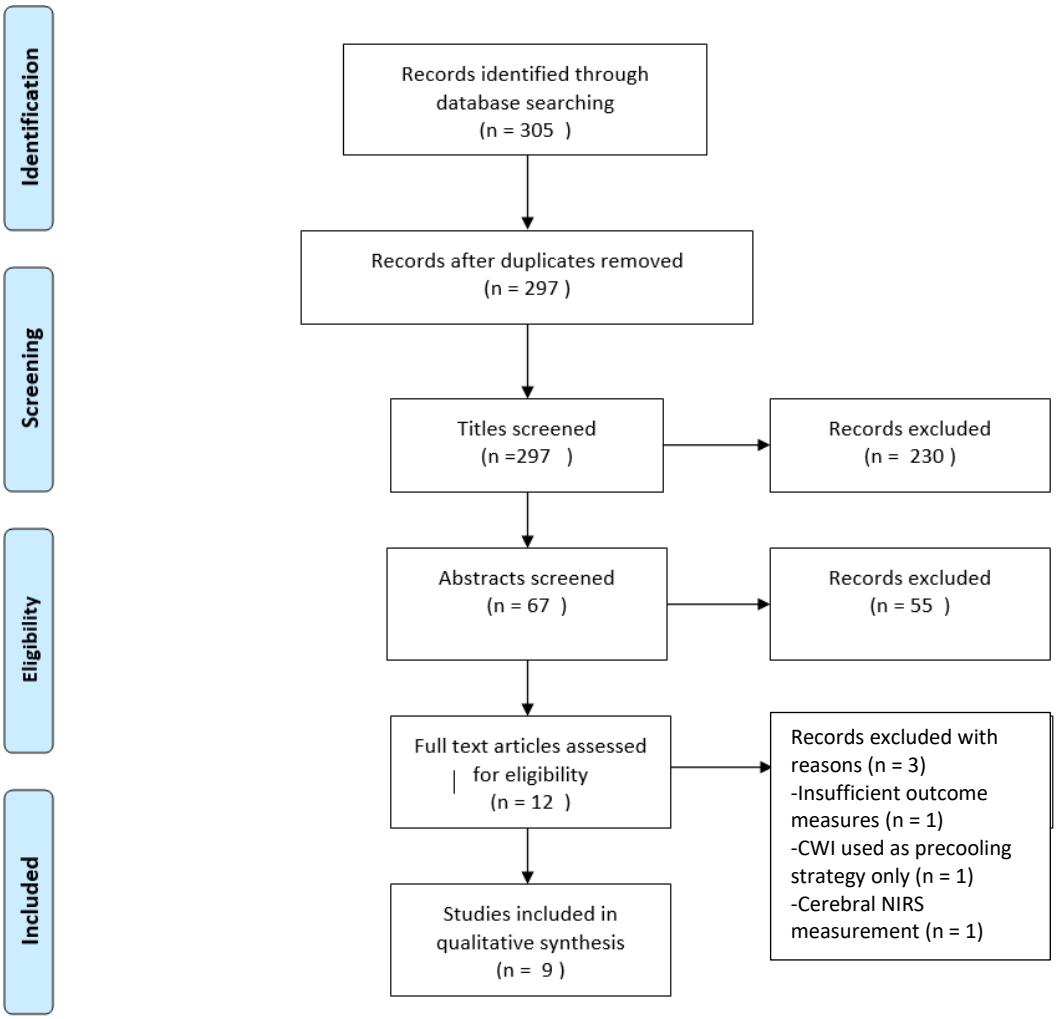

Figure 2.3.1 PRISMA flow diagram illustrating the screening process by which studies were deemed eligible for inclusion within the review.

Table 2.3.2 **Final Physiotherapy Evidence Database (PEDro) Scores for Included Trials**

Study	Criterion no. satisfied	Final PEDro score
Baláš *et al*., (188)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Choo *et al*., (122)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Hohenauer *et al*., (187)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Hohenauer *et al*., (186)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Ihsan *et al*., (185)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Ihsan *et al*., (207)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Roberts *et al*., (121)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☐ 9☐ 10☒ 11☒	4
Stanley *et al*., (174)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5
Yeung *et al*., (189)	2☒ 3☐ 4☒ 5☐ 6☐ 7☐ 8☒ 9☐ 10☒ 11☒	5

Criteria satisfied = ☒, lack of evidence for satisfying criteria = ☐. PEDro scoring thresholds: ≤ 3 = poor, 4-5 = fair, 6-10 = good.

2.3.3 Study characteristics

Table 2.3.3 presents the main characteristics of the included studies. Sample sizes ranged from 9 to 31 with a mean sample size of 16.3 participants (*SD* = 8.12). Three studies performed a power analysis to determine sample size (185,186,189). Six samples included only male participants (121,122,174,185,187,207), one included only female participants (186) and two samples included both sexes (188,189). The age ranged from 21 to 29 years with a mean of 24.7 years (*SD* = 2.63) across all included studies. For participant activity status, five studies (121,185–187,207) consisted of physically active young adults described as regularly participating in endurance or resistance training, two studies (122,189) consisted of untrained young adults and two studies (174,188) consisted of participants specifically trained in rock climbing to an intermediate or advanced ability (208) and endurance trained cyclists, respectively.

There was variation in the specific NIRS device used, however all but one study (189) used a multi-distance continuous-wave (CW) NIRS device. The additional type of device was a multi-distance frequency domain (FD) device. Most CW-NIRS devices (PortaMon, OxyMon, NIRO-200) used the modified Beer-Lambert law to calculate relative concentration changes of oxyhaemoglobin ([O_2Hb]) and deoxyhaemoglobin ([HHb]) and combined these values to give total haemoglobin concentration ([tHb]) (121,122,174,185,188,207). Spatially resolved spectroscopy (SRS) was commonly used to calculated absolute tissue saturation (TSI) (121,122,174,185,188,207). The MOXY monitor CW-NIRS device utilises a technique called Monte-Carlo modelling to estimate muscle oxygen saturation (SmO_2) (186,187), and the FD-NIRS device (ISS Imagent) uses its own specific calculation method (189). The site of attachment of the NIRS probes was mostly on

the lower body, on either the right or left quadriceps *vastus lateralis* (121,122,174,185–187,189) or the medial *gastrocnemius* (207). Baláš *et al.,* (188) attached the NIRS probe to the forearm muscle belly due to the nature of their exercise protocol.

Experimental protocols varied in the number of exercise bouts and recovery periods administered. Most common was a single bout of exercise, followed by a recovery period (121,122,185–187). Other protocols consisted of two bouts of exercise, with a recovery period in between exercise bouts (174,189), or three bouts of exercise, with recovery periods between the first and second exercise bouts (188). Ihsan *et al.,* (207) was a training study utilising three training sessions per week, for four weeks with a recovery period after each training session. Where two (174,189) or three (188) successive exercise bouts were performed, these were separated by a recovery period ranging from ≈15 – 30 min. Transition time from the cessation of exercise to the beginning of the recovery period was commonly reported and ranged from ≈2 – 10 min, when this was not reported it was stated recovery began "immediately post-exercise" (186–188).

Exercise modalities varied in nature, including intermittent handgrip endurance (188), box jumps (186,187), unilateral knee extension (121,189), high-intensity running (185,207) and cycling exercise (122,174).

During all recovery periods, a specific recovery strategy was performed. In all studies one group of participants performed CWI. In certain studies, groups also performed thermoneutral immersion (35 °C) (122), active recovery (121), partial-body cryotherapy (PBC) (186,187) or passive resting in the same posture they maintained

for CWI (122,174,186,188,189). The duration between CWI and alternative recovery strategies was kept the same in all studies.

The duration of CWI protocols ranged from 5 – 18 min, consisting of either a continuous 5 min (122,174) 10 min (121,186,187,189) or 15 min (185,207) immersion, or 3 x 6 min cycles (4 min CWI, 2 min unimmersed) (188). The temperatures used for CWI ranged from 8 – 15 °C; consisting of 8 °C (188), 9 °C (122), 10 °C (121,174,185–187,207), 15 °C (122,188) or 12 – 15 °C (189).

2.3.4 Outcome measures obtained

The reported outcome measures varied and incorporated a combination of physiological, exercise performance related measures (174,188) and muscle damage indices (186,187). Cycling power output, cadence (174) and handgrip endurance (188) were reported during consecutive bouts of exercise. Vertical jump performance (VJP), maximal voluntary contraction force (MVC) and muscle damage indices such as muscle swelling and perceptions of delayed onset muscle soreness (DOMS) were also reported following a specific muscle damaging protocol (i.e. box jumps) (186,187). All studies measured at least one physiological variable concerned with either skin, core, or muscle temperature. Likewise, all studies reported changes in at least one or both of the NIRS measures TSI (%) and [tHb]. Central haemodynamic measures such as stroke volume (SV), heart rate (HR) and cardiac output (Q), and peripheral blood flow measures such as femoral artery blood flow (FABF) and cutaneous vascular conductance (CVC) were also reported.

2.3.5 Timing of when outcome measures were obtained

Outcome measures were obtained at a number of different timepoints; most were obtained during exercise bouts (174,185,188,189), throughout the specific recovery

strategy (e.g., CWI, active recovery, PBC, etc) (121,122,185), and during a follow-up monitoring period after the recovery strategy had been performed (this was usually for ≈60 min) (121,122,186,187). Indices of muscle damage were obtained every 24 h for up to 72 h post-exercise (186,187). Furthermore, the included training study (207) obtained outcome measures before and after a 4 wk. training period.

Table 2.3.3 Summary of key characteristics for included studies.

Study	Participant characteristics	N	NIRS Device	Placement of Device	Exercise Protocol	CWI Protocol	Control/ Alternative recovery strategy
Baláš *et al*., (188)	Trained rock climbers	17 males (27.7 ± 10.2 y); 14 females (26.3 ± 4.6 y)	OxyMon MK III, (Artinis Medical Systems)	Forearm muscle belly.	3 intermittent handgrip trials to exhaustion, 20 min recovery period between each handgrip trial.	• 3 x 6 min (4 min immersed, 2 min unimmersed) at either 8°C or 15°C. • Seated upright, forearm immersion.	• Seated rest for 18 mins.
Choo *et al*., (122)	Untrained	9 males (29.0 ± 9.0 y)	NIRO-200 NX, (Hamamatsu)	Quadriceps VL.	25 min constant paced cycling followed by repeated 30 s sprints until exhaustion. (performed at 32.8 ± 0.4°C)	• 5 mins at either 9°C, 15°C. • Seated upright, immersed to mid-sternal level.	• Thermoneutral immersion at 35°C for 5 mins. • Seated rest for 5 mins.
Hohenauer *et al*., (187)	Recreationally trained (2 h week^{-1})	19 males (25.9 ± 4.4 y)	Moxy, (Moxy Monitor USA)	Right Quadriceps VL.	5 sets of 20 drop-jumps, 2 min rest between sets.	• 10 mins at 10°C. • Seated upright, immersed to mid-sternal level.	• PBC at -60°C for 30 s, then -135°C for 120 s.
Hohenauer *et al*., (186)	Regular endurance activity	28 females (22.5 ± 2.7 y)	Moxy, (Moxy Monitor USA)	Right Quadriceps VL.	5 sets of 20 drop-jumps, 2 min rest between sets.	• 10 mins at 10°C. • Seated upright, immersed to mid-sternal level.	• PBC at -60°C for 30 s, then -135°C for 120 s. • 10 min seated rest in supine position.
Ihsan *et al*., (185)	Physically active	9 males (23.8 ± 3.6 y)	NIRO-200 NX, (Hamamatsu)	Quadriceps VL.	30 min continuous running, followed by 10 sprint intervals.	• 15 min at 10°C. • Immersed one leg to gluteal fold.	• Contralateral leg rested outside the tank for 15 min.
Ihsan *et al*., (207)	Team sport trained (2 –3 h week^{-1})	9 males (22.9 ± 3.3 y)	NIRO-200 NX, (Hamamatsu)	Medial Gastrocnemius.	4 week training programme: 3 sessions week^{-1}. Each consisting of long (6-8 min), moderate (2 min) and short (30 s) running intervals.	• 15 min at 10°C. • Immersed one leg to gluteal fold.	• Contralateral leg rested outside the tank for 15 min.

Roberts *et al*., (121)	Resistance trained (2 – 3 sessions week^{-1})	10 males (21.4 ± 2.0 y)	PortaMon MK II, (Artinis Medical Systems)	Quadriceps VL.	Unilateral knee extension exercise, 10 sets of 20 reps, 2 min between sets.	• 10 min at 10°C. • Seated upright, immersed to level of umbilicus	• AR consisting of 10 mins of low intensity cycling.
Stanley *et al*., (174)	Endurance trained cyclists	14 males (25.0 ± 4.0 y)	PortaMon MK II, (Artinis Medical Systems)	Left Quadriceps VL.	2 x HIIT cycling sessions, 30 min recovery period between sessions.	• 5 min at 10°C. • Standing upright, immersed to level of umbilicus	• Standing upright for 5 min.
Yeung *et al*., (189)	Not reported	10 males, 10 females (22.0 ± 0.52 y)	ISS Imagent, (ISS, USA)	Right Quadriceps VL.	2 bouts of unilateral knee extension and flexion exercise, until decrease <60% peak torque, 10 min recovery between bouts.	• 10 mins at 12-15°C. • Semi-reclined position immersed to iliac crest.	• Seated rest for 10 mins.

AR active recovery, *HIIT* high-intensity interval training, *PBC* partial-body cryotherapy, *VL* vastus lateralis,

2.4 Results of NIRS measures

2.4.1. tHb

Table 2.4.1 summarises the tHb response during CWI, following CWI (e.g., during a follow-up monitoring period after CWI), and during exercise (e.g., HIIT or resistance exercise bouts). The main findings from this table are reported below.

During immersion: Studies monitoring tHb during immersion (121,122,185) observed tHb to either decrease (122,185) or increase (121).

Following immersion: In the follow-up period after immersion studies monitoring tHb observed a reduction as a result of CWI application (121,122,185). There was also no apparent effect of water temperature during CWI on the observed tHb response (122).

During exercise: There was limited studies monitoring tHb during exercise and the results are conflicting due to time of measurement and exercise modality. There was no change in tHb during an intermittent handgrip trial for males and females (188), there was a reduction in tHb during a repeated HIIT cycling bout (174)

2.4.2. TSI (%)

Table 2.4.2 summarises the TSI (%) response during CWI, following CWI and during exercise. The main findings from this table are reported below.

During immersion: Studies monitoring TSI (%) during immersion (121,122,185) reported TSI (%) to remain unchanged (121,122) or to increase (185).

Following immersion: Studies monitoring TSI (%) in the follow-up period after immersion (121,122,186,187) reported TSI (%) to remain unchanged (121,122) or to decrease (186,187).

During exercise: There was limited studies monitoring TSI (%) during exercise and the results are conflicting due to time of measurement and exercise modality. TSI (%) was either found to remain unchanged whilst performing exercise, i.e., handgrip trial and HIIT cycling (174,188). TSI (%) was found to increase during a subsequent resistance exercise bout compared to the first (189).

2.4.3. Other NIRS measures.

Ihsan *et al*., (207) observed significant main effects for time and treatment for reperfusion rate (R-RATE), where incorporating regular CWI during training resulted in significant increases in R-RATE. R-RATE is an index for the reperfusion response following an ischaemic stimulus (e.g., 5 min arterial occlusion); therefore, the authors suggested the regular use of CWI post-exercise might enhance exercise-induced changes in microvascular function, possibly through an enhanced capillarity in the muscle microvasculature (207).

Table 2.4.1 The tHb response during CWI, in the period following CWI, and during periods of exercise.

Study	tHb during CWI	tHb following CWI	tHb during exercise
Baláš *et al*., **(188)**	N/a	20 min recovery (#1): • CWI 8 ˚C and CWI 15 ˚C vs. passive recovery: ↓ tHb (reduction of ≈20 μmol^{-1} vs. ≈10 μmol^{-1}, respectively, $P < 0.05$) • ↔ between males and females. 20 min recovery (#2) • Similar results as above.	ΔtHb during 8 s contraction and 2 s relief phases of handgrip trials: • All groups: ↔ ($P > 0.05$) • ↔ between males and females.
Choo *et al*., **(122)**	CWI 9 ˚C and CWI 15 ˚C vs. passive recovery: ↓ tHb (reduction of ≈150 – 200 $\mu M.cm^{-1}$ vs. 0 –25 $\mu M.cm^{-1}$, respectively, $P < 0.05$).	At 30 min post-immersion: • CWI 9 ˚C vs. passive recovery: ↓ tHb (reduction of ≈150 $\mu M.cm^{-1}$ vs. 0 – 25 $\mu M.cm^{-1}$, respectively, $P < 0.05$). • CWI 15 °C vs. passive recovery: ↔ tHb (reduction of ≈100 $\mu M.cm^{-1}$ vs. 0 – 25 $\mu M.cm^{-1}$, respectively, $P > 0.05$).	N/a
Ihsan *et al*., **(185)**	Cooled limb vs. non-cooled limb: ↓ tHb (at 3 min, and between 9 and 15 min, with peak differences occurring at 15 min, $P < 0.05$).	N/a	N/a
Roberts *et al*., **(121)**	CWI group: ↑ tHb (≈100 – 150 μM) compared to pre-exercise values during the first 4 min of immersion. Reduction in tHb (≈50 μM) for remaining 6 min of immersion, but values remained higher than those obtained in first minute of immersion.	CWI group: ↓ tHb (≈10 μM) compared to pre-exercise values at 5 min ($P = 0.003$) and 20 min post-immersion ($P = 0.033$).	N/a
Stanley *et al*., **(174)**	N/a	N/a	CWI vs. passive recovery: ↓ tHb (reduction of ≈2.6 μM) from HIIT2 to HIIT1 (chance for CWI to be higher/trivial/lower than passive rest: 10/17/73%).

N/a = tHb was not measured during this period of the experiment. ↑ significantly higher, ↓ significantly lower, ↔ no significant difference.

Table 2.4.2 The TSI (%) response during CWI, in the period following CWI, and during periods of exercise.

Study	TSI during CWI	TSI following CWI	TSI during exercise
Baláš *et al*., **(188)**	N/a	20 min recovery (#1): • All groups: ↔ TSI 20 min recovery (#2): • CWI 8 ˚C and CWI 15 ˚C vs. passive recovery: ↓ TSI (≈55% vs. ≈60%, respectively, $P < 0.05$). • TSI was ↓at more timepoints for CWI 8 ˚C than CWI 15 °C when compared to passive recovery.	ΔTSI during 8 s contraction and 2 s relief phases of the handgrip trials (i.e., extent of deoxygenation and reoxygenation). • All groups: ↔ ($P > 0.05$) • ↔ between males and females. The minimum TSI value (TSI_{min}) during contractions: • CWI 8 ˚C and CWI 15 ˚C vs. passive recovery: ↓ for 2nd and 3rd handgrip trials ($P > 0.05$)
Choo *et al*., **(122)**	All groups = ↔ TSI ($P > 0.05$)	All groups: ↔ TSI for 60 min post-immersion ($P > 0.05$)	N/a
Hohenauer *et al*., **(187)**	N/a	CWI vs. PBC: ↓ TSI between 10 and 40 min post-immersion ($P < 0.05$)	N/a
Hohenauer *et al*., **(186)**	N/a	CWI vs. PBC: ↓ TSI at 10 min post-immersion ($P < 0.05$) CWI and PBC vs. passive recovery: ↓ TSI for 60 min post-immersion.	N/a
Ihsan *et al*., **(185)**	Cooled limb vs. non-cooled limb: ↑ TSI (between 9 and 12 min into immersion, $P < 0.05$).	N/a	N/a
Roberts *et al*., **(121)**	CWI group: ↔ TSI compared to pre-exercise values ($P > 0.05$).	CWI group: ↔ TSI compared to pre-exercise values for 40 min ($P > 0.05$).	N/a
Stanley *et al*., **(174)**	N/a	N/a	CWI vs. passive recovery: ↔ TSI from HIIT1 to HIIT2 (chance for CWI to be higher/trivial/lower than passive recovery: 29/70/1%).
Yeung *et al*., **(189)**	N/a	N/a	CWI vs. passive recovery: ↑ TSI (≈10% increase from 1st to 2nd resistance bout, $P < 0.05$)

N/a = TSI was not measured during this period of the experiment. ↑ significantly higher, ↓ significantly lower, ↔ no significant difference.

2.5 Discussion

This review is the first to collectively scrutinize studies which have utilised the NIRS technique during and/or in the acute period (up to 1 h) following post-exercise CWI. Changes in the NIRS derived tHb and TSI (%) measures are discussed in the text; (a) with regard to the proposed physiological mechanisms responsible for the observed changes in these measures and (b) discussing how the observed changes are associated with the mechanisms by which CWI is proposed to aid post-exercise recovery. TSI (%) and tHb are discussed separately; this is because each measure represents a different possible physiologic change, meaning the mechanisms relating to their change are also likely to be different. Three studies monitored the tHb and TSI (%) response during and following immersion (121,122,185). Additionally, three studies obtained tHb and TSI (%) values during subsequent exercise bouts after CWI had been performed (174,188,189). Since the influence of exercise *per se* is expected to affect the observed TSI (%) and tHb response, these findings are reported separate to studies which incorporated no additional exercise.

2.5.1 Muscle blood volume (tHb) changes during and following CWI

Ihsan *et al*., (185) observed a ≈20% reduction in tHb in the *vastus lateralis* (VL) undergoing CWI (15 min at 10 °C to level of gluteal fold) when compared to the contralateral unimmersed leg (resting in same posture for 15 min), with peak differences between legs occurring at the end of the 15 min immersion. A greater reduction in tHb of ≈66% was reported in the VL during full lower-body CWI (5 min at 9 °C or 15 °C to the midsternal level) compared to passive recovery (for the same duration) (122). These two studies implemented different methodologies when determining the change in tHb (ΔtHb) during immersion, such as the initial timepoint

at which the delta (Δ) is calculated from. For example, Ihsan *et al*., (185) determined ΔtHb from the lowest minimum value achieved during continuous running (CR) and compared it with a value obtained every 3 min during immersion. Whereas Choo *et al*., (122) took the value obtained ≈2 min post-exercise and compared it with a value obtained at the start and at the end of immersion. This discrepancy may explain some of the observed difference in ΔtHb between studies.

In both studies, there is evidence that a post-exercise hyperaemic response was present (exercise hyperaemia referring to the increase in skeletal muscle blood flow that occurs during muscular activity in response to increased cellular metabolism (209)); for example, Ihsan *et al*., (185) observed a ≈65 – 75% increase in tHb during a 2 min transition period between the end of exercise and start of immersion; and Choo *et al*., (122) observed an increase in flow measures such as femoral artery blood flow (FABF) and leg vascular conductance immediately post-exercise when compared to resting values. When the initial tHb value used in the Δ calculation is obtained between the end of exercise and beginning of immersion, the magnitude tHb reduction (i.e., ΔtHb) during immersion is expected to be greater. This is a possible reason why Ihsan *et al*., (185) obtained a different ΔtHb during immersion since they did not use an initial tHb value obtained during the aforementioned period of hyperaemia.

Variation in the CWI protocols may further explain the difference in ΔtHb; specifically, the difference in depth of the immersed limb(s). For example, Ihsan *et al*., (185) utilised a standing upright, single leg immersion to the gluteal fold whereas Choo *et al*., (122) incorporated a seated full lower-body immersion to the midsternal level. At the level of the mid-VL there is a greater water depth during seated immersion to the mid-sternal level, consequently the effect of hydrostatic pressure is increased (72).

During water immersion, increased hydrostatic pressures may promote a certain degree of blood redistribution from the lower body regions (i.e., legs) in favour of central circulation (107,109,110). When blood redistribution occurs it likely results in a reduced total blood volume within that region, which is detected by NIRS as a reduction in tHb. In accordance, Choo *et al*., (122) observed a decrease in tHb during thermoneutral (35˚C) water immersion that was not present during passive rest (control), suggesting a hydrostatic effect of water immersion *per se* on the tHb measurement. In Ihsan *et al*., (185), the effect of hydrostatic pressure is not accounted for as no thermoneutral condition was incorporated; however, considering the water depth at the level of VL during upright immersion to the gluteal fold would be ≈0.1 -- 0.2 m, it is likely hydrostatic pressures were minimal and certainly less than those experienced in Choo *et al*., (122). It should be noted the hydrostatic effect of water immersion *per se* should not influence the NIRS devices' themselves, which could mislead interpretation. It's previously been shown that an applied external pressure upwards of 20 mmHg is required to affect the NIRS signal (179). Such pressure would require water immersion to a significantly deeper depth than what is used for CWI. Rather, care should be taken when waterproofing NIRS devices and securing them to the muscle of interest to limit excessive additional pressure, as this would potentially elicit artificial pressure induced ischemia under the device and result in type one errors (179).

Despite methodological differences, both studies observed a reduction in tHb during immersion, suggesting the post-exercise hyperaemic response in the VL was ameliorated by some extent (185). As already mentioned, this could be attributed to a hydrostatic redistribution of blood volume from this superficial muscle region (107,109,110); however, it is more likely caused by the enhanced cooling effect of

CWI on the muscle. This notion is supported by the greater reduction in tHb observed during immersion in cold water temperatures (9 ˚C and 15 ˚C) compared to during thermoneutral (35 ˚C) immersion (122). Furthermore, tHb decreased in a leg undergoing CWI but increased in the contralateral unimmersed leg (representing hyperaemia) (185). Since the hydrostatic pressure acting on the VL during immersion was minimal in this study, it was likely the greater cooling effect on the muscle during CWI which was primarily responsible for the observed reduction in tHb (185). Taken together, the aforementioned findings suggest the greater degree of cooling during CWI (i.e., not present in thermoneutral immersion) induces a physiological response which modulates tHb.

During limb cooling cutaneous blood flow (CBF) is reduced via peripheral vasoconstriction. Upon cold exposure, the initial response is a sympathetically-mediated peripheral cutaneous vasoconstriction, and a reduction in CBF in favour of a central pooling of blood in the torso and deep body core (98,198,199). This reduces convective heat transfer between the body's core and shell (e.g., skin, subcutaneous layer and skeletal muscle), effectively increasing insulation by the body's shell (198). However, due to the large surface area-to-volume ratio, heat is still lost from the exposed body surface faster than it is replaced; therefore, skin temperature (T_{skin}), and eventually intramuscular (T_{muscle}) and core body temperature (T_{core}) will steadily decline, and is commonly observed to do so following CWI (123,125,137,198). Thus, the vasoconstrictor response to cold exposure helps retard heat loss and defend core temperature but at the expense of a decline in peripheral tissue temperature (198). Following prolonged steady state exercise, vasoconstriction begins when T_{skin} falls below ≈35˚ C and becomes maximal when T_{skin} is 31 ˚C or less (210). In accordance, overall reductions in thigh T_{skin} of ≈10 – 15

˚C from post-exercise (≈35 – 37 ˚C) to the end of immersion (≈19 – 27 ˚C) were observed in two studies (122,185), as well as an ≈2 – 3 ˚C reduction in deep (2 – 3 cm) VL T_{muscle} (122). Additionally, reductions in cutaneous vascular conductance (CVC) were observed during CWI (9 ˚C and 15 ˚C) when compared to thermoneutral immersion and passive rest (for which there was an increase) (122). CVC is calculated from the perfusion units obtained by laser doppler flowmetry (LDF) and indirectly estimates local CBF (112,149), therefore its reduction during cooling is commonly associated with the occurrence of cutaneous vasoconstriction (112,150). Similar reductions in tissue temperature and CVC have been observed previously during post-exercise CWI (111,150). Whilst a degree of cutaneous vasoconstriction appeared to take place (122), the observed reductions in tHb (122,185) also suggests a vasoconstrictive response can occur within the local microvasculature of the superficial muscle during CWI. In this regard, the reduction in tHb, representing reduced muscle blood volume, may have occurred due to reduced blood supply to this region.

The novel findings such as observing a reduction in muscle blood volume during CWI are unique to the NIRS technique; however, vasoconstriction as a result of cooling has been observed within similar muscle regions using other techniques. For example, utilising an isotope clearance technique (injected at a depth of ≈2 - 2.5 cm), local muscle blood perfusion of the VL was observed to decrease following a period of post-exercise cold exposure (20 min ice pack application) (211). Furthermore, utilising positron-emission topography (PET), capable of distinguishing muscle perfusion in the individual deep and superficial muscles of the quadriceps, a decreased muscle perfusion was demonstrated in the superficial *rectus femoris* (RF) and VL muscles of the quadriceps following CWI (10 min in 8 – 15 ˚C) (154).

Compared to these techniques, NIRS offers the benefit of non-invasive assessment and the methodological advantage of application during immersion. Therefore, the onset of vasoconstriction (i.e., decrease in tHb) and resultant muscle blood volume response can be continuously monitored during immersion. This could aid understanding for how specific CWI protocols may elicit differences in the blood flow response to cooling.

In contrast, Roberts *et al*., (121) observed an increase in tHb, representing increased muscle blood volume, in the VL during CWI (10 min at 10 °C to the umbilicus). To the authors knowledge, only one other study (212) utilising the NIRS technique has previously observed a similar increase in tHb during cold exposure (15 min of ice pack application); however, the difference in experimental protocols (e.g., prior exercise and cooling modality), and muscle of interest (forearm vs. VL) makes direct comparison difficult. It may be speculated that shivering was responsible for the increase in tHb during CWI. It's proposed the benign shivering response during cold exposure begins from deep muscles to maintain T_{core} (213). However, utilising surface electromyography (EMG) to assess changes in superficial muscle recruitment, at the whole-body level as average T_{skin} and T_{core} decreases, shivering thermogenesis (ST) increases mainly in the proximal trunk and upper leg muscles (213,214). Additionally, high-intensity bursts of ST are linked to the recruitment of type II muscle fibres (215), of which the VL possesses a larger quantity than deeper leg muscles (216). Any involuntary twitching of the muscle fibres during ST stimulates an increased metabolism and O_2 consumption, with an increase in blood flow required to meet this increased metabolic demand (217). While it was acknowledged the participants may have shivered to some extent during immersion (121), without explicit monitoring of VL recruitment, the occurrence of ST cannot be

attributed to the increase in tHb since it remains unclear whether a high contraction rate occurred within this muscle and stimulated an increase in blood flow.

An alternative mechanism associated with an increase in peripheral blood flow is the occurrence of cold-induced vasodilation (CIVD). The phenomenon of CIVD is well established in acral (nonhairy) areas of the skin, such as the extremities (218,219) where, despite an overall drive for vasoconstriction in the cold, following a brief period of lowered T_{skin}, a transient increase in blood flow and rewarming occurs in areas such as the fingertips, toes, face, and feet (220–222). The ensuing rise and fall in blood flow occurs in a cyclic fashion and was originally termed the “hunting response” (218) - but is now commonly referred to as the CIVD phenomenon (219). In these peripheral regions CVID is proposed to maintain tissue temperature and have a potential protective role against cold injury (223). More recently, CIVD was observed to occur in the cutaneous region of the thigh, as represented by an immediate and sustained (2 – 10 min) increase in CVC during CWI, before a gradual vasoconstriction occurred (112). Furthermore, the increase in CVC was present only in colder water immersion (8 ˚C) and was associated with a greater reduction in T_{skin} when compared to a 22 ˚C immersion (112,149). In acral skin, it is the periodic opening and closing of the arteriovenous anastomoses (ASA’s) which is thought to play a role in mediating CIVD (218). However, ASA’s are not thought to be present (or are present in low numbers) in nonacral (hairy) skin (224) and therefore are unlikely to promote the biphasic response in CBF observed in Gregson *et al*., (112) (225). Indeed, the precise mechanisms mediating such changes in nonacral skin have yet to be elucidated; however, a direct inhibitory effect on the normal vasoconstrictor response may be involved (226). The increase in tHb during CWI reported by Roberts *et al*., (121) suggests the occurrence of CIVD within the VL,

thereby causing an increased blood supply to this region. Previously, cyclic fluctuations in T_{muscle} have been observed within the forearm muscle during long-duration CWI (3 h), suggesting the occurrence of CIVD and a significant contribution of muscle vessels during this response (227). Collectively, this limited evidence suggests CIVD may occur both within cutaneous and skeletal muscle regions during CWI. It is likely that CIVD is induced by a significant reduction in T_{skin} and possibly T_{muscle}, however the magnitude of decrease in tissue temperature which may elicit CIVD requires further investigation. The observed reduction in T_{muscle} (≈7 – 8 ˚C) and T_{skin} (≈12 ˚C) during immersion reported by Roberts *et al*., (121) was comparable to that observed (≈4 ˚C and ≈15 ˚C, respectively) during an 8 ˚C immersion, where it was suggested cutaneous CVID occurred (112). Therefore, a similar threshold in temperature reduction may be required in order to induce CIVD in cutaneous and superficial muscle regions.

A further uncertainty presents itself when considering the difference in tHb response (i.e., vasoconstrictive vs. vasodilatory) between studies utilising CWI. Variation in the type of exercise performed and the effect on thermal load prior to immersion may be a possible explanation. Early in the local cooling response (i.e., within the first 10 mins of cooling), vasoconstriction is primarily mediated by nor-epinephrine and the α_2 – adrenergic receptor (228). It is proposed that exercise-induced increases in T_{skin} and/or T_{core} associated with heat stress may attenuate cutaneous α- adrenergic-mediated vasoconstriction responsiveness (229). The influence of prior exercise on vasoconstrictor responsiveness has previously been inferred from the comparison of two similar CWI protocols (10 min in either 8 ˚C or 22 ˚C), on the resultant FABF and CVC response during immersion (111,112). Under resting conditions, an increase in CVC with a concomitant decrease in FABF was observed in the colder (8 °C)

immersion (112), suggestive of the occurrence of cutaneous CIVD. Conversely, a similar decrease in CVC and FABF was observed between both immersion temperatures following exercise, despite further T_{skin} and T_{muscle} reductions in the colder (8˚ C) immersion (111). It was suggested the exercise-induced increase in limb and body temperature (and attendant skin blood flow) prior to immersion caused a reduction in the degree of cutaneous vasoconstriction (111). This does not necessarily mean vasoconstriction did not take place but rather was attenuated, thereby preventing CBF reaching a low enough level for the associated onset of CIVD (112). As such, a sustained cutaneous vasoconstriction occurred in both immersion temperatures following post-exercise CWI (111). Similar patterns of reduction in CVC and FABF have been observed during immersion (temperatures ranging 8 – 22 ˚C) following resistance exercise (150) and endurance exercise (111,122). On the other hand, following muscle damaging exercise (i.e., box jumps), an increase in CVC has been observed immediately post-immersion, followed by a gradual decrease over the next 20 min (186,187). This may be more reflective of the biphasic response previously observed under resting conditions (112), however, since CVC was not actually monitored during the immersion period, it cannot be ascertained whether the increase in CVC began during cooling. Collectively, these findings demonstrate the mixed and inconclusive findings regarding the CBF response during post-exercise CWI, with differences in the experimental designs (e.g., group design), prior exercise, and timepoints of assessment making it difficult to compare studies. Nevertheless, where a sustained reduction in CVC is observed post-exercise compared to rest, this may be attributed to an attenuation in vasoconstrictor responsiveness, therefore preventing CVID (111).

Utilising NIRS to assess muscle blood volume changes has revealed similar variation in apparent CIVD (i.e., increase in tHb) (121) and vasoconstrictive (i.e., decrease in tHb) (122,185) responses during CWI. Therefore, it is possible an attenuation in vasoconstrictor responsiveness may also modulate blood flow within the local muscle microvasculature. In this regard, the difference in exercise modality (endurance (122,185) vs. resistance (121)) and ambient conditions (normothermic (121) vs. hyperthermic (122)) between studies could explain differences in the observed tHb response during CWI. For example, high-intensity endurance activity is typically associated with a greater level of systemic (i.e., core temperature) hyperthermia and different metabolic perturbations than resistance exercise (230–232). Physiologic data from the included studies also suggests a greater post-exercise hyperaemic response was present following endurance exercise; for example, there was increased superficial muscle (i.e., tHb) and limb (i.e., FABF) blood flow observed (122,185). Arguably, the accumulated thermal strain was higher following endurance exercise, as evidenced by significantly elevated skin (i.e., CVC) blood flow following high-intensity cycling in hyperthermic ambient conditions (122). In addition, T_{muscle} was significantly greater following endurance exercise (122), versus resistance exercise (121), (≈4 ˚C at 3 cm depth vs. ≈2 – 3 ˚C at 1 – 2 cm depth, respectively), representing an increase in limb temperature following endurance exercise. The greater perturbations within these parameters prior to immersion following endurance exercise may have attenuated vasoconstrictor responsiveness; conversely, the smaller increase in thermal strain and limb temperature following resistance exercise may promoted CIVD at the onset of immersion, in order to maintain blood flow and temperature within the superficial muscle.

When tHb was observed to increase during CWI, an ≈10% decrease in tHb ensued for the following 20 min post-immersion (121). This reflects an increase in muscle blood volume during immersion, followed by a decrease upon exit. A similar biphasic response has been observed for CBF when CWI was performed under resting conditions (112). The underlying mechanisms mediating this response are yet to be established in either nonacral cutaneous or skeletal muscle regions. It is possible that both CBF and muscle blood flow are maintained during a sustained period of CIVD; however, the greater contact time between the blood within these peripheral regions and cooling medium (i.e., water) as a result of CIVD leads to significant reductions in T_{skin}, $T_{muscle,}$ and T_{core}. Consequently, vasoconstriction possibly occurred following immersion to compensate for the reductions in limb and core temperature, and attempt to regain thermoregulatory homeostasis (98). Indeed, there was a very gradual increase in T_{skin} and T_{muscle} back towards baseline such that values were still below baseline 60 min post-immersion. This possibly reflects the vasoconstrictive response and reduction in blood flow to these peripheral regions, thereby delaying the rewarming of the tissue.

In contrast, following endurance exercise a continued reduction in tHb for up to 30 min post-immersion was observed (122). Furthermore, the tHb reduction was greater in the colder (9 ˚C) immersion compared to the 15 ˚C immersion (63% vs. 30%, respectively), suggesting the 9 ˚C immersion caused a prolonged vasoconstrictive response within the superficial muscle (122). Despite the difference in tHb reduction between temperatures, $T_{skin,}$ T_{muscle}, and T_{core} responses were similar at all timepoints (122). Previously, a 10 min CWI at 8 ˚C caused greater reductions in T_{skin} and T_{muscle} compared to 22 ˚C (111); also, application of a cooling pad for 30 min reduced T_{skin} and T_{muscle} of the ankle dorsiflexors to a greater extent when colder temperatures (0,

10 ˚C) were used (233). Therefore, a longer immersion duration than 5 min may have been necessary to observe differences in these parameters in Choo *et al*., (122), particularly given the hyperthermic ambient conditions (35 ˚C) which were incorporated. Since, greater increases in core body and limb temperatures are observed following hyperthermic exercise (234), a longer immersion duration would be required to overcome this response by allowing time for convective and conductive heat exchange to occur within the limb (130). Given sufficient time, the greater cooling effect of 9 ˚C immersion should permit further reductions in T_{skin}, T_{core} and T_{muscle}. Nevertheless, this study suggests that the use of colder immersion temperatures may lead to greater reductions in muscle blood volume, likely through a prolonged vasoconstrictive response limiting blood flow to the muscle microvasculature. However, in the case of this study, and indeed most CWI literature, it is important to note that such changes may be unique to the specific experimental context, and other forms of exercise (e.g., resistance) and ambient conditions (normothermic vs. hyperthermic) may influence the physiological response to CWI in a different way (49).

The inclusion of multiple techniques for assessing relative, limb, muscle, and cutaneous blood flow during cooling is useful for providing insight into the blood flow response within each of these distinct compartments and the potential association between them. For example, Choo *et al*., (122) reported a reduction in CVC, FABF and tHb as a result of cooling (9 ˚C and 15 ˚C). Since superficial muscles still contribute to a large proportion of the bulk skeletal muscle mass, it is possible the observed reduction in tHb (i.e., superficial muscle blood flow), and CVC (i.e., skin blood flow) primarily contributed to the reduction in FABF (i.e., overall limb blood flow). Interestingly, and similar to previous studies performing CWI post-exercise

(111,150), the magnitude of reduction in FABF and leg vascular conductance was similar between immersion temperatures, despite the greater reductions in tHb in the colder (9 ˚C) immersion. This reflects the potential difference in blood flow kinetics between bulk flow (i.e., to the limb) and blood flow within the microvasculature (i.e., small arteries, veins, and capillaries) (151,152). A combined approach for monitoring blood flow utilising the Doppler and NIRS techniques, along with the common thermoregulatory measures (i.e., T_{skin}, T_{muscle}, T_{core}) should become the objective of future studies in order to gain further understanding into the different blood flow changes within the cooled limbs, and the physiologic mechanisms responsible.

2.5.2 Muscle oxygenation changes (TSI) during and following CWI

An increase in TSI (%) was observed during CWI alongside a concomitant decrease in local muscle blood volume (122,185). TSI (%) provides an overall measure of tissue O_2 saturation (171,177,235), considering both O_2 demand and delivery. Therefore, with an apparent reduction in O_2 availability (i.e., reduction in tHb), the increase in TSI (%) was suggested to represent a decrease in O_2 utilisation due to a cold-induced reduction in muscle metabolic activity (185). The notion for a reduced O_2 utilisation within the muscle was further supported in Ihsan *et al*., (185), with the individual oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb) traces during CWI demonstrating a relative increase in O_2Hb concentration and decrease in HHb concentration (185), thereby suggesting a reduction in O_2 extraction from the local muscle blood volume. Previously, decreases in tissue metabolism during cold exposure have largely been investigated in animal (73,155) and surgical/medical studies that performed cryotherapy on amputated and stored limbs and organs (131,157,236). Within these specific anatomical contexts, it is primarily the cold-induced reduction in tissue temperature which facilitates a reduction in metabolism.

In accordance with the Q_{10} effect (155), it is proposed cryotherapy may slow muscle cellular metabolism through a reduction in T_{muscle} (131,155,200), which could then reduce O_2 demand during oxidative phosphorylation (58). Ihsan *et al.,* (185) did not explicitly monitor T_{muscle} during CWI, however there was a significant reduction in thigh T_{skin}, indicating that the underlying muscle was likely cooled to a significant extent. Indeed, similar reductions in T_{skin} following 20 min of full lower-body CWI have resulted in large reductions in T_{muscle} (126). Choo *et al*., (122) on the other hand did monitor T_{muscle} and recorded a significant decrease (≈2 – 3 °C) during CWI at both temperatures (5 min in 9 ˚C or 15 ˚C). It should be noted, whilst this study also observed a trend for TSI (%) to increase (≈2%) during cooling (compared to thermoneutral immersion or passive rest), the TSI (%) increase was minimal, and the differences in TSI (%) was not statistically significant between groups. Comparing this to Ihsan *et al*., (185) who reported significant differences in TSI (%) between the cooled and non-cooled limbs after 9 min into immersion, it is possible that Choo *et al*., (122) required a longer immersion duration in order to observe a similar change in TSI (%), as this would have allowed a greater reduction in tissue temperature to have occurred. Indeed, reductions of 7 – 8 °C in T_{muscle} have been observed when 30 min of CWI in 10 °C was performed (137). Nevertheless, it is difficult to directly compare the magnitude of tissue cooling between the included studies due to variation between the methods of cooling, (i.e., local limb immersion vs. full lower body immersion). Previous research has also shown greater reductions in tissue temperature (i.e., T_{muscle}) are achieved as a result of local limb cooling (e.g., ice pack application) compared to full-body CWI (133,134,155). Therefore, it is possible a greater reduction in T_{muscle} may have occurred during the single limb immersion in Ihsan *et al.*, (185), significantly altering muscle metabolic activity, as reflected by the

greater change in TSI (ΔTSI). However, since T_{muscle} was not monitored within Ihsan *et al*., (185), the magnitude of reduction between the two CWI methods cannot be ascertained. Aside from directly altering cellular metabolism, cold-induced reductions in tissue temperature (i.e., muscle and skin) may also modulate muscle blood flow through vasoconstrictive and vasodilatory mechanisms (see section 2.4.1). Indeed, a reduction in local muscle blood volume (tHb) was observed during CWI suggesting a vasoconstrictive response may have occurred. Consequently, muscle O_2 utilisation may then decrease to match any reduction in O_2 delivery which could arise due to vasoconstriction (237).

Following CWI, TSI (%) remained unchanged (121) or reduced (186,187) in comparison to baseline (i.e., obtained before exercise) values. Specifically, TSI (%) was reduced by ≈10 – 15% during a 40 min post-immersion period following muscle damaging exercise (i.e., box jumps) (186,187). These findings for TSI (%) suggest O_2 saturation within the local muscle was reduced during this 40 min period. However, the aforementioned studies did not report the change in either tHb, O_2Hb or HHb. Without these further NIRS measures, the reason for the reduction in TSI (%) (i.e., O_2 saturation) can only be speculated upon. One possible scenario for the TSI (%) reduction is a cold-induced reduction in O_2 supply (i.e., due to vasoconstriction within the muscle), without any concomitant change in O_2 extraction from the available blood volume- this would inevitably reduce overall muscle O_2 saturation. In this regard, the reduction in TSI (%) would not represent a decrease in muscle O_2 demand or metabolic activity, but rather reflect the change in muscle blood flow as a result of cooling. Obviously, this notion would require further evidence in the form of other NIRS measures (i.e., tHb, O_2Hb or HHb).

Overall, caution should be applied when using the change in TSI (ΔTSI) as a proxy

for skeletal muscle metabolism. The TSI (%) measurement can only realistically provide information on the adequacy of local muscle O_2 saturation on a 0 – 100% scale, it does not enable quantification of muscle O_2 consumption. As such, it remains to be established if a change in TSI (%) represents any significant alteration in muscle metabolic activity, and this information is only further limited when there is no observed change in TSI (%). Through inspecting changes in TSI, tHb, O_2Hb, and HHb at the same timepoints, further information regarding O_2 extraction from the blood volume can be determined, thereby allowing some interpretation with regard to a potential decrease in muscle O_2 demand and cellular metabolism. Future studies should report all primary NIRS measures (i.e., TSI, tHb, O_2Hb, and HHb) not only to aid understanding but also enable comparison in the observed responses between studies. Since studies only choose to report certain NIRS measures during and following CWI, it's efficacy for reducing muscle metabolism remains inconclusive.

2.5.3 Muscle blood volume (tHb) and muscle oxygenation (TSI) during exercise.

The previous sections have explored the tHb and TSI (%) response during and following post-exercise CWI. This section will now explore these measures during exercise in order to investigate how the cold-induced changes in muscle blood flow and oxygenation influence subsequent activity. During exercise there was variation in the local muscle blood volume response (tHb). For example, during high-intensity cycling (e.g., 1 – 4 min short intervals), tHb values tended to be lower in a subsequent bout after CWI (5 min at 10 ˚C) had been performed compared to passive recovery (174). Although tHb was not monitored during or following CWI leading up to the subsequent exercise bout, it was suggested that the occurrence of vasoconstriction within the superficial muscle was responsible for the reduction in

tHb at the onset of subsequent exercise. This also appeared to be the case in Baláš *et al.,* (188) where during a 20 min rest period prior to performing handgrip exercise (repeated forearm contraction at 60% MVC until exhaustion), CWI's (3 x 4 min in either 8 ˚C or 15 ˚C) were performed and resulted in a similar reduction in tHb of ≈20 μmol^{-1} between temperatures when compared to passive recovery. However, during the intermittent handgrip exercise, tHb was also monitored continuously and a similar magnitude of change was observed during each 8 s contraction phase between all groups (8 ˚C CWI, 15 ˚C CWI, and passive recovery) (188). Therefore, microvascular function was quicky restored within the forearm muscle once exercise began in response to the metabolic demands of the muscle, and the lower T_{skin}, (and likely T_{muscle}) which was observed in the CWI groups did not have an effect on muscle blood perfusion during exercise (188). TSI (%) was also recorded in both the aforementioned studies during exercise which enables useful insight with regards to O_2 utilisation. For example, Stanley *et al.,* (174) observed no changes in TSI (%) or the rate of deoxygenation between groups during subsequent cycling exercise. Therefore, since muscle O_2 availability (i.e., tHb) was reduced, yet O_2 saturation of the blood (TSI) was similar it was suggested that CWI led to a reduction in muscle O_2 utilisation during subsequent exercise (174). In tentative support of this notion, the anaerobic contribution to exercise was measured during the first cycling interval of each exercise bout and was observed to increase in the subsequent bout when CWI had been performed (174). Nevertheless, the proposed reduction in O_2 utilisation did not affect subsequent exercise performance, as performance measures (e.g., VO_2, cadence, power output) were observed to be similar between groups. Similarly, during resistance exercise (unilateral knee exercise at 60% peak torque) Yeung *et al*., (189) observed an increase in TSI (%) during a subsequent

bout after CWI (10 min at 12 – 15 ˚C) had been performed compared to passive recovery. During the same bout of exercise, an increase in O_2Hb and a concomitant decrease in HHb (reported as % of baseline) were also reported. Collectively, these NIRS measures may interpreted as a reduction in O_2 extraction from the blood, similar to what Ihsan *et al*., (185) observed during post-exercise CWI, which may be due to a reduction in muscle metabolic activity and O_2 demand. The short time period (≈10 min) between the end of CWI and the start of subsequent exercise in Yeung *et al*., (189) may be responsible for influencing O_2 utilisation during subsequent activity. Similar to Stanley *et al*., (174) the potential reduction in O_2 utilisation induced by CWI did not limit muscle function during subsequent exercise, with similar reported values for peak torque, work done and fatigue rate when compared to a passive rest control condition (189). Therefore, within these studies, the observed changes in the NIRS measurements (e.g., TSI, O_2Hb, HHb) may not have represented a significant enough alteration in muscle metabolism and O_2 utilisation to negatively affect muscle function and performance during subsequent exercise. Conversely, Baláš *et al*., (188) monitored ΔTSI during the contraction phases and observed the minimum value for TSI (TSI_{min}) achieved during exercise was lower after both CWI groups compared to passive recovery (188). Given that muscle blood perfusion (tHb) was similar between all groups, this was interpreted as a greater O_2 extraction in the cooled muscle and could've been a potential mechanism leading to the improvement in handgrip performance (i.e., prolonged time to failure). However, the greater TSI (%) decrease was not the only mechanism leading to improved performance (i.e., prolonged time to failure), as there was a decrease in performance in the 3rd and final handgrip trial following 8 ˚C CWI, despite a low TSI_{min}. It also was speculated that a greater subjective pain and

temperature perception during CWI recovery may have also played a role in subsequent performance improvements (188). On the other hand, the likely greater reduction in T_{muscle} in the colder (8 ˚C) immersion group may have lowered nerve conduction velocity and prolonged contraction-relaxation time (55,238), thereby explaining the decrease in performance in the final handgrip trial.

2.5.4 Importance of findings for athletic recovery.

The current review has demonstrated that NIRS can inform upon changes in tHb (ΔtHb) and TSI (ΔTSI) during and following CWI. A number of physiological mechanisms have been proposed in an attempt to explain these observed changes; with ΔtHb primarily associated with either a cold-induced vasodilatory or vasoconstrictive response, and ΔTSI (alongside other NIRS measures) reflecting a possible alteration in muscle metabolic activity. The potential for CWI to modulate muscle blood flow and metabolism form some of the rationale for its application following strenuous (i.e., muscle damaging) exercise (47). Since the negative symptoms associated with EIMD last into the following days (generally observed to peak around 24 – 48 h) following exercise (35,59), CWI is commonly administered shortly following exercise in an attempt to ameliorate these negative symptoms, hasten recovery and promote return to athletic activity (36,48). The legs, particularly the superficial muscles of the upper leg (i.e., VL, RF), are a common site in which EIMD occurs due to the higher proportion of damage vulnerable (239,240) fast-twitch (type II) muscle fibres which they possess (216). Therefore, these superficial muscles likely play a significant role in causing the negative symptoms associated with EIMD (e.g., DOMS, reduction in ROM and muscle function) and it may be of benefit in attempting to reduce the extent of damage which occurs within these muscles. It is proposed one way which CWI aids post-exercise recovery is through a

reduction in blood flow to the muscle; this may attenuate the infiltration of pro-inflammatory cells (contained within the blood) to the site of muscle damage, thereby potentially limiting secondary enzymatic injury to the surrounding uninjured cells (47,58,72). Theory also suggests that cold application is beneficial in reducing oedema manifestation particularly during the initial inflammatory stage due to its ability to produce vasoconstriction and reduce arteriolar blood flow, thereby reducing membrane permeability and capillary infiltration (241). A decrease in microvascular permeability and capillary infiltration limits the flow of proteins and fluid into the interstitial spaces (which when excessive leads to oedema manifestation) (241). The presence of oedema increases compression of the capillaries, causing an inadequacy of fuel substrate, O_2 delivery and waste product removal for affected muscle cells, thereby contributing to secondary ischaemic injury (58). By utilising the NIRS technique, studies were able to observe how CWI reduced tHb in the VL both during (122,185) and for up to 40 min post-immersion (121,122). This finding reflects a reduction in total blood volume within the local muscle microvasculature and is interpreted as a vasoconstrictive response to cold exposure. Since the primary purpose of the aforementioned studies was to investigate the physiological response to post-exercise CWI, it remains to be elucidated if such reductions in tHb had a beneficial effect on recovery indices. Based off the current proposed mechanisms, the observed reduction in tHb, representing a possible reduction in acute microvascular blood flow, may aid in ameliorating symptoms of EIMD in the days following exercise via facilitating reductions in acute inflammation and oedema. However, whether the observed ΔtHb was significant enough to alter these processes within the muscle would require further investigation with studies which monitor the NIRS physiological response during post-exercise CWI, and also assess

indices of muscle damage (e.g., DOMS, ROM, muscle function tests, blood inflammatory markers). Whilst an acute reduction in microvascular blood flow may facilitate improvements in recovery, there are implications for CWI performed shortly prior to exercise. For example, two studies observed a prolonged reduction in tHb was observed for up to 40 min following CWI (121,122). Subsequent exercise performed within this timeframe may then be negatively impacted through a reduction in O_2 and substrate availability to the active musculature (47). In this regard, CWI may increase the reliance on anaerobic metabolism during subsequent exercise; Stanley *et al*., (174) provided support for this notion by reporting a greater anaerobic contribution during the initial stages of subsequent HIIT cycling.

In contrast to the aforementioned included studies, one study observed a rise in tHb in the VL during CWI (121). Similarly, this response has previously been observed in the forearm muscle during a 15 min ice pack application (212). An increase in tHb may reflect a cold-induced vasodilatory response, and increased blood flow to the local muscle microvasculature. In this regard, an opposing response to that outlined above may occur, in which the application of CWI accentuates the acute inflammatory response and oedema manifestation within the superficial muscle, thereby possibly delaying recovery. In support, Tseng *et al*., (212) observed an increase in the muscle damage markers CK and myoglobin concentration as well as subjective feelings of muscle pain and fatigue at 48 – 72 h following eccentric elbow exercise, when cooling was compared to non-cooling (control).

Overall, the NIRS measure of TSI (%) produced varying results, with some studies reporting no significant changes during or following CWI (121,122). One study (185) observed an increase in TSI (%) during CWI alongside a concomitant decrease in tHb, which was suggested to reflect a decrease in O_2 utilisation due to a cold-

induced reduction in muscle metabolic activity. The opposing directional changes in O_2Hb (i.e., increased) and HHb (i.e., decreased) during immersion (185) also seemed to support this notion by demonstrating the apparent reduction in O_2 extraction from the local blood volume. Taking the findings of Ihsan *et al*., (185), a reduction in muscle metabolic activity during cold exposure may aid in reducing the inflammatory response, thereby limiting secondary damage to the affected muscle cells. However, similar to the observed tHb response during CWI, it remains to be established whether the observed changes in TSI (%) represent a significant enough physiological change within the muscle which would then benefit recovery following EIMD, i.e., would contribute to improving the recovery of muscle damage indices. Furthermore, other studies failed to report both TSI (%) and tHb, thereby making it difficult to interpret the ΔTSI with regard to any potential alteration in muscle metabolism. The TSI (%) measurement represents the dynamic balance between O_2 delivery and extraction within the muscle and can be jointly affected by either parameter, with an inability to discriminate between the two. Therefore TSI (%) can only realistically inform upon overall muscle O_2 saturation and cannot quantify muscle O_2 consumption. However, when TSI (%) is reported alongside other primary NIRS variables (tHb, O_2Hb, HHb), it allows interpretation with regards to changes in O_2 extraction taking place within the muscle, with tHb used as a proxy measurement for muscle blood flow.

In the physiological assessment of CWI, it is important to observe how different protocols (i.e., immersion temperatures and durations) affect the NIRS measures, as this could improve understanding regarding protocol optimisation for athletic recovery. Overall, comparison between the included studies of this review was difficult due to heterogeneity in the exercise protocols used (endurance vs

resistance), and timepoints of NIRS assessment (during/ post-CWI) which likely has an effect on the observed outcome measures. Where a reduction in tHb occurred, CWI durations and temperatures ranged from 5 – 15 mins and 8 – 15 °C, respectively (122,185,188,242). However, a 15 min immersion at 10 °C resulted in a lower magnitude of tHb reduction when compared to a 5 min immersion at 9 °C and 15 °C. Therefore, as previously explained within the text (section 2.5.1), this disparity may arise from the variation in prior exercise, the initial timepoint at which ΔtHb was calculated from during CWI, or other contributing factors such as hydrostatic effect during different immersion protocols. Nevertheless, the aforementioned studies suggest a reduction in tHb is achievable in when a ≈10 – 15 °C immersion is performed for ≈10 mins following strenuous exercise. Two studies (122,188) included more than one immersion temperature in their physiological assessment. Most notably, Choo *et al.,* (122) did not observe significant differences in tHb during a 5 min immersion at either 9 °C or 15°C; however, at 30 mins post-immersion tHb remained lower in the 9 °C immersion compared to the 15 °C. As such, for targeting a prolonged reduction in muscle blood flow, the use of colder immersion temperatures may be required.

The TSI (%) response was also difficult to compare between CWI protocols due to the large variation in research methodologies. Most notably, a significant increase in TSI (%) was observed with a single limb 15 min immersion in 10 °C, alongside a concomitant reduction in tHb (185). Conversely, other studies utilising CWI durations ranging from 5 – 10 min and temperatures of 9 – 15 °C did not observe any significant difference in TSI (%), despite significant reductions in tHb. Therefore, it is possible that a CWI duration of at least 15 mins is required to influence metabolic changes within the muscle.

2.6 Limitations of the reviewed studies.

From the research presented within this review a number of generic limitations are offered, which should be considered when interpreting the findings. There is a lack of research which incorporates NIRS measurements alongside CWI, meaning there is limited evidence to support the NIRS-related findings from individual studies. Next, variation existed in the timepoint at which NIRS measurements were obtained; with studies either monitoring during immersion, during exercise or during a post-immersion period. Only two studies monitored the change in tHb and TSI (%) both during the immersion period and in the post-immersion period (121,122). Continuous monitoring during and post-CWI enables a longer time-course of the tHb and TSI (%) response to be observed, which is desirable for aiding understanding of the physiological mechanisms involved, observing when changes occur during CWI (e.g., vasoconstriction/vasodilation), and how long these persist post-immersion. In particular, the changes which occur post-immersion may have important implications for CWI performed close to subsequent exercise, through facilitating negative effects on performance. Therefore, future studies should look to obtain NIRS measures both during CWI and in the post-immersion period in order to guide effective implementation. Of the included studies, variation in the experimental protocol (e.g., modality of exercise, CWI protocol) may alter the observed physiological response to CWI (49). As such, comparisons between studies is limited when considering the heterogenous research methodologies.

There are also a number of limitations which are more specific to individual studies. Firstly, Ihsan *et al*., (185) utilised a single leg CWI, with the contralateral unimmersed leg acting as the control. This arguably does not reflect real-world athletic application of post-exercise CWI since there are limited scenarios where only a single leg would

need to be immersed as opposed to both following exercise. It has been shown that cooling one leg can also lower T_{muscle} of the contralateral non-cooled limb (243), thereby limiting the rationale for a single limb immersion. Secondly, as already mentioned, a limited number of the reviewed studies actually obtained NIRS measurement during water immersion. For those that did, to improve validity, a degree of pre-testing is warranted to ensure the method of waterproofing does not affect the NIRS signal, this however was not explicitly mentioned within any study. Assuming the waterproofing method does not affect the NIRS signal, this eliminates the potential concern for further variation between studies. Furthermore, different NIRS devices have also demonstrated greater average reductions particularly in the TSI (%) signal during exercise (179), which could be misled as a greater muscle deoxygenation. Such variation may also arise in the TSI (%) signal during CWI between NIRS devices. In order to overcome this intrinsic limitation, along with other sources of inter-subject variation including adipose tissue thickness, a maximum range of all NIRS measures should be obtained (e.g., physiological calibration), with Δ values calculated as a percentage on this “true” 0-100 scale (168,179,233,244).

2.7 Conclusion

This review presents the novel findings from studies which have monitored the NIRS-derived tHb and TSI (%) measurements during and following CWI. There is evidence for both a vasoconstrictive and vasodilatory response during and following CWI, as represented by a reduction or increase in muscle blood volume (tHb), respectively. Conversely, the TSI (%) measurement rarely allows interpretation regarding changes in muscle metabolism during and following CWI. This is either due to no observed change in its value, or studies reporting it as a standalone measure without assessing further primary NIRS variables (tHb, O_2Hb, HHb).

Overall, caution should be applied when inferring changes in muscle blood flow and muscle metabolic activity when assessing ΔtHb and ΔTSI, respectively, as these measures cannot actually quantify such parameters. Nevertheless, when NIRS measures are combined alongside other blood flow (CVC, FABF) and thermoregulatory (T_{skin}, T_{core}, T_{muscle}) measures an extensive physiological profile during and following CWI can be obtained within a limb of interest. Such an approach is a positive step in improving understanding of the physiological response to post-exercise CWI, and how this coincides with the proposed mechanisms of recovery in the days following EIMD. Therefore, future work should aim to adopt this approach. Furthermore, in order to investigate how CWI protocols can be optimised to produce a desired physiological change within the muscle, there is the requirement for further studies monitoring the physiological response during different CWI durations and temperatures. Moreover, the main advantage of NIRS is its capability to non-invasively monitor continuously during and following an immersion period, offering insight into the physiological changes within superficial muscle regions pre-, during- and post-CWI.

3

Chapter 3: The Effect of Cold Water Immersion on Lower Limb Muscle Blood Flow and Muscle Oxygen Consumption.

3.1 Introduction

Exercise-induced muscle damage (EIMD) is the result of mechanical and/or metabolic disruption to the muscle sarcomeres (57,245,246). The initial muscle damage attained from exercise leads to an increased inflammatory response and oedema manifestation, both of which contribute to secondary cell damage and/or cell necrosis. The resulting swelling and damage to the muscle contractile structures compounds muscle soreness and leads to loss of muscle function in the days following exercise (35,57,63,245,246). Cooling of the exercised limbs following strenuous activity is proposed to limit the extent of secondary damage via temperature-induced reductions in tissue metabolism and microvascular blood flow to the damaged muscle (47,53,54,63,72,131); for a in depth explanation of how cooling modulates these physiological changes, please refer to sections 1.6 -- 1.7. As such, the rationale for application of cold-water immersion (CWI) post-exercise lies in its ability to rapidly cool whole limbs and large muscle groups (49,106), suggested to facilitate reductions in muscle blood flow and muscle metabolism. Previously whole limb (117,147) and main conduit artery (111,112) measurements of blood flow have shown contrasting results in response to CWI, with no change (147) and decreases (111,112,117) reported. Correspondingly, differences exist in the techniques utilised to provide measurements of blood flow i.e., strain gauge plethysmography, doppler ultrasound and magnetic resonance imaging (MRI) via intravascular tracer. These measurements provide useful insight into the peripheral blood flow response following CWI. However, the obtained measurements of “bulk” flow may not accurately represent skeletal muscle blood flow (152). Furthermore, assessment of the tissue metabolic response to cooling has largely been inferred from animal models (73,155) and medical/surgical studies that performed

cryotherapy on amputated and stored limbs and organs (131,157) which suggest large decreases in tissue temperature are required to significantly alter metabolic activity. These recommendations however lack evidence in human skeletal muscle, for which the tissue temperature response on muscle metabolism remains largely unestablished. Therefore, accurate physiological measurements are required to understand the changes in tissue metabolism, blood flow and temperature as a result of CWI. Arguably, this understanding is needed before gross application across sporting disciplines and athlete groups.

Chapter 2 of this thesis explored the use of near-infrared spectroscopy (NIRS) during post-exercise CWI; for an in depth explanation of the NIRS technique please refer to section 1.8. Changes (Δ) within NIRS-derived measures of tHb and TSI (%) have previously been used as a proxy for muscle blood flow and muscle metabolic activity, respectively (167,247–250). As such, Chapter 2 reviewed studies utilising NIRS during immersion and in the acute period (0 – 60 min) following immersion (121,122,185–187). To summarise, a post-exercise reduction in tHb was observed during CWI (122,185); in the acute period following immersion (0 – 60 min) (121,122) and during the initial phases of subsequent exercise when CWI is performed ≈30 min prior (174). Collectively, these findings were interpreted as a reduction in local muscle blood flow due to a vasoconstrictive response. The TSI (%) response to CWI was less clear, with studies observing no change (121,122) or an increase (185) during immersion. In the period (0 – 60 min) following immersion, TSI (%) was reported as unchanged (121,122) or reduced compared to baseline up to 60 min post-immersion (186,187). Monitoring ΔTSI (%) alongside ΔtHb measurements enables insight regarding changes in muscle metabolic demand (174,185). For example, Ihsan *et al*., (185) observed an increase in TSI (%) during immersion

alongside a concomitant decrease in tHb, suggesting this reflected a decrease in O_2 utilisation and muscle metabolic demand.

Whilst chapter 2 demonstrated the capability of the NIRS technique to monitor local muscle haemodynamics and muscle oxygenation both during and following CWI, conflicting findings and interpretation of ΔtHb and ΔTSI (%) have been shown. Additionally, the use of proxy measurements for muscle blood flow and muscle metabolic activity is problematic. tHb and TSI (%) signals can only provide indirect and relative changes in local muscle blood volume and muscle O_2 saturation, respectively. As such, they cannot be used to accurately quantify changes in either muscle blood flow or muscle O_2 consumption (mVO_2) the latter previously been used as a measure of muscle oxidative function (161,167,244).

The information obtained from NIRS signals can be quantified by applying a physiologic intervention in the form of a venous (VO) and arterial occlusion (AO), thereby enabling direct evaluation of muscle blood flow (mBF) and mVO_2 (see section 1.10). It is the manipulation of blood flow entering/ leaving the limb during VO and AO which allows quantification of mBF and mVO_2 to occur (163). Previously, mBF in the forearm and lower leg has been estimated at rest and post-exercise by measuring ΔtHb during the initial period of VO (163,178,180,191–193). mVO_2 in the same muscles has been estimated at rest and post-exercise by measuring Δ in either O_2Hb, HHb or Hb_{diff} during AO (163,178,180,191,193). Utilising the NIRS-occlusion methodology, it will be possible to observe how CWI influences mBF and mVO_2. These measures will provide accurate insight into the physiological processes occurring and could improve recommendations for CWI implementation as a recovery strategy for EIMD.

Chapters 1 and 2 have both highlighted gaps in knowledge regarding CWI protocol optimisation. Indeed, since no specific guidelines exist, there was heterogeneity in the CWI protocols incorporating NIRS measurement, thereby limiting comparison of outcome measures. The most common CWI durations and temperatures that have generally shown positive effects on the recovery of performance, are between 10 – 15 min and 10 – 15 °C, respectively (48,197). Additionally, a limited number of studies have investigated the influence of different CWI protocols (e.g., temperatures and durations) on physiological measures of muscle blood flow and/or muscle metabolism (112,122); these suggest colder immersion temperatures (e.g., 9 °C) lead to a prolonged vasoconstrictive response (i.e., reduced tHb) following CWI (122), but observed no significant effect of water temperature on muscle metabolic activity (i.e., TSI % remained unchanged). Therefore, the purpose of this study was to investigate the effects of CWI at two different water temperatures (10 °C and 15 °C) upon NIRS calculated measures of mBF and mVO_2. Given that colder water temperatures lead to greater reductions in tissue temperature (section 1.7.1); that a greater reduction in tissue temperature is suggested for reducing tissue metabolic rate (137,155); NIRS tHb data suggests colder water temperatures lead to a greater magnitude of reduction in local muscle blood volume, the following hypothesis were made; (1); the application of CWI would cause a reduction in mBF and mVO_2 post-immersion, (2); the colder water temperature (10 °C) will lead to greater reductions in mBF and mVO_2 compared to the 15 °C.

3.2 Methods

3.2.1 Participants

Eleven adult male participants (age = 23.4 ± 3.4 years, height = 1.8 ± 0.1 m, body mass = 68.8 ± 10.7 kg) volunteered to take part in this study. Participants were recruited via email (distributed by the University athletics email) after initially obtaining consent from the coaches for their athletes to take part. Mean adipose tissue thickness beneath the NIRS device was 6.7 ± 2.7 mm. All participants were university level long-distance runners (NCAA Division 2) competing for the same University athletics team. Participants were requested to refrain from consuming caffeine and alcohol for 48 h before attending each testing session. Participants were included if they had no history of lower limb surgery or musculoskeletal injury in the prior month before the study taking place, had no history of skin hypersensitivity or other conditions affected by cold exposure, and possessed a subcutaneous adipose tissue thickness of less than 15 mm at the gastrocnemius muscle. This maximum value for subcutaneous adipose tissue thickness was necessary to ensure effective NIRS light penetration to the underlying muscle tissue. Participants were screened for this inclusion/ exclusion criteria by including this information within the recruitment email and skinfold thickness was assessed on the first visit. All participants were fully informed of the requirements and risks associated with the study, and a written informed consent was obtained before participation. This study was approved by the University of Essex Research sub-committee.

3.2.2 Experimental Design

A randomised, crossover design was implemented to assess the influence of cold-water immersion (CWI) on measures of muscle blood flow and muscle oxygen

consumption following exercise. Each participant was required to take part in two testing sessions, separated by at least 24 h. Participants attended the university sports rehabilitation centre at the same time of day where they were randomly assigned to one of two recovery interventions, a 10 °C or 15 °C CWI for 20 min. The selected immersion temperatures and duration were chosen for the following reasons; 1) there is currently no consensus for CWI temperature or duration; 2) 10 °C or 15 °C are however the most commonly used temperatures within literature (48,251); 3) a sufficient period of immersion is required to reduce tissue temperature (133,134), however duration length must be feasible/ practical for the user. Before and after the CWI, a venous and arterial occlusion of the leg undergoing NIRS assessment was performed. All participants underwent both recovery interventions on separate days. An overview of the experimental protocol can be observed in Figure 3.2.2.

3.2.3 Preparation

The NIRS device was placed on the midportion of the medial gastrocnemius muscle belly. Placement site was located through measurement of the lower leg from the lateral epicondyle to lateral malleolus and 1/3 of this distance was marked. Next, the widest part of the main gastrocnemius head (medial) was measured, and the midpoint was found and marked, the NIRS device was placed where these two points met, with the middle of the placed device over this point. To ensure accuracy of repeat device placement, an outline of the device was drawn onto each subject's leg using a surgical marker pen. Subjects were asked to maintain the outline during the testing period. Additionally, any bodily hair within the device placement area was removed from each subject's skin. Leg skinfold thickness was measured at the site of device attachment using Harpenden skinfold callipers (HSK BI, Baty International,

West Sussex, United Kingdom) and divided by two to determine the thickness of the subcutaneous fat layer. The leg undergoing assessment was randomly assigned, with half of the participants being assigned to either right or left, to account for possible physiological bias arising from measuring one leg.

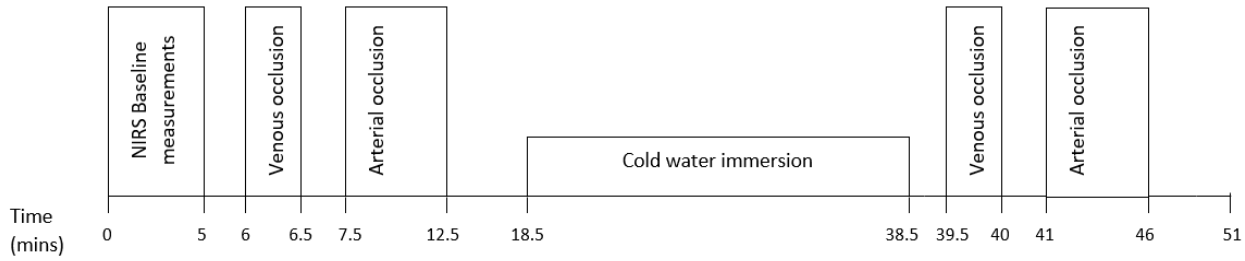

Figure 3.2.2. Experimental protocol illustration.

3.2.4 CWI protocol

For CWI, participants remained in a seated, upright position in an immersion bath (Whitehall manufacturing, California, USA, Bath model: S-85-S)(Figure 3.2.4) which was filled with water to the level of the navel. Water temperature was maintained within ± 0.3 °C of 10 °C or 15 °C using a digital aquarium thermometer and stirred every two minutes using an inbuilt whirlpool machine. Participants wore shorts and were instructed to keep as still as possible and not activate their lower limb muscles for the duration of the 20 min immersion. Following immersion participants would exit the recovery bath and quickly towel dry before transitioning to the post-CWI occlusions, this transition period took no longer than 120 s. The NIRS device remained attached throughout the experimental procedure (pre, during and post immersion).

Figure 3.2.4. Displays the immersion bath used for CWI.

3.2.5 Occlusion protocol

The occlusion protocol remained identical for both testing sessions (10 °C or 15 °C CWI), Participants lay in a supine position upon a sports therapy couch next to the CWI bath. The protocol consisted of one venous occlusion, followed by a 1 min recovery, then one arterial occlusion, followed by a 5 min recovery, participants then transitioned to CWI. Post CWI, participants returned to the sports therapy couch and the procedure was repeated. Venous occlusions were performed for 30 s and at a pressure of 70 mmHg. Arterial occlusions were performed for 5 min at a pressure of 250 mmHg (192,207), as this has been shown to be sufficient to produce a nadir (252–254). A pressure cuff was attached to the participants leg just above the knee joint and manually inflated to the corresponding pressure for either venous or arterial occlusion. The location for cuff placement was chosen to minimise individual discomfort and avoid motion artefact in the NIRS signal.

3.2.6 NIRS measurements

A miniaturised (83 x 52 x 20 mm) and lightweight (84 g) portable NIRS device (PortaMon; Artinis Medical Systems BV, The Netherlands) was placed and fixed with black adhesive taping over the marked measurement site to prevent signal contamination from external light sources. To make the device waterproof, it was vacuum sealed in an optically clear, transparent, thin plastic material before placement as per Jones *et al*., (183). The same researcher attached each device and applied the adhesive taping for all testing procedures. In order to account for the external pressure applied to the muscle belly (179), a standardised approach (i.e., single fold strapping) was utilised.

The PortaMon is a dual wavelength continuous-wave system, which simultaneously uses the modified Beer-Lambert law and spatially resolved spectroscopy (SRS) methods. Changes in tissue oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb) were measured using the difference in absorption characteristics of wavelengths at 760 and 850 nm. Values for O_2Hb, HHb, and tHb are reported as a concentration change from baseline (5 min averaging before each test with participants in supine position) in micromolar units (μM), utilising a differential path length factor of 4.0. Haemoglobin difference (Hb_{diff}) was calculated $[Hb_{diff}] = [O_2Hb] – [HHb]$ and reflects the difference in concentration between O_2Hb and HHb. The tissue saturation index (TSI) was expressed in % and calculated as $[O_2Hb]/([O_2Hb] + [HHb]) \times 100$. TSI is independent of NIR photon path length in muscle tissue and was calculated using the SRS method (176). This particular device consists of three light optodes and one light detector, with an interoptode spacing between the light sources and detector of 30, 35, and 40 mm. In the present study, chromophore concentrations were detected using the furthest light-emitting

diode (40 mm). Assuming the penetration depth of NIR light into tissue is approximately half the distance between the light sources and the detector (≈2 cm) (255), in our sample of participants with a low adipose level, a significant fraction of the light will interrogate the underlying muscle. Data was collected offline at a sampling rate of 10 Hz, and subsequently graphically displayed and analysed by proprietary NIRS software (Oxysoft; Artinis Medical Systems BV). The NIRS data were collected continuously throughout the experimental protocol.

3.2.7 Muscle blood flow (mBF)

Estimates of mBF were assessed as the [ΔtHb] signal during the first 10 s of VO and analysed using simple linear regression as previously described (163,180,192,196). Changes in [tHb] were expressed in micromolars per second ($\mu M \cdot s^{-1}$) and were converted to millilitres blood per minute per 100 millilitres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using a total haemoglobin concentration of 9.95 mmol·L for males (256). The molecular mass of haemoglobin (64.458 $g \cdot mol^{-1}$) and the molecular ratio between Hb and O_2 (1:4) were taken into account (163). The full equation used to calculate mBF is shown below (Equation 2.)

Equation 2:

$$\text{mBF} = \frac{(\left((\Delta\text{tHb x } 60) \div (([HB]\ x\ 1000) \div 4)\right) x\ 1000)}{10}$$

3.2.8 Muscle oxygen consumption (mVO_2)

mVO_2 was calculated by evaluating the linear decrease in [Hb_{diff}] ([Hb_{diff} = [O_2Hb] – [HHb]) with the assumption that [tHb] is constant (163). In instances where tHb does not remain constant, Hb_{diff} is suggested as preferable to O_2Hb for calculating mVO_2 since it is currently unknown whether the increase in blood volume originates from the arterial or venous site, and therefore remains unclear if the true consumption is reflected by a decrease in O_2Hb or the increase in HHb. Choosing the wrong variable (O_2Hb or HHb) for the calculation of mVO_2 increases the measurement error compared to using Hb_{diff} (163). The linear decrease in Hb_{diff} over the first 3 min of AO was used as this represented the best fit for linear regression for all the participants. Concentration changes of Hb_{diff} were expressed in micromolars per second ($\mu M \cdot s^{-1}$) and converted to millilitres O_2 per minute per 100 grams tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$). A value of 1.04 $kg \cdot L^{-1}$ was used for muscle density (163). The full equation used to calculate mVO_2 is shown below (Equation 3.)

Equation 3:

$$mVO_2 = \frac{\left(\left(\left(\frac{\Delta Hbdiff}{2}\ x\ 60\right) \div (10\ x\ 1.04)\right) x\ 4\right) x\ 22.4}{1000}$$

3.2.9 Statistical Analysis

All results are reported as mean ± standard deviation (SD) unless otherwise stated. The final analysis included ten participants, due to one participant acquiring insufficient data and therefore was excluded from the final analysis. The values for muscle blood flow and muscle oxygen consumption were log-transformed, and the

normality assumption for all data was checked using the Shapiro-Wilk test. Mean values for O_2Hb, HHb, tHb, Hb_{diff} and TSI were calculated for the entire 20 min CWI and for 5 min segments during immersion. A paired samples T-Test was performed on the mean values for the entire 20 min CWI between both temperatures for all NIRS measures. Furthermore, a two-way repeated measures ANOVA was conducted for all NIRS measures obtained at each 5min segment of immersion; the two within subject-variables were time (5min, 10min, 15min and 20min) and temperature (10°C and 15°C). Wherever a significant main effect of time was observed, post-hoc analysis were conducted to compare the effect of time within each temperature using a Bonferroni correction factor. Partial eta squared (η^2) was used to report effect size where η2 = 0.01 indicates a small effect; η2 = 0.06 indicates a medium effect; η2 = 0.14 indicates a large effect. A two-way repeated measures ANOVA was conducted for muscle blood flow and muscle oxygen consumption; the two within-subject variables were time (pre- and post- CWI) and temperature (10 °C and 15 °C). The delta for muscle blood flow and muscle oxygen consumption (difference between pre- and post) was calculated and plotted against skinfold thickness for each temperature (10 °C and 15 °C). The r^2 correlation coefficient between these two variables was calculated (data not included). Level of significance was set at $P < 0.05$. All statistical analysis was performed using SPSS (Version 27; IBM SPSS statistical software package).

3.3 Results

3.3.1 O_2Hb, HHb, tHb and Hb_{diff} during pre- and post-CWI venous occlusion.

Figure 3.3.1 displays the O_2Hb, HHb, tHb and Hb_{diff} signals from a representative participant during the experimental protocol. A similar response was seen in the

group data, (see Appendix A and B for individual traces). During both pre- and post-CWI venous occlusions (VO) there was little to no increase in O_2Hb, HHb, tHb and Hb_{diff} concentrations (see Appendix G and H for individual traces).

3.3.2 O_2Hb, HHb, tHb and Hb_{diff} response during pre- and post-CWI arterial occlusion.

Pre and post arterial occlusion (AO) exhibit an overall similar response. At the onset of AO (Figure 3.3.2), a steady and progressive drop in O_2Hb concomitant with an increase in HHb concentration is present throughout the course of the 5 min occlusion which reflects depletion of local O_2 stores within the static compartment of blood (arterial inflow and venous outflow eliminated). A more rapid drop in Hb_{diff} concentration is present at the onset of occlusion. Hb_{diff} represents the difference in concentration between O_2Hb and HHb and therefore provides clearer observation of the extent of desaturation taking place during occlusion. As can be observed in figure 3, during pre AO a nadir or flattening was reached in the O_2Hb, HHb and Hb_{diff} signals indicating complete desaturation. During pre AO for both temperatures, a nadir was achieved in 7 participants, occurring between the 3rd and 4th minutes of occlusion, meaning the remaining 3 participants continued to desaturate for entire 5 min period. During post AO, 4 participants achieved a nadir in the 15 °C temperature, occurring in the final minute of occlusion, only 1 participant achieved a nadir in the 10 °C temperature, (see Appendix E and F for individual traces).

Figure 3.3.3 shows the overlapped O_2Hb, HHb, tHb and Hb_{diff} signals pre and post AO for a representative participant. During post AO, there is a noticeably slower rate of decrease in both O_2Hb and Hb_{diff} concentrations over the course of the 5 min occlusion, (the slope value for the line, as calculated by linear regression, confirms

this). Similar end concentration values are achieved. This may reflect a slower depletion of local O_2 stores during the post AO. Following cuff release (of both AO's), there is a rapid resaturation which can be observed as an increase in O_2Hb and Hb_{diff} and a concomitant decrease in HHb. Restoration of all signals can be seen after 1-2 minutes. A noticeably faster rate of resaturation was observed in the post AO as seen by the steeper incline of O_2Hb and Hb_{diff} concentrations (the reoxygenation rate from the TSI trace has been calculated to evidence this). Interestingly, there were slight decreases in tHb concentration during arterial occlusions followed by an increase upon release. These observations may be attributed to residual pressure gradients causing movement of heme chromophores in and/or out of the NIRS field of view during arterial occlusion (195).

3.3.3 TSI (%) response during pre- and post-CWI arterial occlusion

Figure 3.3.4 displays the TSI (%) signal during the experimental protocol. A similar response was seen within the group data, (see Appendix C and D for individual traces). Figure 3.3.5 shows the overlapped TSI (%) signal for both pre and post AO, a post-occlusion hyperaemic response can be observed following *both* pre- and post-CWI AO, which is characterised as an increase in TSI (%) above resting values, followed by a decrease back to resting values over the next 2 min. Visual inspection of individual traces revealed the post-occlusion hyperaemic response is greater for post AO compared to pre AO. Rapid desaturation is present throughout the 5 min AO with a nadir attained in the pre but not post AO, which occurred in a high proportion of participants as previously mentioned.

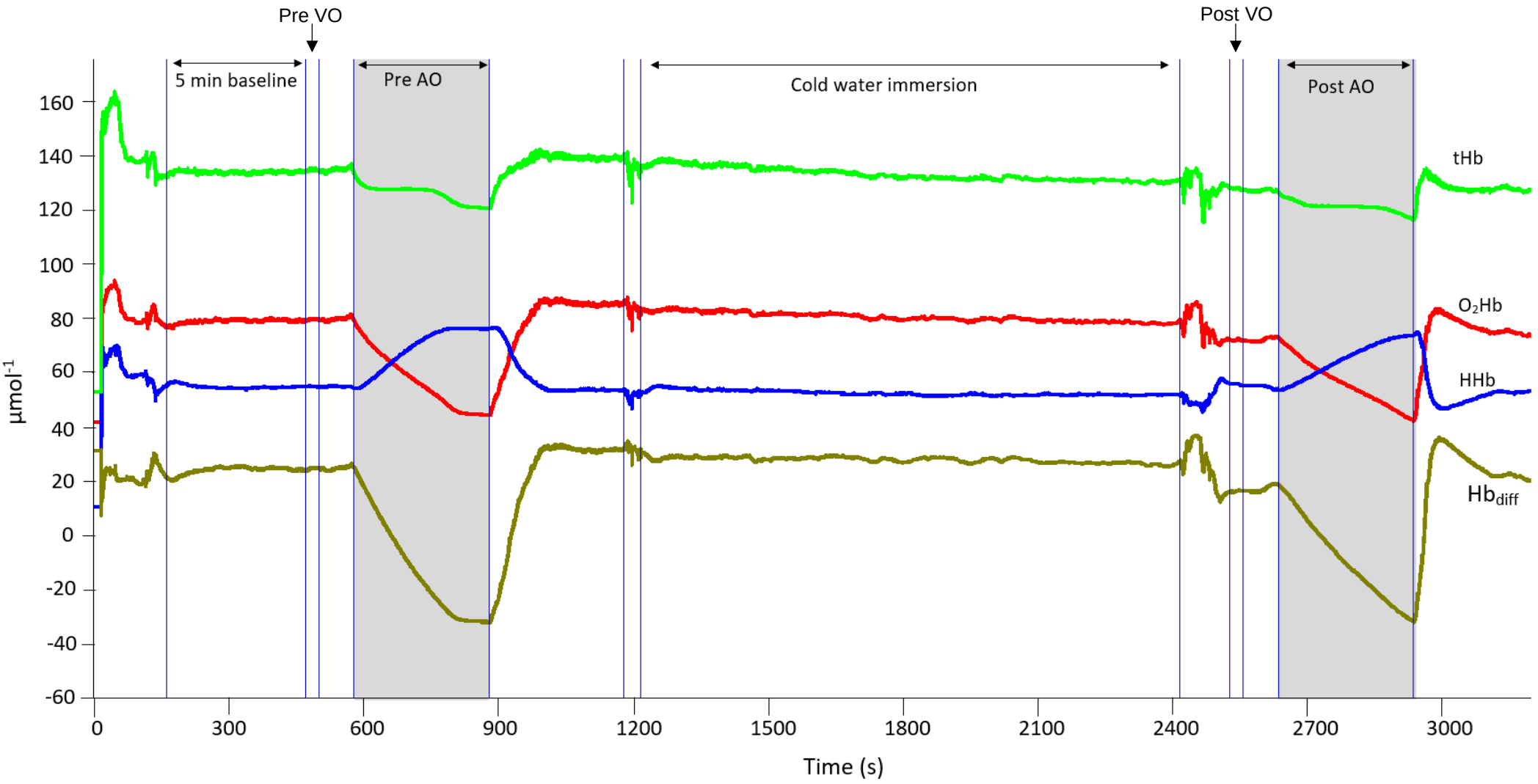

Figure 3.3.1 A representative example of the O_2Hb, HHb, tHb, and Hbdiff traces during the experimental protocol. Shaded regions represent the time period when arterial occlusion took place. The colour of each trace represents the following: green = tHb, red = O_2Hb, blue = HHb, brown = Hb_{diff}.

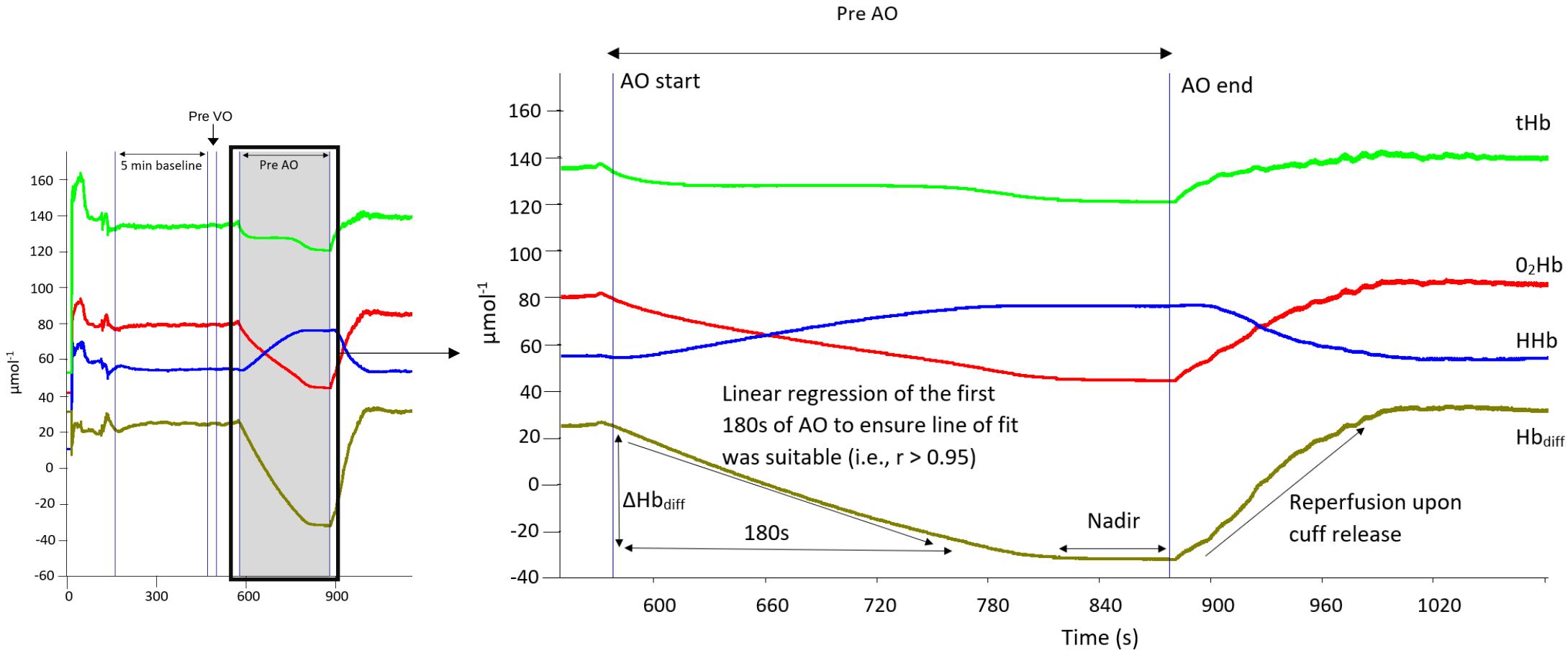

Figure 3.3.2 A representative example of the O_2Hb, HHb, tHb, and Hb_{diff} traces during a 5 min arterial occlusion and following cuff release (AO end). The experimental approach used to calculate mVO_2 has been annotated on the Hb_{diff} trace. This same method was utilised for both pre- and post-AO's. Due to a nadir, ΔHb_{diff} was calculated over the first 180 s of AO. The colour of each trace represents the following: green = tHb, red = O_2Hb, blue = HHb, brown = Hb_{diff}.

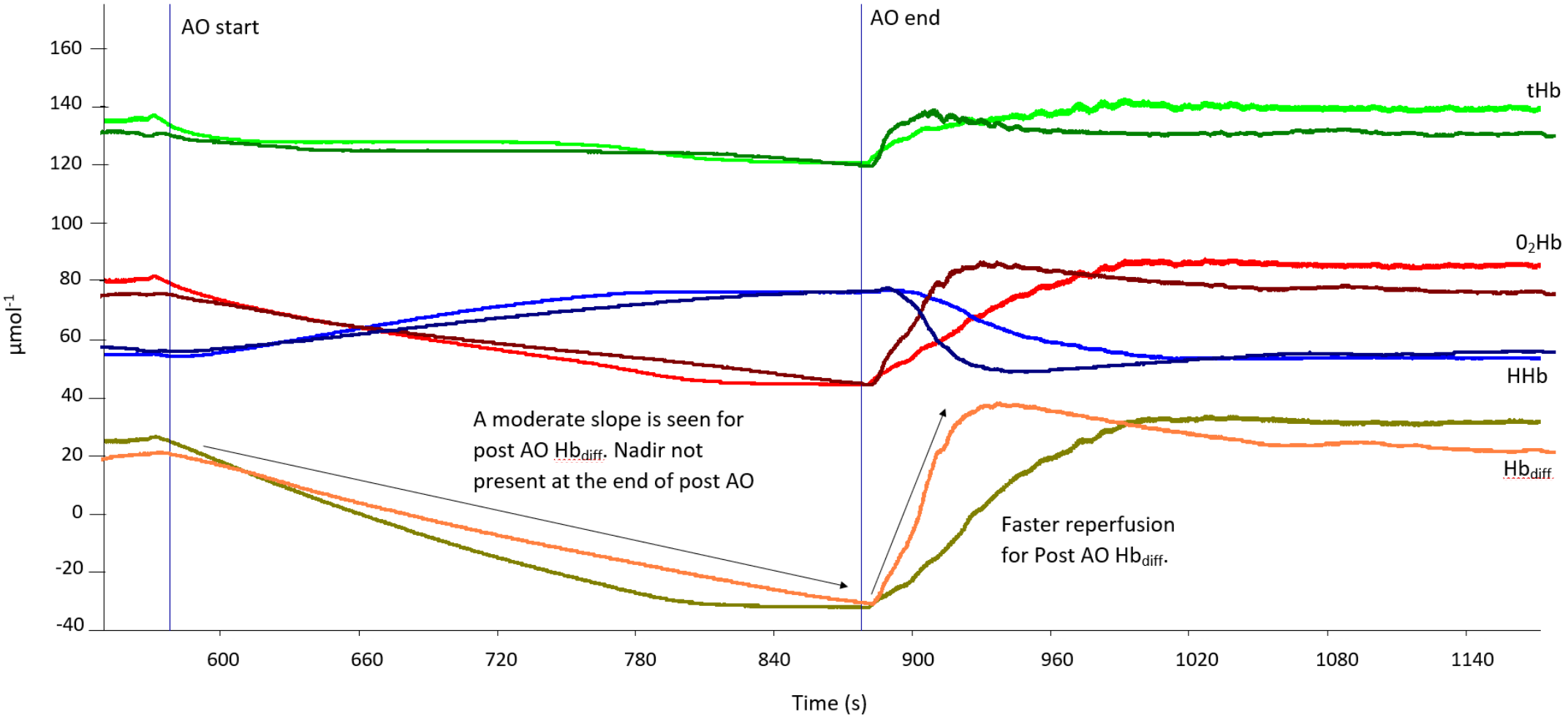

Figure 3.3.3 A representative example of the O_2Hb, HHb, tHb, and Hb_{diff} traces during a 5 min AO and following cuff release (AO end), with the traces for pre- and post-AO overlapped for visual comparison. The colour of each trace represents the following: light green = pre AO tHb, dark green = post AO tHb, light red = pre AO O_2Hb, dark red (burgundy) = post AO O_2Hb, light blue = pre AO HHb, dark blue = post AO HHb, brown = pre AO Hb_{diff}, light orange = post AO Hb_{diff}.

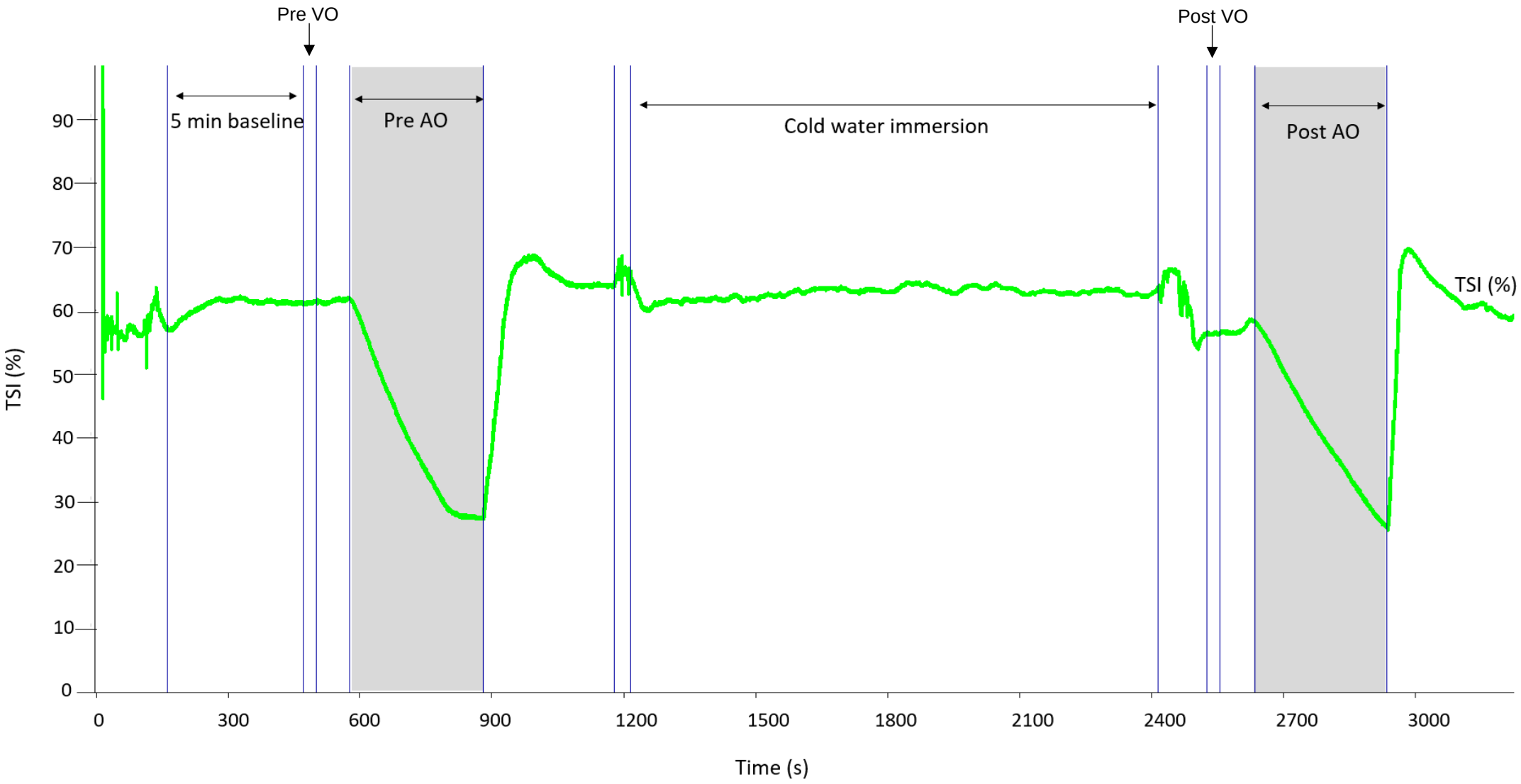

Figure 3.3.4 A representative example of a TSI (%) trace during the experimental protocol. Shaded regions represent the time period when occlusions took place.

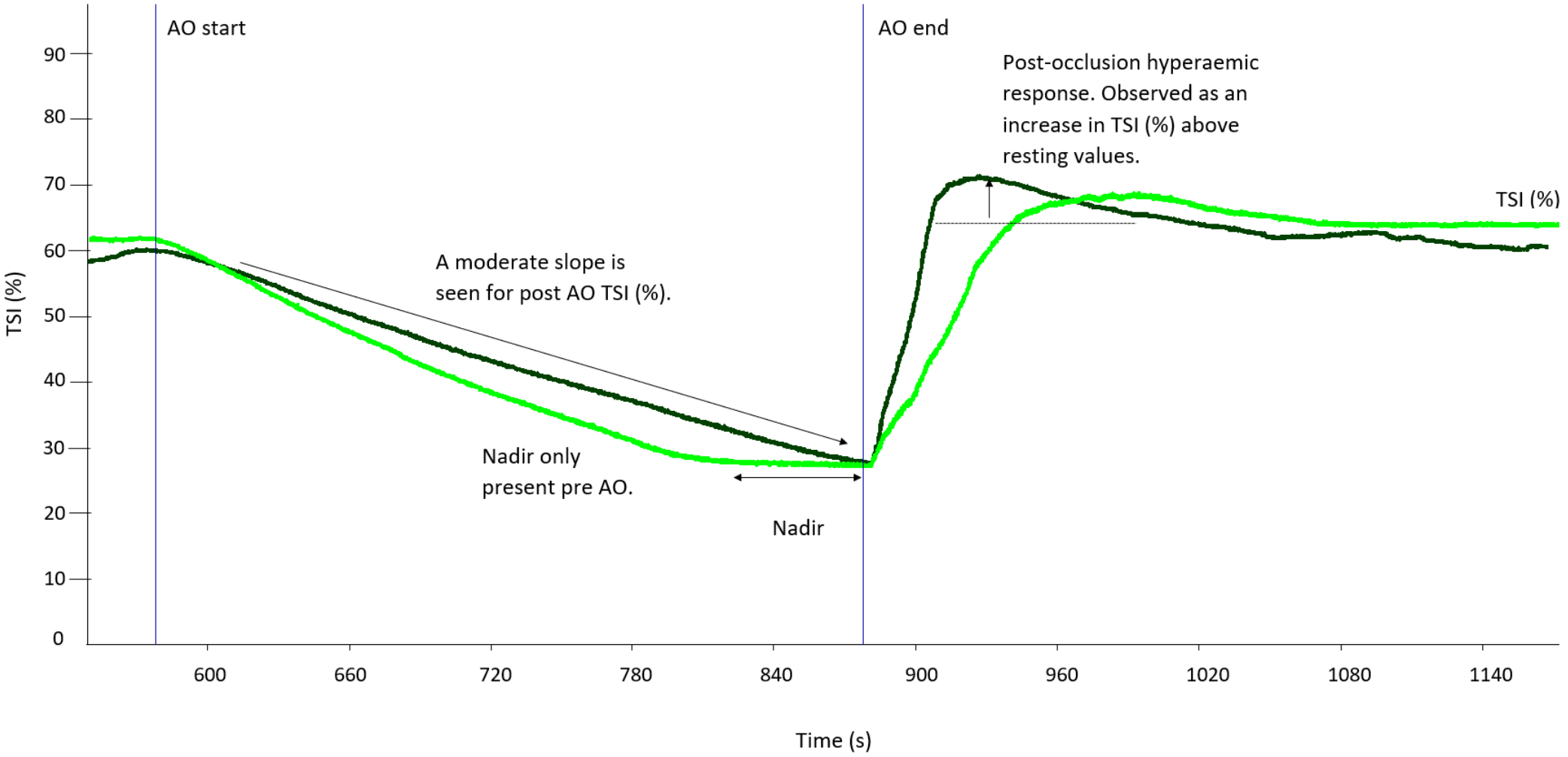

Figure 3.3.5 A representative example of a TSI (%) trace during a 5 min AO and following cuff release (AO end). The TSI (%) trace for pre- and post-AO are overlapped for visual comparison. The colour of each trace represents the following: light green = pre AO TSI (%), dark green = post AO TSI (%).

During the 20 min immersion at both temperatures there were significant changes in the O_2Hb, tHb and Hb_{diff} signals. There were no significant changes in either the HHb or TSI (%) signals during immersion at either temperature. Firstly, mean values for all NIRS measures (O_2Hb, HHb, tHb, Hb_{diff}, TSI) obtained during the entire 20 min CWI period were compared between temperatures and revealed no significant difference; O_2Hb $t(9) = 0.6104$, $P = 0.562$; HHb $t(9) = 0.023$, $P = 0.981$; tHb $t(9) = 0.348$, $P = 0.735$; Hb_{diff} $t(9) = 0.690$, $P = 0.507$; TSI (%) $t(9) = 0.685$, $P = 0.510$. When mean values for all NIRS measures (O_2Hb, HHb, tHb, Hb_{diff}, TSI) for each 5 min period of the immersion were analysed, there was a significant main effect of time for O_2Hb, tHb and Hb_{diff}; O_2Hb ($F(1,3) = [12.06]$, $P = 0.04$, $\eta^2 = 0.838$); tHb ($F(1,3) = [21.26]$, $P = 0.01$, $\eta^2 = 0.901$); Hb_{diff} ($F(1,3) = [5.53]$, $P = 0.02$, $\eta^2 = .703$), in which they decreased over the course of the 20 min immersion. Post-hoc pairwise comparison analysis revealed where the significant differences existed between these timepoints (i.e., 5 min, 10 min, 15 min, 20 min) within each temperature. It was within the last 5 min segment of immersion (15 – 20 min) where the significant reductions were found in comparison to the earlier segments of immersion (0 – 5 mins) for: tHb at 10 °C (MD = -3.45, P = 0.02); and O_2Hb (MD = -2.56, P = 0.04), Hb_{diff} (MD = -1.20, P = 0.01) and tHb (MD = -3.35, P = 0.01) at 15 °C. Additionally, tHb at 10 – 15 mins was shown to be significantly different to 0 – 5 mins in the 15 °C (MD = -2.63, P = 0.05). The reductions in O_2Hb, tHb and Hb_{diff} represented a change of ≈2-4 μmol^{-1}. Table 3.3.1 summarises these findings.

Table 3.3.1 Displays the mean values for each NIRS variable during 20 min CWI, and at 5 min intervals during CWI.

Variable	CWI 10 °C					CWI 15 °C				
	5 min	10 min	15 min	20 min	Entire 20 min	5 min	10 min	15 min	20 min	Entire 20 min
O_2Hb (μmol^{-1})	81.2 ± 14.5	80.0 ± 13.3	79.4 ± 12.7	78.6 ± 12.7	79.8 ± 13.3	80.7 ± 14.2	79.5 ± 13.3	79.1 ± 13.3	**78.1 ± 12.8** $^{*\,\wedge\,\#}$	79.4 ± 13.3
HHb (μmol^{-1})	60.9 ± 15.2	60.1 ± 14.9	59.9 ± 15.0	60.0 ± 15.0	60.2 ± 15.0	60.7 ± 14.3	60.1 ± 14.2	59.9 ± 14.4	60.1 ± 14.5	60.2 ± 14.3
tHb (μmol^{-1})	142.1 ± 29.4	140.1 ± 27.8	139.3 ± 27.4	**138.7 ± 27.4** $^{*\,\wedge\,\#}$	140.1 ± 28.0	141.6 ± 28.2	139.6 ± 27.2	**139.0 ± 27.0** *	**138.3 ± 27.0** $^{*\,\wedge\,\#}$	139.6 ± 27.3
Hb_{diff} (μmol^{-1})	20.2 ± 4.4	20.0 ± 5.0	19.6 ± 4.7	18.6 ± 4.3	19.6 ± 4.4	20.1 ± 4.0	19.4 ± 3.9	19.2 ± 4.5	**18.0 ± 4.7** $^{\#}$	19.2 ± 4.1
TSI (%)	58.9 ± 3.0	59.9 ± 3.2	59.8 ± 3.5	59.3 ± 3.4	59.4 ± 3.3	58.8 ± 2.6	59.1 ± 2.9	59.4 ± 3.1	59.0 ± 3.3	59.1 ± 2.9

Values are mean ± SD. n =10 for all variables. * significant difference when compared to 0-5 min, $^{\wedge}$ significant difference when compared to 5-10 min, $^{\#}$ significant difference when compared with 10-15 min. Significant when $P < 0.05$. Variables shown are; Tissue oxyhaemoglobin (0_2Hb), deoxyhaemoglobin (HHb), and total haemoglobin (tHb), Haemoglobin difference (Hb_{diff}) and Tissue Saturation Index (TSI).

The calculated values for mBF and mVO_2 pre- and post-CWI for both temperatures are displayed in Table 3.3.2 There was no significant main effect or interaction effect (*P* = 0.434) present for mBF and temperature, however there was a trend for mBF to increase post-CWI in both temperatures (F(1,9) = [3.635], *P* = 0.089, $\eta^2 = 0.288$). A statistically significant main effect of time (F(1,9) = [7.727], *P* = 0.021, $\eta^2 = 0.462$) was observed, showing $mV0_2$ decreased as a result of CWI. There was no significant main effect for temperature or interaction effect respectively; (F(1,9) = [0.283], *P* = 0.608, $\eta^2 = 0.031$); (F(1,9) = [2.587], P = 0.142, $\eta^2 = 0.223$).

Table 3.3.2 Displays the mean values for mBF and mVO_2 pre- and post-CWI.

Variable	CWI 10 °C		CWI 15 °C	
	Pre	Post	Pre	Post
Muscle blood flow (mBF) ($ml \cdot min^{-1} \cdot 100ml^{-1}$)	0.26 ± 0.09	0.50 ± 0.35	0.29 ± 0.14	0.43 ± 0.34
Muscle oxygen consumption (mVO_2) ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.065 ± 0.03	**0.055 ± 0.04***	0.061 ± 0.02	**0.05 ± 0.01***

Values are mean ± SD. n = 10 for all variables. * statistically significant difference between pre and post CWI.

Figures 3.3.6 and 3.3.7 show the delta values (Δ) for mBF and mVO_2 (representing the difference between pre and post CWI) for each temperature, plotted against individual skinfold thickness. There is no correlation between ΔmBF values and skinfold thickness (Figure 3.3.6), our participants mostly had skinfolds in the range 4-7 mm, with the exception of one who had 14 mm. The ΔmBF for this participant was similar to those for participants with lower skinfolds, therefore the greater skinfold did not appear to have an effect. Within the skinfold range 4-7 mm, which encompasses

most of our participants, there is variation in ΔmBF (ranging from ≈0.2 to 1.1 ml· min^{-1}·100ml^{-1}), however this variation does not appear to be correlated with skinfold thickness. There is also no effect of temperature, as a similar variation in ΔmBF within the skinfold range 4-7 mm can be seen in both temperatures. ΔmVO_2 values also do not appear to be correlated with skinfold thickness (Figure 3.3.7). In the 10 °C temperature, there is one participant who achieves a significantly greater mVO_2, which does not follow the trend for the rest of the participants, for which there is largely a reduction in mVO_2 from pre to post-CWI. However, for ΔmVO_2 calculated in the remaining participants, variation does exist which does not appear to be correlated with skinfold thickness.

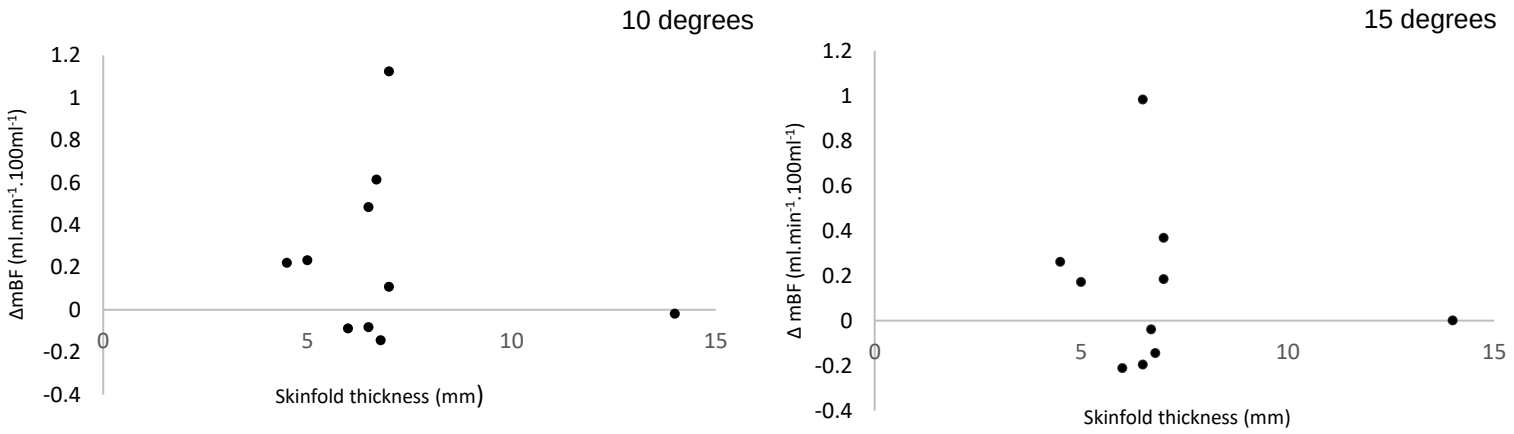

Figure 3.3.6 Displays ΔmBF plotted against skinfold thickness for each participant.

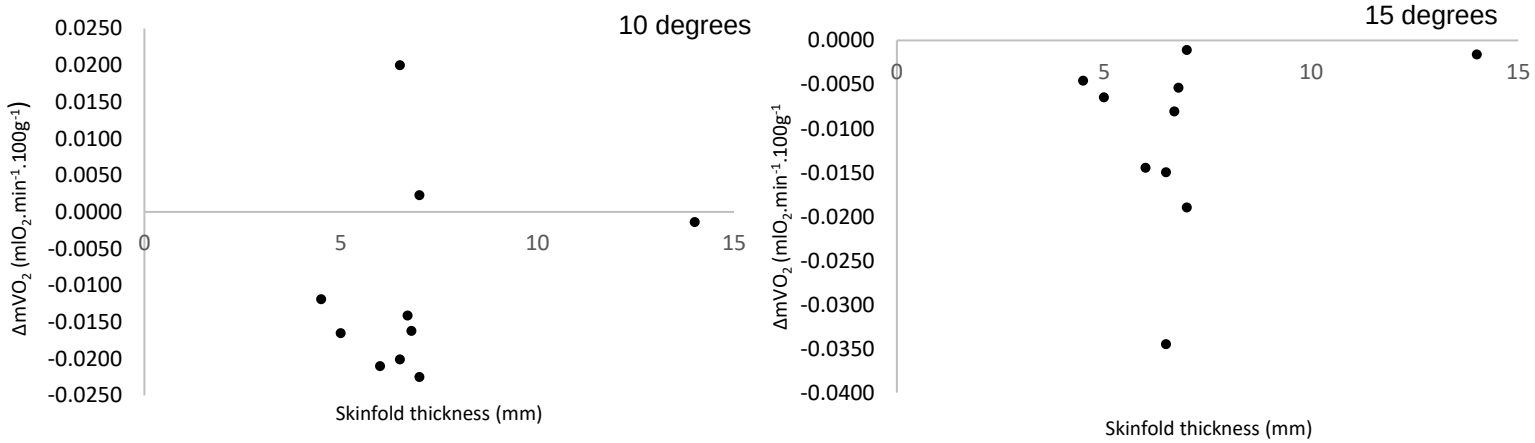

Figure 3.3.7 Displays ΔmVO_2 plotted against skinfold thickness for each participant.

3.4 Discussion

The present study investigated the effect of a 20 min cold-water immersion (CWI) at two different water temperatures (10 °C and 15 °C) on gastrocnemius muscle blood flow (mBF) and muscle oxygen consumption (mVO_2), as measured by near infrared spectroscopy (NIRS). The main findings of this study were (1); CWI at 10 °C and 15 °C significantly decreased mVO_2 (2); the magnitude of mVO_2 decrease did not differ between immersion temperatures. These findings partially support our initial hypothesis that CWI would cause a reduction in mVO_2 post-immersion, however the cooler immersion temperature (10 ˚C) did not enhance this response.

CWI is the most commonly used recovery intervention by elite and amateur athletes to reduce the negative symptoms associated with EIMD (29,30,32). CWI is suggested to achieve this primarily through temperature-induced reductions in microvascular blood flow and tissue metabolism (47,63), which may help minimise oedema manifestation, inflammation, and secondary muscle damage (47,50,73,118,131). Previous analysis of mBF and tissue metabolism via NIRS has been inferred from changes in local muscle blood volume (ΔtHb) or muscle O_2 saturation (ΔTSI), respectively. Unfortunately, the tHb measurement cannot accurately quantify mBF, and the TSI % measurement is also limited as it cannot discriminate between oxygen supply and oxygen demand (161,167), restricting physiological interpretation. To further convolute current understanding, differences in muscle blood flow and metabolism responses have been observed in response to CWI (see discussion of chapter 2). Therefore, the present study combined NIRS measures of haemodynamic response (O_2Hb, HHb, tHb, Hb_{diff} and TSI (%)) with the addition of a simple venous and arterial occlusion procedure to more accurately quantify mBF and mVO_2 following CWI.

3.4.1 Muscle blood volume, tHb.

NIRS derived measurement of muscle blood volume (tHb) have shown blood volume reductions during (122,185), and following post-exercise CWI (121,122). The change (Δ) in muscle blood volume (tHb) during immersion was used as an indirect and proxy measure of muscle blood flow (mBF) (121,122,185). Collectively, these results were interpreted as a reduction in mBF during and following CWI (111,122,150,185). In the present study, a reduction in muscle blood volume (tHb) was observed during 20 min CWI. This reduction represented an overall change of ≈4 μmol^{-1} in tHb during the entire 20 min immersion. Specifically, tHb was significantly lower 15 – 20 min during immersion compared to all other measured timepoints (0 – 5 min, 5 – 10 min, 10 – 15 min); and the reduction in tHb was similar in both temperatures (10 ˚C and 15 °C). More substantial reductions in tHb (22% and 66%) have previously been observed during a 15 min single leg immersion and 5 min full lower body immersion, respectively (122,185). Given that CWI was performed shortly after (≈2 – 10 min) exercise in these studies (122,185), post-exercise hyperaemia was likely present within the muscle. Therefore, since CWI is proposed to attenuate microvascular blood flow through a vasoconstrictive response, a significant reduction in tHb relative to post-exercise values occurs during and following CWI when a hyperaemic response is present (122,185). This effect was not present in the current studies as participants performed CWI in a rested rather than immediate post-exercise state and may explain the smaller magnitude of change in tHb during CWI. During the current CWI protocol, peak changes in tHb occurred within the last 5 min of immersion (15 – 20 min), with minimal changes observed prior. Furthermore, the 5 °C difference in immersion temperature did not have an effect on the magnitude of tHb reduction, this supports previous work which suggested the reduction in tHb

during a cooling protocol was non-dependent on temperature (233). The present study therefore suggests that a small reduction in muscle blood volume (tHb) can be achieved in either a 10 °C or 15 °C CWI following at least 15 min of immersion. These findings may have implications for selecting immersion durations, which currently range from 30 s – 30 min (48,197). Our findings are also in congruence with Machado *et al*., (197) who have reported a dose-response relationship indicating immersion times between 11 – 15 min as the most effective for alleviating muscle soreness symptoms. Observing a reduction in tHb during immersion is suggested to represent the occurrence of peripheral vasoconstriction. However, it remains to be established whether an optimum, or indeed any reduction in tHb during and following CWI contributes to a significant improvement in post-exercise recovery. It should be noted that a change of ≈4 μM is considered minimal and could be attributed to noise within the NIRS signals; however, the noise-signal ratio is automatically adjusted within the Oxysoft software (from which the signals are obtained), and visual inspection of the traces (figure 3.3.1) does not suggest there was large variation during immersion.

3.4.2 Muscle blood flow, mBF.

The NIRS derived measure of muscle blood volume (tHb) is an estimation for the total volume of blood within the local region of muscle interrogated (162–164) and is therefore likely affected by reductions/ increases in blood supply to the muscle microvasculature. However, it is unknown whether changes in tHb as a result of CWI represent significant changes in “actual” blood flow to the muscle without performing venous occlusion (VO). In the current study, VO’s were performed pre- and post-

CWI to assess its influence on the quantitative measure of muscle blood flow (mBF). VO prevents the outflow of venous blood in the limb, while leaving arterial blood flow unaffected (163,191,192). The blood impounded by cuff inflation causes the venous blood volume to rise at a rate proportional to arterial inflow. mBF is therefore determined by the linear increase in tHb during the initial moments of VO, where tHb represents muscle blood volume (163,192). In this regard, calculating mBF using this method should theoretically provide a more accurate quantification of the “actual” flow of blood into the muscle. The current study found that mBF was not significantly affected by CWI and based on the calculations there was an observed trend for mBF to increase from pre- to post-CWI in both temperatures (10 °C and 15 °C), with this increase approaching statistical significance (P = 0.089). Visual inspection of the VO indicates that a VO most likely did not occur. Please see Appendix I for a comparison of the current data vs. a typical VO trace. Insufficient pressure may have resulted due to the use of a manual handheld sphygmomanometer and relatively short occlusion period (in comparison to the AO). An inability to observe the real time trace due to the off-line measurement during water immersion, restricted researchers from identifying this at the time. Interpretation of the findings is perhaps mute, however the trend for an increase in mBF values post-CWI suggests a peripheral vasodilatory response may have occurred as a result of CWI. Cold-induced vasodilation has previously been observed in cutaneous (112,149) and superficial muscle regions (212,242) following cold exposure and may be associated with a significant reduction in tissue and limb temperature, thereby evoking an inhibitory effect on the vasoconstrictor response (226), although the exact mechanisms are yet to be fully elucidated (112).

At present, it remains unconfirmed as to how potential changes in mBF influence

post-exercise recovery. From a mechanistic standpoint, a prolonged period (up to 60 min) of peripheral vasoconstriction following cooling may help limit oedema manifestation and the infiltration of pro-inflammatory cells, thereby reducing the extent of secondary muscle damage (47,58,63,72). In contrast, an increase in mBF due to a vasodilatory response to cooling may actually exacerbate inflammation following muscle damage (212) by promoting an opposing response to that mentioned above. Following EIMD, the accompanying inflammation, the progression of injury and the subsequent repair are different to other types of injury (e.g., direct trauma) (58), which alters the rationale for cooling post-injury and the mechanisms involved for post-exercise recovery. A biphasic recovery pattern of muscle performance has been reported after EIMD, i.e., there is a rapid recovery related to metabolic fatigue subsidence within the first hours after exercise, followed by a secondary slower phase of recovery during subsequent days where muscle repair takes place (257). Therefore, further research is necessary to determine the optimal time after EIMD to implement recovery strategies in order to produce a beneficial recovery response. CWI is commonly administered shortly following exercise, within this acute recovery period (i.e., within the first hour post-exercise) CWI may modulate changes in mBF, however it remains to be established whether such transient alterations play a significant role in reducing secondary muscle damage, and the potential this has for improved recovery of muscle damage indices (e.g., muscle function, muscle soreness) following EIMD.

3.4.3 Tissue saturation index, TSI (%).

A small number of studies have utilised the TSI (%) response during and following CWI (121,122,185–187), with ΔTSI (%) considered a proxy for changes muscle

metabolic activity (174,185). In the present study there were no significant changes in TSI (%) during the 20 min CWI and marginal reductions in tHb at the end of immersion. No change in TSI %, despite a small reduction in local muscle blood volume tentatively suggests O_2 utilisation decreased during this period, possibly reflecting a reduction in muscle metabolic activity. This finding and interpretation has been reported by Ihsan *et al*., (185), whilst Choo *et al*., (122) observed no significant change in TSI (%) values during CWI despite a reduction in tHb, again suggested as a decrease in O_2 utilisation, if ΔtHb is considered a proxy for mBF. However, as previously noted, caution should be applied when interpreting the TSI (%) signal in relation to O_2 utilisation since this cannot be directly quantified.

3.4.4 Muscle oxygen consumption, mVO_2.

In order to accurately quantify mVO_2, arterial occlusions (AO) were performed pre- and post-CWI. During AO, both venous outflow and arterial inflow in the limb are temporarily arrested, creating a “static” compartment of blood within the muscle (163). The linear decrease in O_2Hb or Hb_{diff} directly reflects the rate at which the pre-existing O_2 store in the blood compartment is consumed (163). As such, this method has the advantage of quantifying the O_2 extraction element of the TSI (%) measurement, whilst preventing the influence of further O_2 delivery. In the current study, it was found that mVO_2 was significantly reduced as a result of 20 min CWI. This finding suggests that CWI reduced O_2 metabolic demand within the muscle, which is consistent with the mechanism of recovery by which CWI is proposed to prevent inflammation and secondary hypoxic injury following EIMD (47,50,58,73). The reduction in muscle metabolism associated with CWI is proposed to be mediated through reductions in tissue temperature (185). In the current study, tissue

temperature was not measured. However, it is likely that a reduction in tissue temperature did occur, as similar CWI protocols used in the literature have observed significant reductions during and following immersion (116,121,126,133,137). Meeusen and Lievens (131) recommended that a decrease in tissue temperature of 7 – 10 °C is needed to decrease tissue metabolism by 50% based off the Q_{10} principal (2 – 3 fold reduction in tissue metabolism following 10 °C reduction in tissue temperature). Animal models and surgical studies utilising cryotherapy on amputated limbs and organs suggest temperatures between 5 – 15 °C are required for metabolic reductions to be facilitated (155). In the current study, the 5 °C temperature gap between immersions did not have an effect on the magnitude of reduction in mVO_2. This is an interesting finding and contrasts the initial hypothesis. Similar gaps in immersion temperature have previously led to a significantly lower tissue temperature being achieved (111,112). Assuming that a similar difference in temperature was achieved between temperatures in the current study, this suggests there may be a threshold tissue temperature at which reductions in tissue metabolism occur, and below which colder temperatures lead to greater reductions in metabolism. This would then explain why no significant difference was achieved between temperatures in the current study, as it may be tissue temperatures were not low enough to surpass this threshold. In support of this notion, Wakabayashi *et al*., (258) observed the effect of decreasing muscle forearm temperature (via cooling pads) upon NIRS derived mVO_2, indicating higher mVO_2 values at higher tissue temperatures. To the best of our knowledge this is the first study to employ the AO technique with NIRS to establish mVO_2 changes in response to different CWI temperatures. Our findings of reduced mVO_2 support previous interpretations of reduced muscle metabolism (122,185). Whilst an accurate quantification of muscle

metabolism is certainly useful and extends previous findings, it is still unknown what threshold (if any), or extent of reduced muscle metabolism is required to be beneficial in aiding recovery from EIMD. For context, the mVO_2 values seen within the present study are comparable to those reported by Van Beekvelt (163) and Ihsan *et al.,* (207) at rest utilising the same mVO_2 measurement approach. It may be that an “optimal” reduction value exists, however establishment of that value is outside the constraints of the present study. Recently, Ihsan *et al.,* (207) has shown mVO_2 changes in response to regular CWI application during training, albeit, mVO_2 increased post-training and there was no difference between CWI and control conditions. Moreover, measurement of muscle oxidative capacity has been shown to differentiate between trained and untrained individuals (259) and healthy and disease states (260). Collectively these findings indicate a strong premise for use of this measurement within CWI and recovery applications. A NIRS derived mVO_2 may provide a more accurate assessment of CWI as a therapeutic modality for muscle damage recovery.

3.5. Limitations

There are several recognised limitations both device and sample based which should be discussed within the context of this study. Firstly, the portable NIRS device used in this study only interrogates a small (2 to 6 cm^3) volume of muscle tissue. The calculated values for mBF and mVO_2 are taken to represent values for the entire muscle. However, given the limited coverage of the gastrocnemius and the heterogeneity of fibre type, muscle metabolism and muscle blood flow (261,262) across the muscle length, it is likely the observed changes are not representative of the entire muscle (162). In order to obtain representative estimations, multi-site

mVO_2 measurements are required. Secondly, the influence of the sub-adipose tissue layer on the CW-NIRS signal has been well documented (163), with previous studies reporting a significant influence of ATT on mVO_2 (163,195). Sub-adipose tissue has a significantly lower metabolic demand in comparison to the underlying skeletal muscle tissue. As such, influence of this region on the NIRS signal may lead to an underestimation of mVO_2 and/or mBF. Within the current study the upper range of ATT (14 mm) was low, and no relationship was observed between mVO_2, mBF and ATT (figures 3.3.6 and 3.3.7) Thirdly, it is suggested that skin blood flow can significantly influence the NIRS (O_2Hb and tHb) signals. For example, during whole-body heating, an elevated skin blood flow has been demonstrated to significantly influence the NIRS signal (263,264), however, this effect during cooling has not readily been examined. Choo *et al*., (122) suggested that during the cooling conditions (CWI at 9 °C and 15 °C), skin blood flow had a notably lower influence on the NIRS signal when compared to non-cooling (i.e., passive rest or thermoneutral immersion). Cooling has been well-documented to reduce local cutaneous blood flow (111,112,122), therefore theoretically the contribution of cutaneous blood flow to the NIRS signal should be less during cooling than during heating or even resting or exercise conditions, where cutaneous blood flow is higher in comparison. Finally, the study sample was exclusively male and comprised of elite long-distance runners. Therefore, extrapolation to female and other sporting populations may require further study.

3.6 Perspectives

The present study is the first to assess mVO_2 and mBF pre- and post-CWI, comparing two immersion temperatures. Theoretically, the physiological challenge that is imparted by VO and AO on a limb allows a quantitative and more informative

measure to be obtained by consulting the rate of increase/decrease in NIRS derived signal of tHb and Hb_{diff}. The present study demonstrates that the NIRS-occlusion approach can detect significant changes in mVO_2 pre- and post-CWI and could provide future investigators with a simplistic tool by which muscle metabolic activity can be assessed. Whilst this study documents how CWI influences this measure, the significance of a reduction in mVO_2 as a result of CWI is yet to be established. A key finding from the present study was that there was no significant difference in mVO_2 between immersion at 10 °C and 15 °C. This is difficult to explain since a measure of tissue temperature was not obtained. However based off previous research a greater reduction in tissue temperature has been achieved in the colder temperature (e.g. 8 °C) when a similar temperature gap was used (111,112). Therefore, it may be that a cooler immersion temperature than that which was utilised in the present study may be required to elicit further reductions in muscle metabolic activity. This may not be pleasing for athletes who cannot tolerate cold exposure. Indeed, previous studies have found significant differences in ratings of thermal comfort and thermal sensations between 8˚C and 22˚C CWI (154). Furthermore, as explained, it is likely that VO did not successfully occur within the present study therefore the mBF response to CWI remains largely unestablished and requires further investigation.

Establishing the role of mBF and mVO_2 within post-exercise recovery protocols is necessary before investigation into an optimal immersion time or temperature during CWI in which to alter these parameters. Indeed, the application of CWI as a recovery tool is still controversial with evidence to suggest its regular use can ameliorate training induced changes following resistance training (265–267), with more beneficial findings found with endurance based training (265)

Future research should look to provide an entire physiological profile, (mBF, mVO_2 and thermoregulatory responses) before, during and following immersion, and continue to monitor physiological indices alongside measures of muscle function and soreness in the days following exercise. This will add to the growing body of literature investigating how CWI influences the physiological response in muscle and shed light on the mechanisms involved in post-exercise recovery.

3.7 Conclusion

The present study demonstrated a 20 min CWI at 10 ˚C and 15 °C led to significant reductions in mVO_2. The reduction in mVO_2 suggests a decrease in muscle metabolic demand for O_2, which is reported to limit the extent of secondary tissue damage and is consistent with the mechanism of recovery by which CWI may reduce inflammation in the period following muscle damage. The NIRS-occlusion technique may offer further insight into muscle blood flow and muscle metabolic responses, beyond what is attainable from observing the NIRS primary signals

4

Chapter 4: Discussion of Findings and Recommendations for Future Research.

4.1 Discussion of findings and recommendations for future research.

This thesis comprised of three chapters which contribute in synergy to the thesis's overall aim: to explore the utility of near-infrared spectroscopy (NIRS) as a measurement tool during cold-water immersion (CWI). Chapter 1 introduced the application of CWI as a post-exercise recovery strategy, with a predominant focus on the proposed mechanisms by which it benefits recovery, and an overview of the associated physiological responses. Specifically, a large proportion of the reviewed literature discussed the effectiveness of CWI for improving recovery from exercise-induced muscle damage (EIMD) as athletes commonly implement CWI for this objective. The rationale for CWI as a recovery strategy originally stems from the application of cryotherapy as a treatment modality for soft-tissue injury. However, the aetiology of soft-tissue injury such as direct trauma or crush injury is undoubtedly different to EIMD, meaning the efficacy for cryotherapy may not necessarily be transferable between the two types of injury. EIMD is associated with an increase in oedema manifestation, inflammation and secondary muscle damage, the combination of which are attributed to alterations in muscle function and an increase in delayed onset muscle soreness (DOMS) in the days following exercise. CWI is proposed to aid recovery following EIMD by promoting a cold-induced reduction in microvascular blood flow (i.e., peripheral vasoconstriction) along with a reduction in tissue metabolism, thereby ameliorating oedema, inflammation, and the extent of secondary muscle damage. However, while the isolated effects of cold exposure (e.g., via ice packs) and water immersion (e.g., thermoneutral immersion) have been well documented, the physiological response to CWI which incorporates both effects in conjunction is less established. Blood flow responses have been assessed using whole limb or conduit artery measures of bulk flow, with utilised techniques offering

limited insight into changes within the muscle microvasculature. Alterations in tissue metabolism are associated with a direct cooling effect on the underlying tissues, including skeletal muscle, yet current best guidelines for target tissue temperatures are largely inferred from animal models. It is important to investigate the influence of CWI on these parameters in order to provide evidence-based rationale for its inclusion in post-exercise recovery. Chapter 1 progressed on to introduce NIRS: a non-invasive technique capable of providing insight into skeletal muscle haemodynamics and muscle oxygenation *in vivo*. The capability of NIRS to provide insight into the muscle physiological response to exercise has been demonstrated in a multitude of sport disciplines, including in aquatic environments. Therefore, NIRS also has potential to provide similar insight during a recovery strategy such as CWI. A small number of studies to date have incorporated NIRS measurement during CWI. Chapter two collectively identified and reviewed these studies. Changes in the NIRS-derived tHb and TSI (%) measures, representing local muscle blood volume and muscle O_2 saturation respectively, were observed during CWI and in the acute period (e.g., up to 60 min) following immersion. The physiological responses associated with a change in tHb and TSI (%) were discussed, where ΔtHb and ΔTSI (%) were taken as proxy measures for changes muscle blood flow and muscle metabolic activity. For example, a decrease in tHb during CWI was explained as a reduction in blood flow to the muscle microvasculature, due to a cold-induced vasoconstrictive response. Overall, the review demonstrates the utility of NIRS as a measurement tool during CWI, with the technique being capable of providing insight into the muscle physiological response during and following immersion. However, while the observed tHb and TSI (%) responses arguably provide evidence in line with the proposed mechanisms by which CWI aids recovery, the information provided by

these measures is limited. The information obtained from these NIRS signals can be quantified via a physiologic challenge in the form of venous occlusion (VO) and arterial occlusion (AO). This methodology is well-established in NIRS literature for obtaining measures such as muscle blood flow (mBF) and muscle oxygen consumption (mVO_2) in healthy participants under resting conditions and during rhythmic exercise, and with clinical populations in which blood flow and oxidative metabolism may be impaired. However, NIRS-occlusion methodology had yet to be incorporated in CWI recovery.

The main aim of the present study (chapter 3) was to investigate the effects of different CWI temperatures (10 °C and 15 °C) on lower leg (i.e., gastrocnemius) mBF and mVO_2. A simple NIRS occlusion procedure was incorporated to quantify these physiological measures pre- and post-CWI. It was found in the present study was that mVO_2 was reduced as a result of CWI, which was explained as a potential reduction in muscle cellular O_2 demand due to a reduced rate of oxidative metabolism. This is in congruence with the proposed mechanism of recovery by which cooling limits inflammation and secondary muscle damage following EIMD (47,50,58,63,73,131), and supports similar interpretation from previous studies incorporating NIRS with CWI (185). Arguably, the mVO_2 measurement provides a more accurate estimation of O_2 extraction in the muscle than can be attained through monitoring changes in the TSI (%) signal, (ΔTSI (%)). This is because the TSI (%) measurement represents the dynamic balance between O_2 delivery and extraction within the muscle and can be jointly affected by either parameter, with an inability to discriminate between the two. Therefore TSI (%) can only realistically inform upon overall muscle O_2 saturation. The previous review (chapter 2) highlighted the inadequacy of ΔTSI (%) as a proxy measure for estimating alterations in muscle

metabolic activity, with studies either observing no change during or following CWI (121,122), or reporting TSI (%) as a standalone measure (186,187). In these circumstances, failure to report all primary NIRS signals (i.e., O_2Hb, HHb, tHb, TSI %) limits interpretation since the influence of O_2 supply on the TSI (%) measurement cannot be ascertained. Performing AO is preferable since it provides a method to quantify the O_2- extraction element of the TSI (%) measurement, whilst preventing the influence of further O_2 delivery. Whilst an accurate quantification of muscle metabolism is certainly useful and extends previous findings, it remains unknown what threshold (if any), or extent of reduced muscle metabolism is required to beneficial in aiding recovery from EIMD. The mVO_2 values seen in the present study are comparable to those reported by others (163,207) under resting conditions. Recent work has shown mVO_2 changes in response to regular CWI application during endurance training (207), albeit the primary aim of this study was to investigate microvascular adaptations to regular CWI as opposed to monitoring the acute metabolic response through a single CWI application. Elsewhere, inclusion of the mVO_2 measurement to inform upon muscle oxidative capacity has been shown to differentiate between trained and untrained individuals (259) and healthy and disease states (260). Moreover, work has demonstrated good test-retest reliability of mVO_2 under resting and exercise conditions (163,268). As such, when situated within wider NIRS application, there is a strong premise for use of the mVO_2 measurement within CWI and recovery applications.

It is acknowledged that more recently, performing repeated transient (e.g., ≈15 s) AO's to measure the recovery rate of mVO_2 is now a common method for enabling direct insight into a muscles maximal oxidative capacity (178,195,269–271). The time required for this testing procedure is short enough not to pose methodological

constraint, could be conducted pre- and post-CWI and is arguably preferrable to a single continuous 5 min AO such as utilised in the present study, as it may reduce discomfort for the subject. However, while the method is shown to differentiate muscle oxidative capacity between endurance trained and untrained individuals (259,272,273) and been used in individuals with certain disease states (260,274), its yet to be implemented in post-exercise recovery regimens. Future studies may wish to adopt this methodology when assessing the influence of CWI on muscle oxidative metabolism.

In contrast to mVO_2, measures of mBF did not show a significant difference as a result of CWI. As already discussed in chapter 3, there was complication when applying the VO pre- and post-CWI. Visual inspection of the individual traces indicated that VO did not occur, as typical changes in the NIRS signals were not observed during VO (see appendix 4). The use of a handheld manual sphygmomanometer, along with an inability to observe the real time trace due to offline NIRS measurement are methodological constraints of the present study, which affected the researcher's ability to successfully conduct VO. As a result, interpretation of the mBF response is mute and the efficacy of CWI for altering this measure requires further investigation. Nonetheless, the rationale for conducting VO to obtain mBF measures pre- and post-CWI remains the same, as theoretically it provides a more valid approach for these interpretations compared to previous research which has used the change in muscle blood volume (ΔtHb) as a proxy measure. The previous review highlighted the capability of NIRS to monitor changes in tHb during immersion (121,122,185) and in the acute period (e.g., up to 60 min) following immersion (121,122). With changes explained as either a cold-induced vasoconstrictive (i.e., tHb decrease) or vasodilatory (i.e., tHb increase) response

within the superficial muscle region. The present study also reported a small decrease in tHb in the final 5 min of a 20 min CWI, thereby supporting the notion that CWI can modulate changes in local muscle blood volume. While it is likely that an alteration in blood flow to the muscle microvasculature will be detected as a change in tHb, the measure cannot actually quantify arterial inflow to the muscle unless VO is applied to the limb. Furthermore, similar to mVO_2, it is currently unknown what threshold (if any), or extent of reduction in blood flow to the muscle microvasculature is required to be beneficial in improving recovery from EIMD. Therefore, being able to quantify a value for mBF may aid in the identification of a threshold value since it is easily comparable between studies. Indeed, the previous review highlighted variations in the method for calculating ΔtHb during CWI, which tended to restrict comparison between studies. Therefore, the use of the VO method is superior when assessing muscle haemodynamics in response to CWI and should be adopted in future studies.

In following, recent work has suggested a number of methodological factors which should be considered when obtaining mBF measurement. For example, Cross *et al*., (192) studied the change in mBF during successive cardiac beats (detected using EMG) and found that mBF was highest when calculated over the first cardiac beat following VO cuff inflation. More importantly, the inclusion of further cardiac beats in the calculation of the tHb upslope led to progressive underestimation of mBF, regardless of VO being performed at rest or during reactive hyperaemia (192). Future studies incorporating mBF measurement may wish to account for this effect in order to obtain a more accurate estimation of mBF. It is worth nothing, the brief time window (<1 s) suggested in the aforementioned calculation of mBF requires rapid cuff inflation to attain sufficient pressure instantaneously and identify an exact point

at which VO was initiated on the trace. Therefore, a pneumatic rapid cuff-inflation device such as the Hokanson E-20 rapid cuff inflator (D.E. Hokanson Inc) and also a NIRS device with high sampling frequency will be required for this procedure.

In summary, the overarching aim of this thesis was to explore the applicability and utility of NIRS as a measurement tool during CWI. There are a limited number of studies which have incorporated NIRS measurement with CWI (chapter 2). Also, variation in the specific exercise and recovery contexts inevitably makes comparison difficult. Indeed, the type of prior exercise and CWI protocol utilised are likely significant factors which influence the observed physiological response (48,49). Nevertheless, the previous review demonstrated the capability of NIRS to monitor changes in tHb and TSI (%) during and following CWI. NIRS devices, once waterproofed and attached to the muscle of interest, offer the methodological advantage of continuous physiological monitoring during the immersion period; this cannot be achieved with other techniques such as strain gauge plethysmography or Doppler Ultrasound. However, while the tHb and TSI (%) measures certainly provide insight into the muscle physiological response, the information they provide on muscle blood flow and muscle metabolism is limited and should be interpreted with caution. The present study aimed to extend upon previous NIRS findings by implementing a simple occlusion procedure to quantify mBF and mVO_2 from the NIRS signals pre- and post-CWI. This methodology is well-established in NIRS literature (163,178,180,191,244) but had yet to be incorporated within CWI recovery. Utilising the NIRS occlusion procedure showed reductions in mVO_2 as a result of CWI; however, it is important to emphasise the present study only aimed to provide insight into the acute muscle physiological response to CWI. The significance of these findings in the wider context of athletic recovery remains to be established.

Applied research has shown conflicting evidence regarding the efficacy of CWI for improving the recovery of muscle damage indices (see section 1.5.1); albeit variation in the type of exercise used to induce muscle damage, along with training status of the subjects may explain some of the heterogeneity in the findings. In order to understand the underlying mechanisms associated with a beneficial recovery effect, there is a need to characterise the physiological response to CWI. Overall, this thesis demonstrates the utility of the NIRS measurement in fulfilling this requirement. Future research should aim to investigate the association between the physiological response to CWI, and the subsequent effect on the recovery of muscle damage indices. Obtaining a "full" physiological profile including acute changes in mBF, mVO_2 and thermoregulatory (e.g., limb and core temperature) measures following CWI, with follow-up assessment of the recovery of muscle damage indices in the days following exercise may be an approach to achieve this. Ultimately, the goal is to improve the evidence based rationale for CWI implementation following muscle damaging exercise, since this is primarily when athletes use it during recovery (48,197,257). However, the application of CWI as a recovery tool is still controversial, with evidence to suggest its regular use can ameliorate training induced changes following resistance training (265–267). Improving the knowledge surrounding the physiological response to CWI should clarify its efficacy for aiding post-exercise recovery and provide directional progression for researchers and practitioners alike. Arguably, this is needed before gross application across sporting disciplines and athlete groups. Finally, as already mentioned, a question that arises from this study, and others investigating the physiological response to CWI, is the existence of a "threshold", or extent of reduction in the obtained measures (i.e., mBF/ mVO_2) for achieving a desired physiological response and/ or producing a beneficial

effect on recovery. The reduction in tissue temperature that accompanies cooling likely modulates changes in mBF and mVO_2 through mechanisms such as cold-induced vasoconstriction, and a reduction in the rate of cellular metabolic reactions. The present study investigated two different immersion temperatures (10 °C and 15 °C) on mBF and mVO_2, with the important finding being no observed difference in the magnitude of mVO_2 reduction despite the 5 °C temperature gap. It is likely that differences in tissue temperature occurred between the two immersion protocols, suggesting that a threshold value may exist for tissue temperature, below which greater reductions in mVO_2 may occur. However, thermoregulatory responses were not assessed in the present study meaning the extent of reduction in either CWI temperature could not be established. Future studies should investigate the notion of a threshold or target reduction in mBF and mVO_2 and explore the association between these physiological measures with tissue temperatures during CWI. Utilising different CWI protocols (i.e., temperatures and durations) to elicit a different magnitude of tissue temperature reduction is needed for such investigations. Documenting the variation that arises between CWI protocols will guide effective CWI optimisation to achieve the desired physiological response or target a specific recovery objective.

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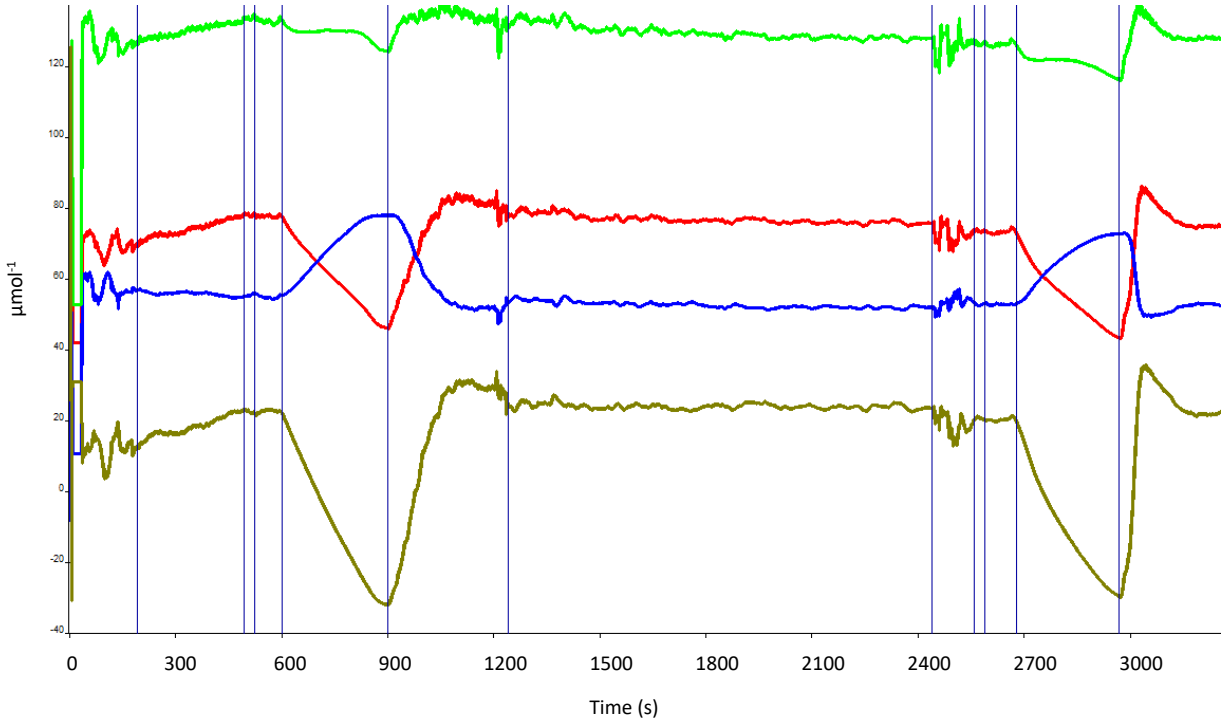
6. Appendices

Appendix A: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during experimental protocol for all participants, (15 °C CWI temperature)

(a)



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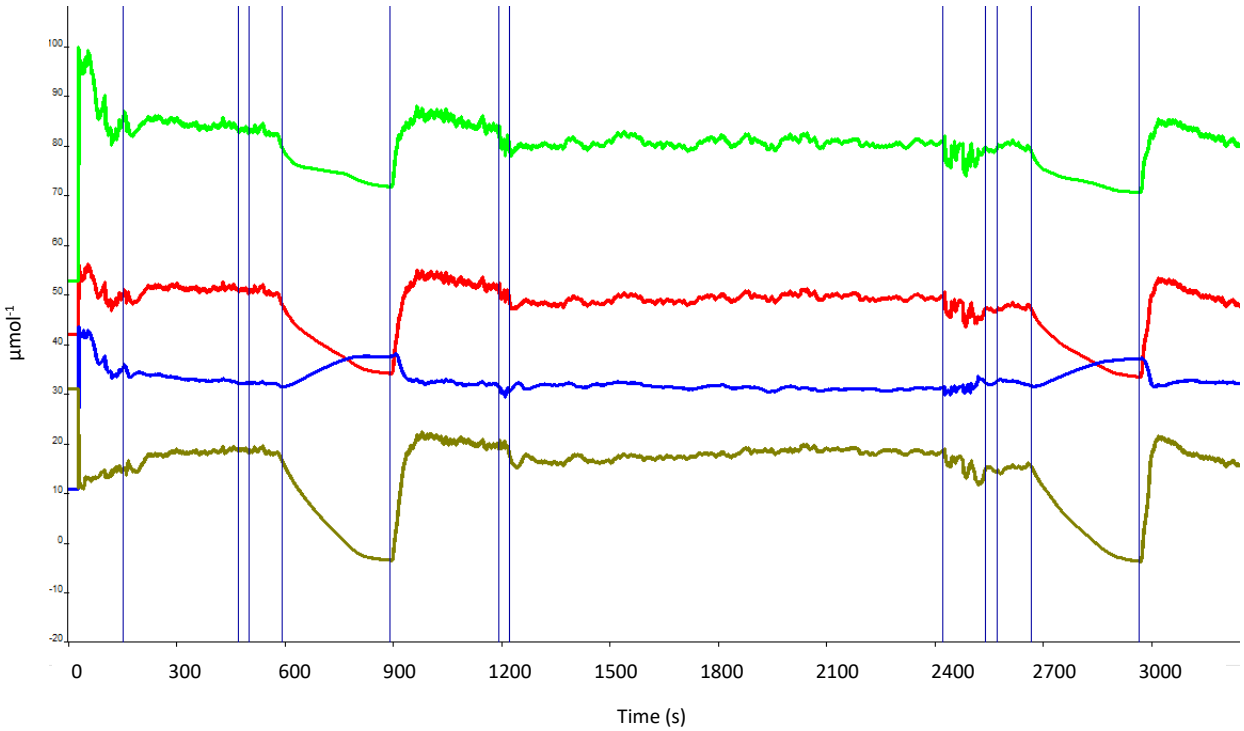

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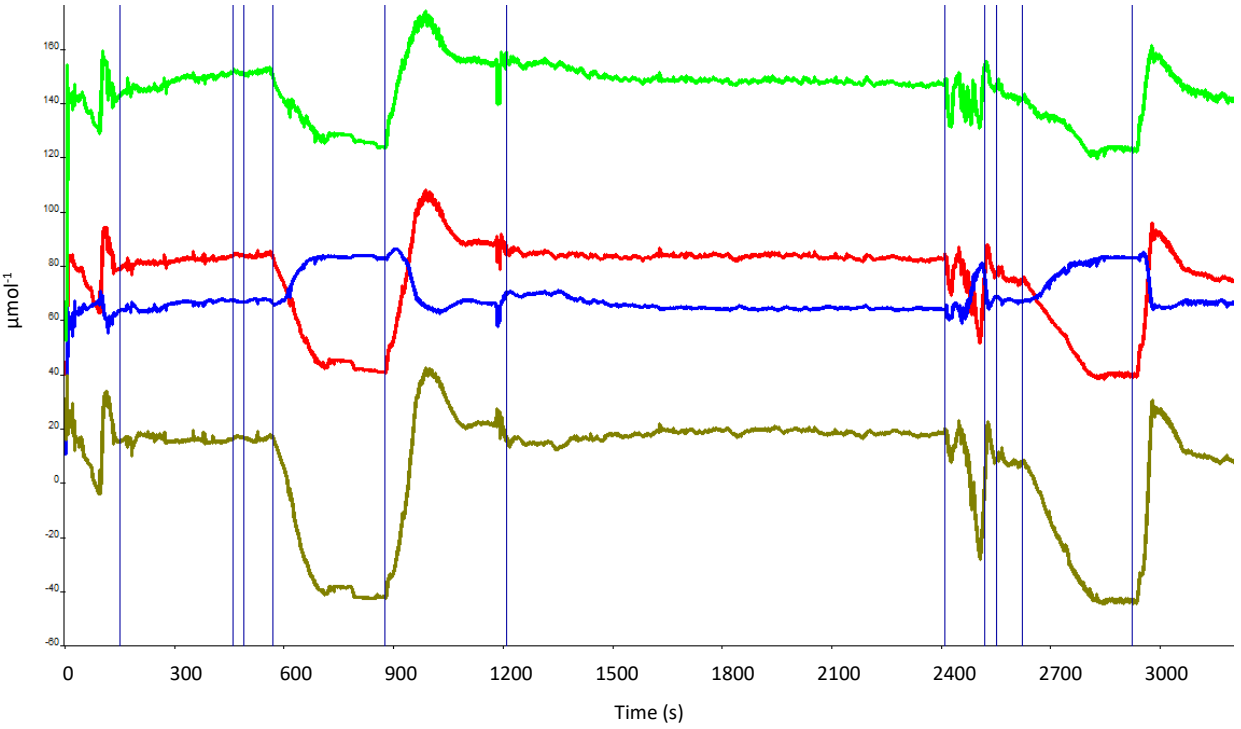
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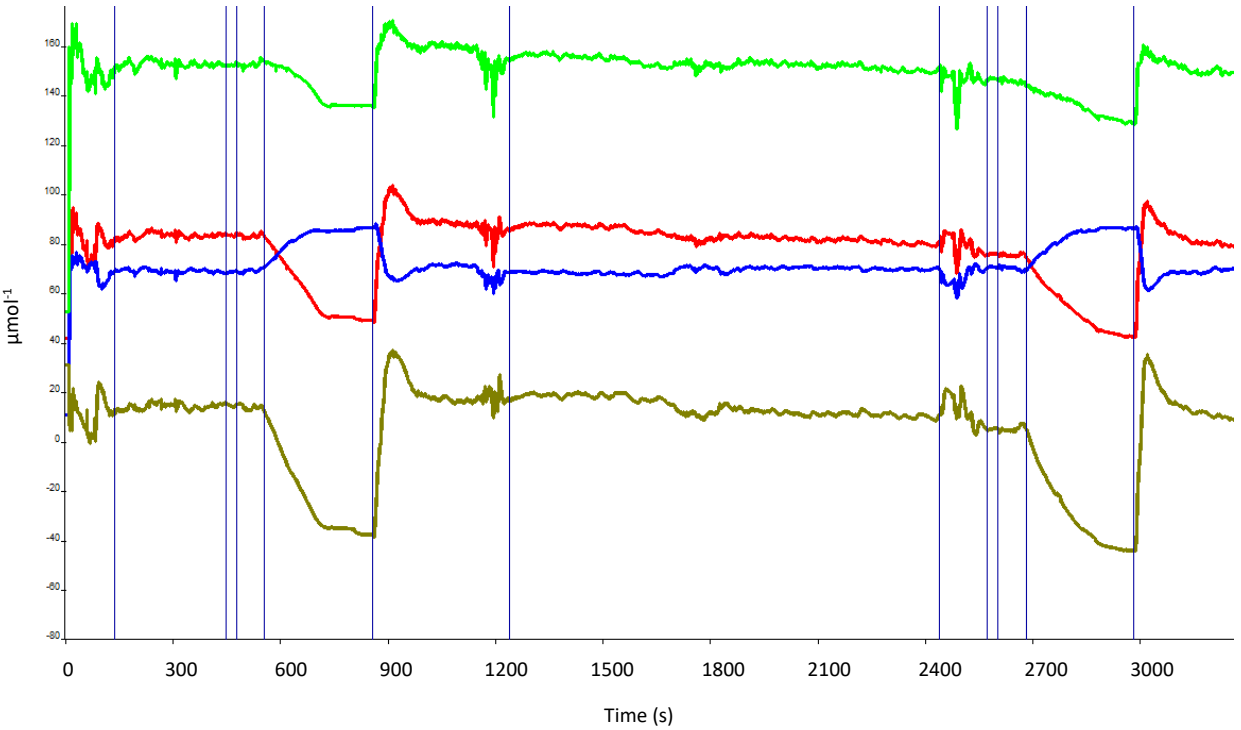
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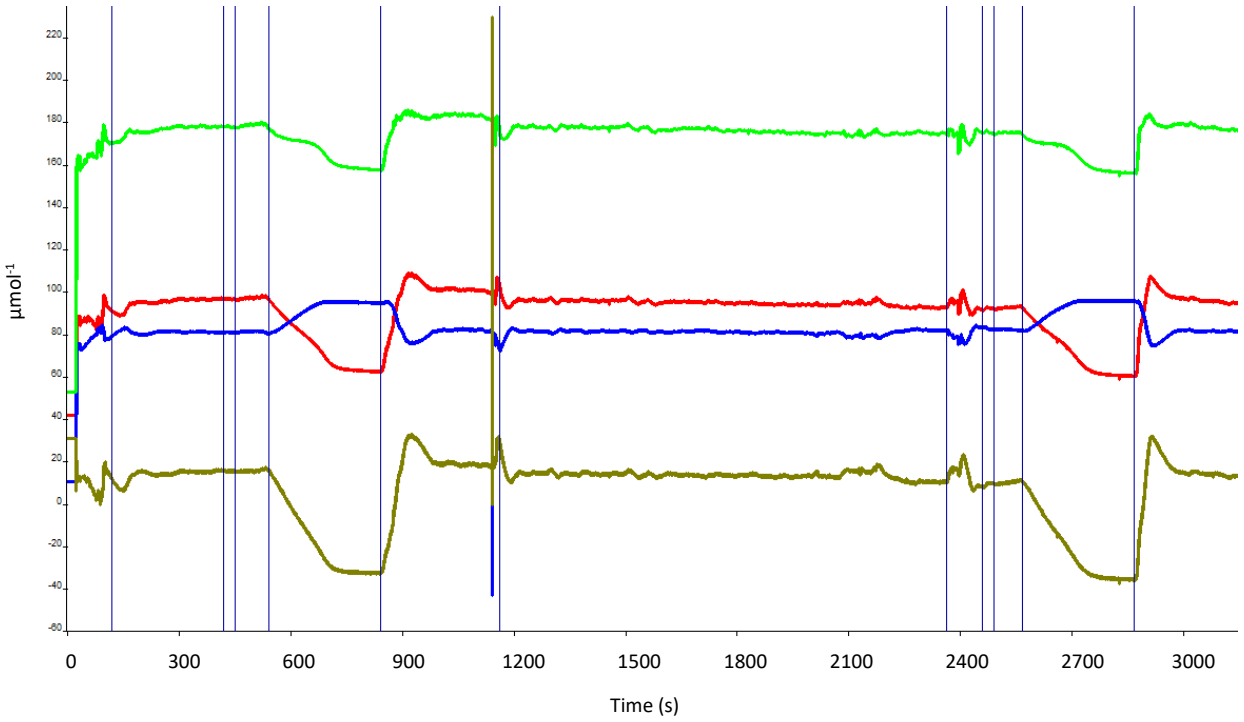
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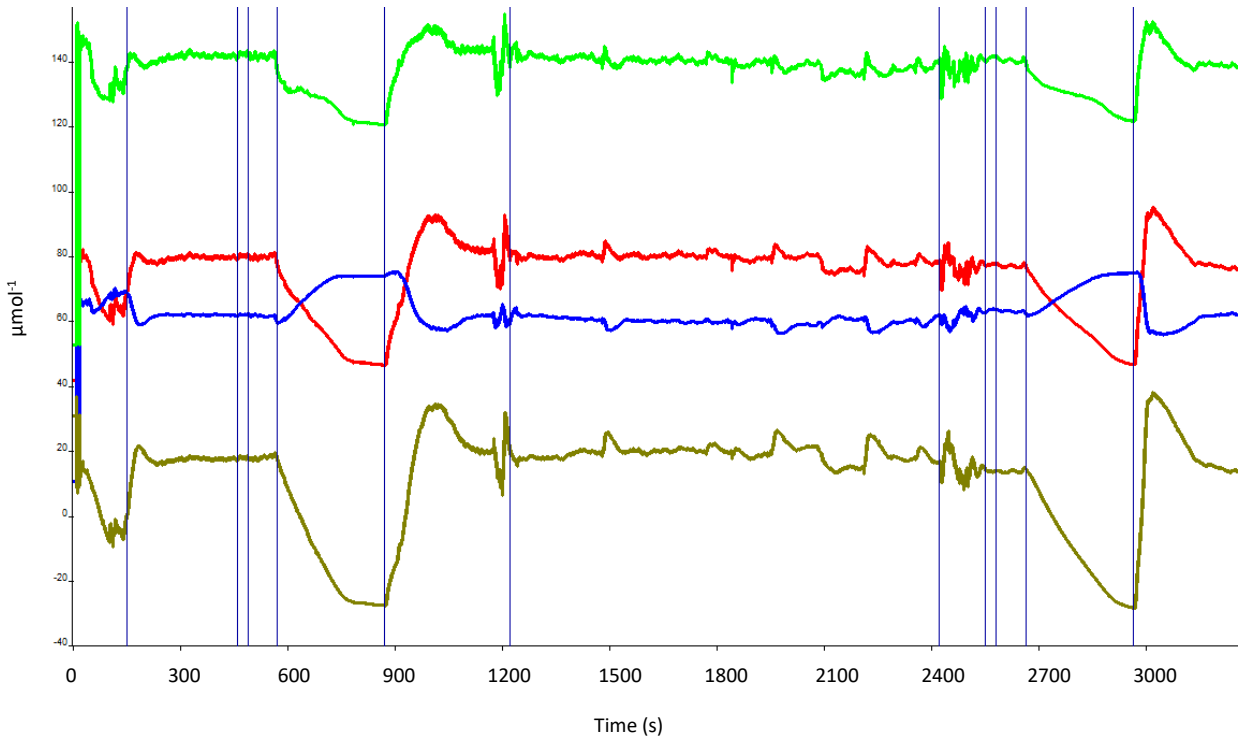
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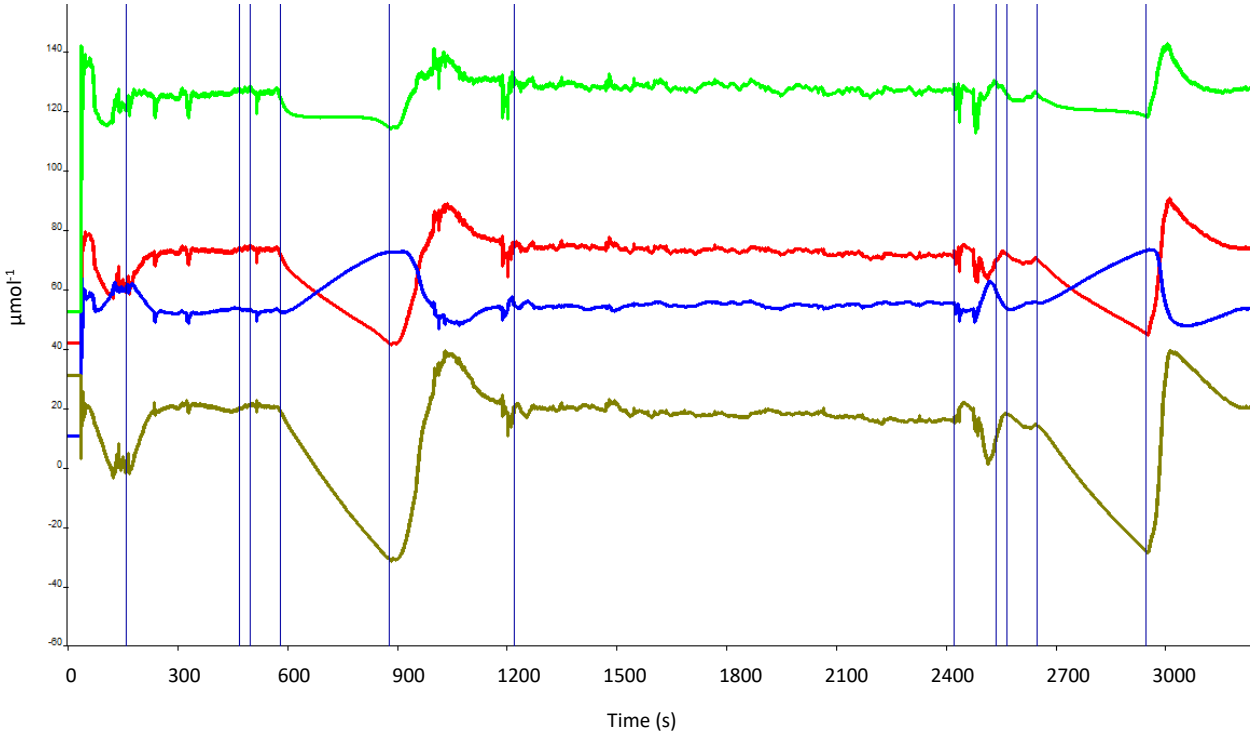
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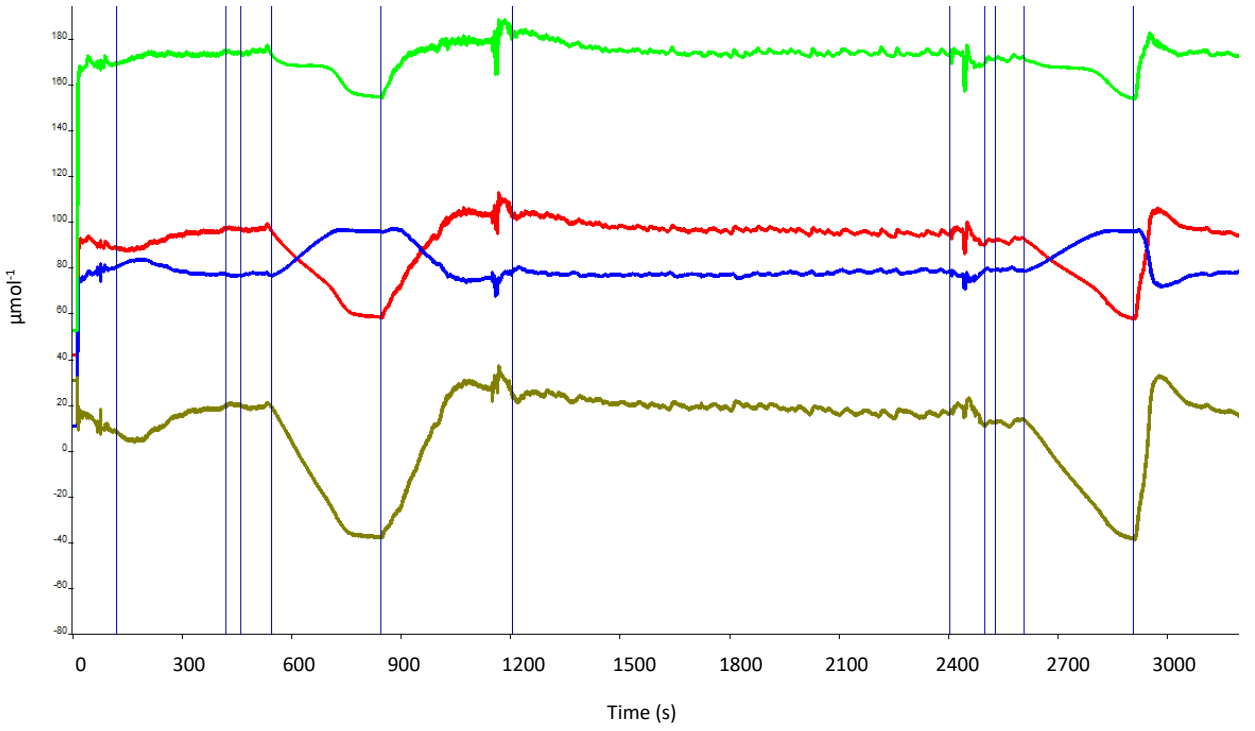
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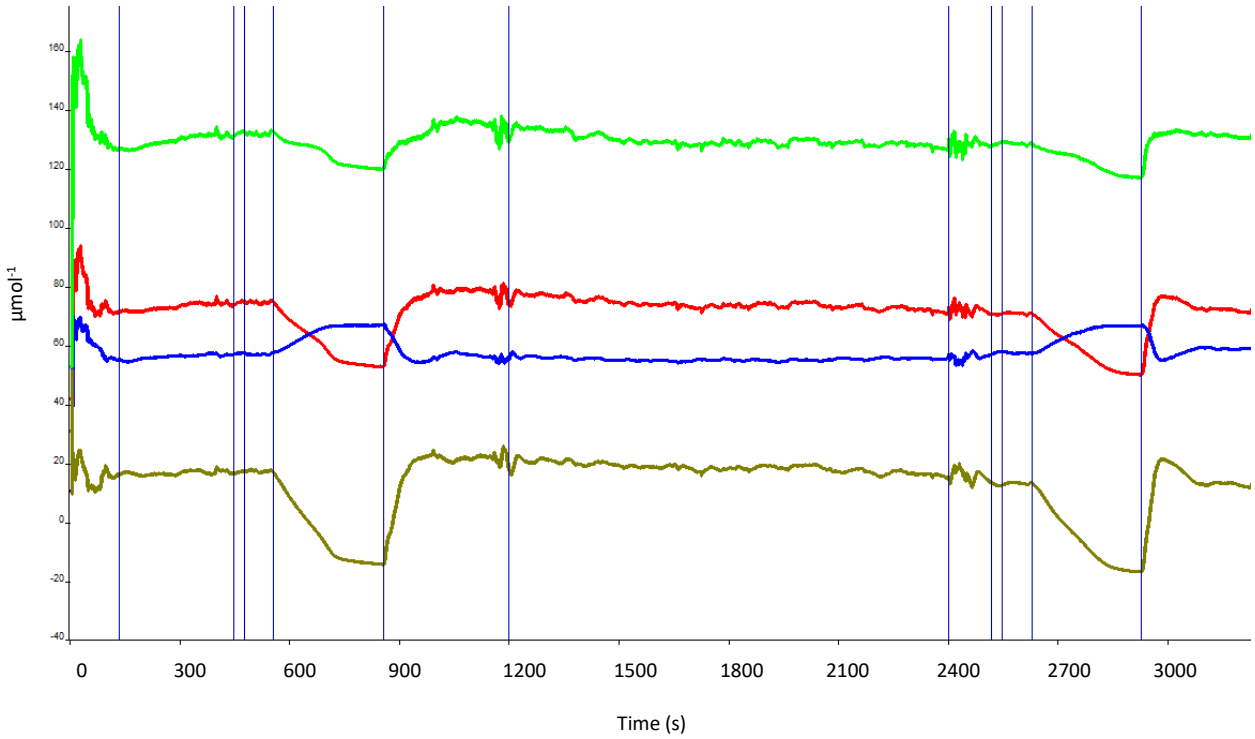
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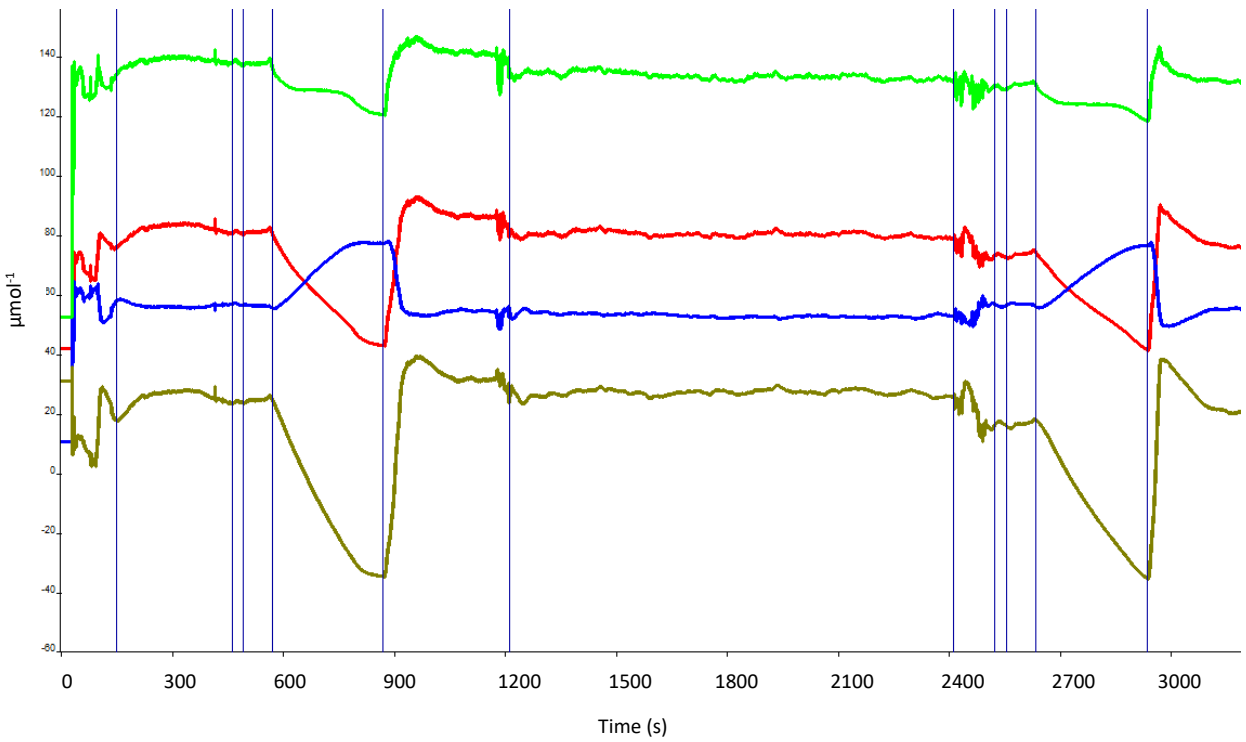
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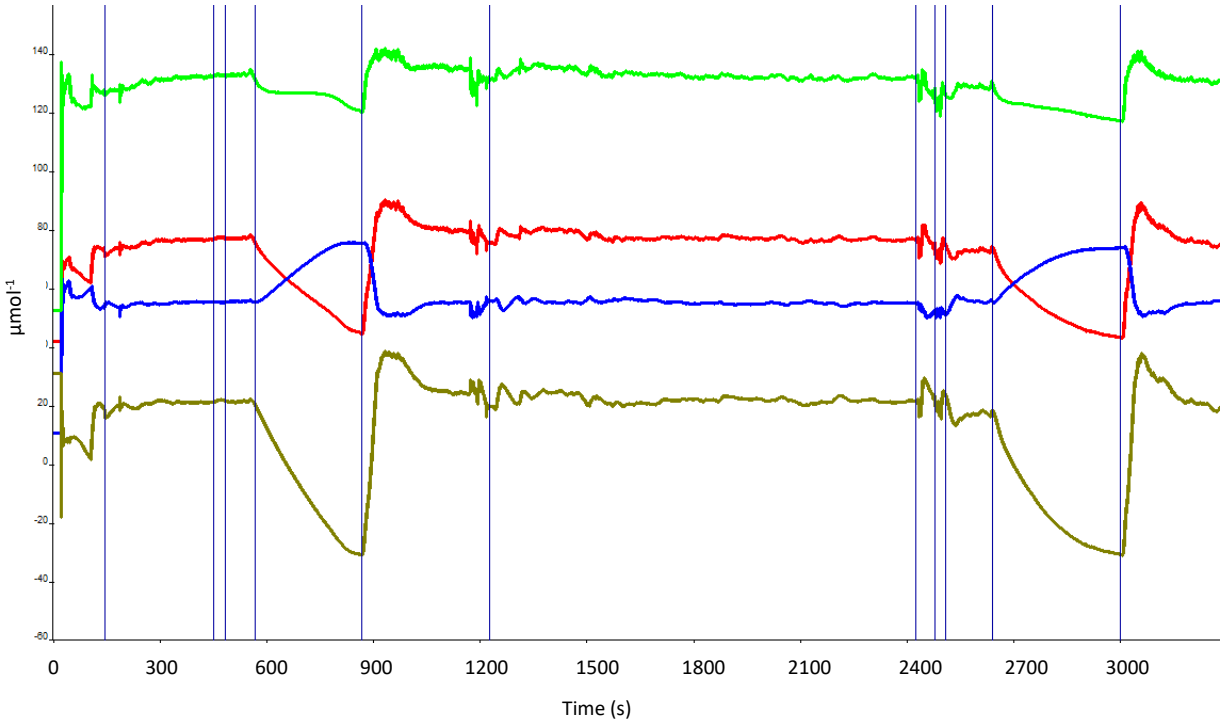
Appendix B: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during experimental protocol for all participants, (10 °C CWI temperature)

(a)



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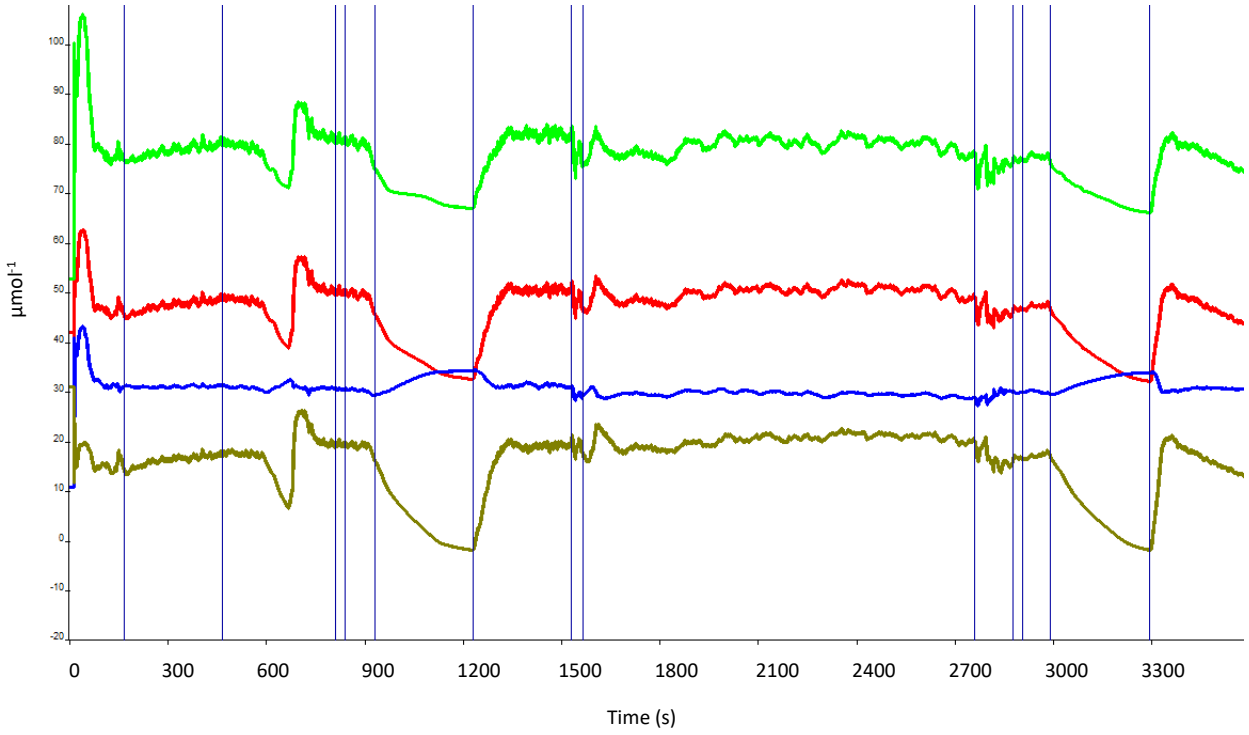

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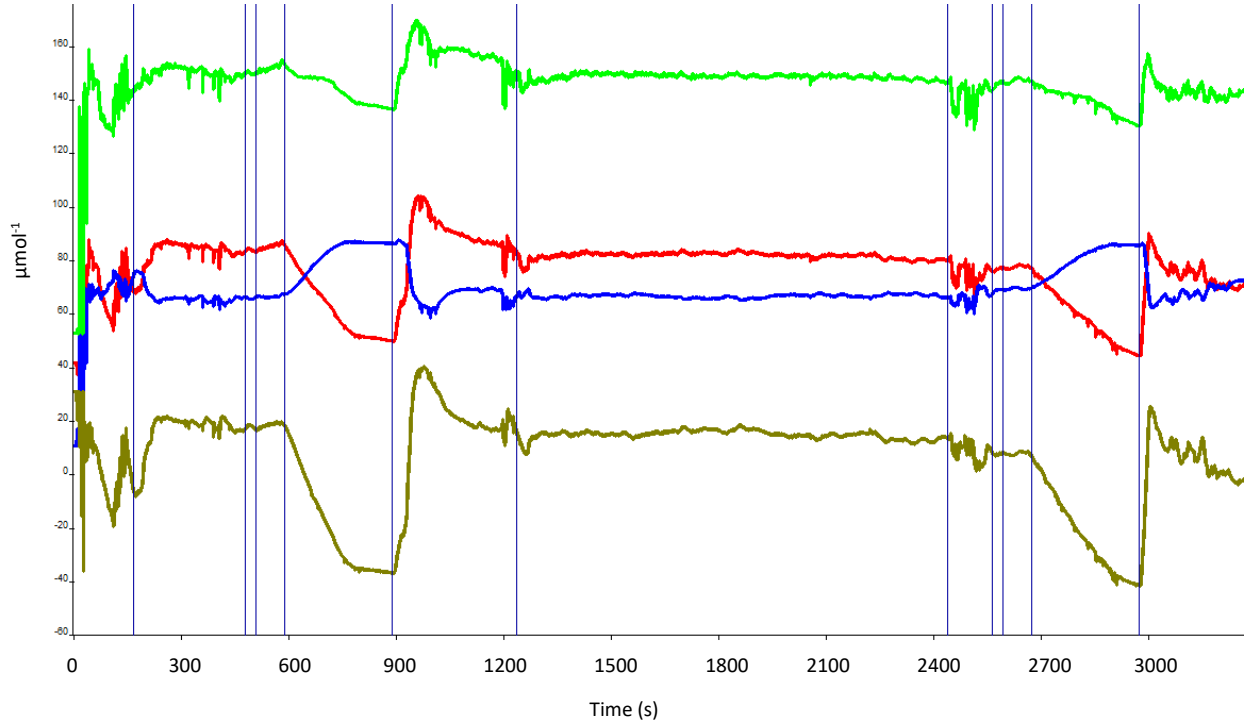
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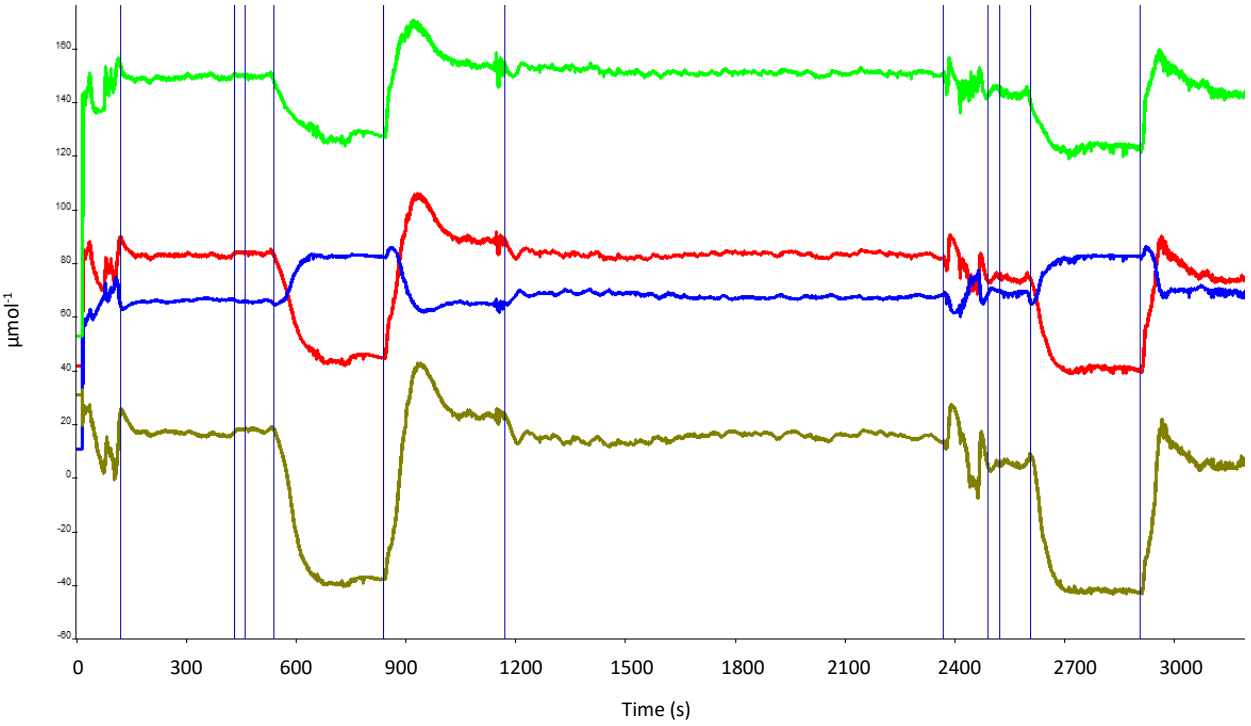
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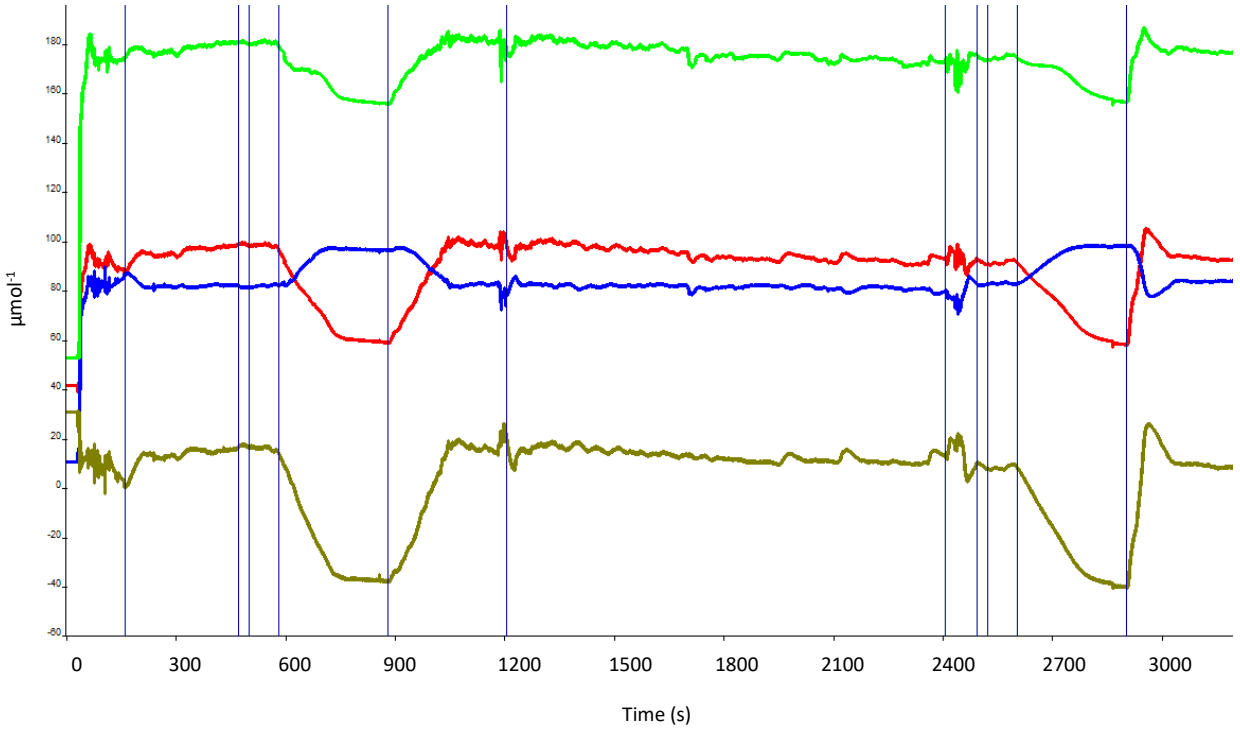
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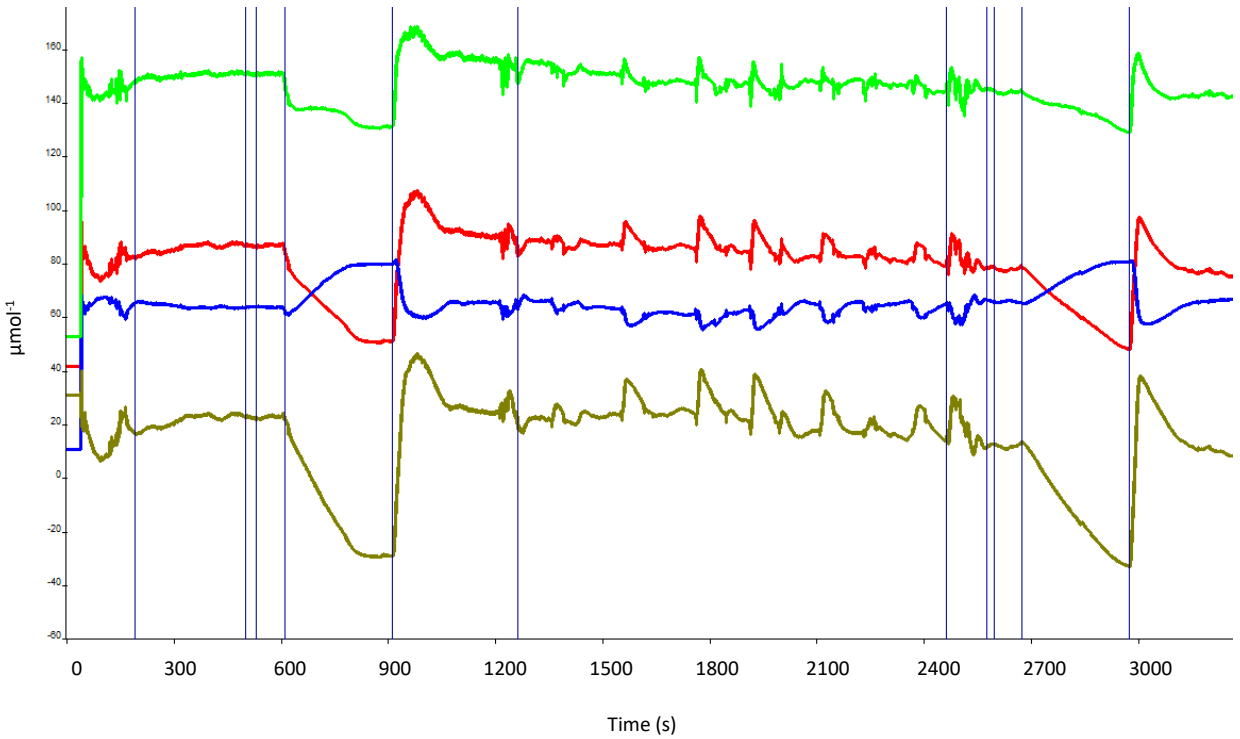
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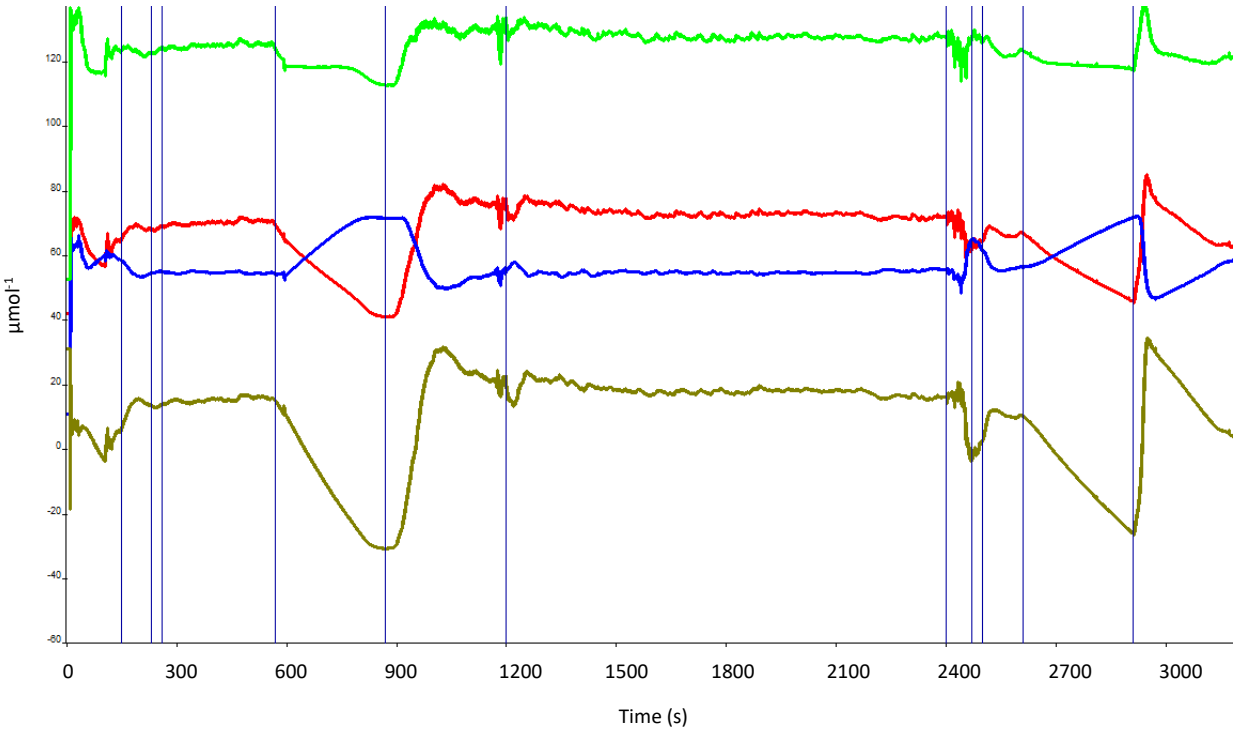
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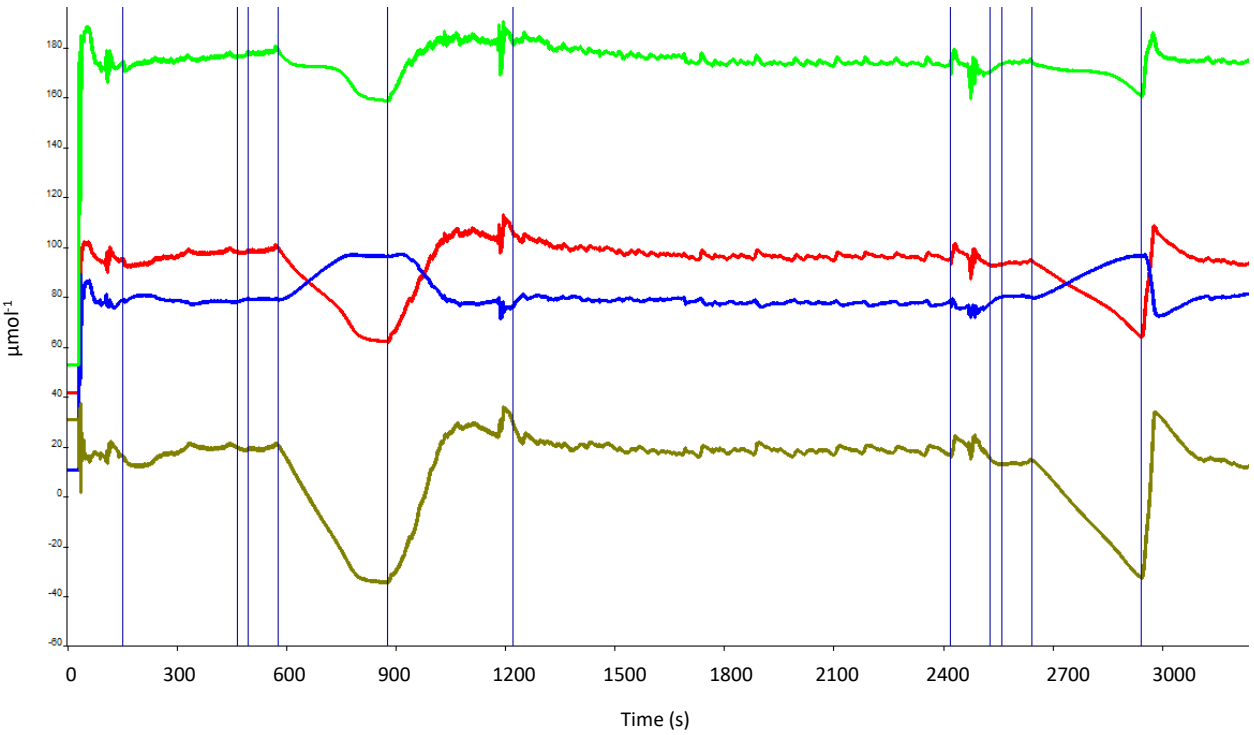
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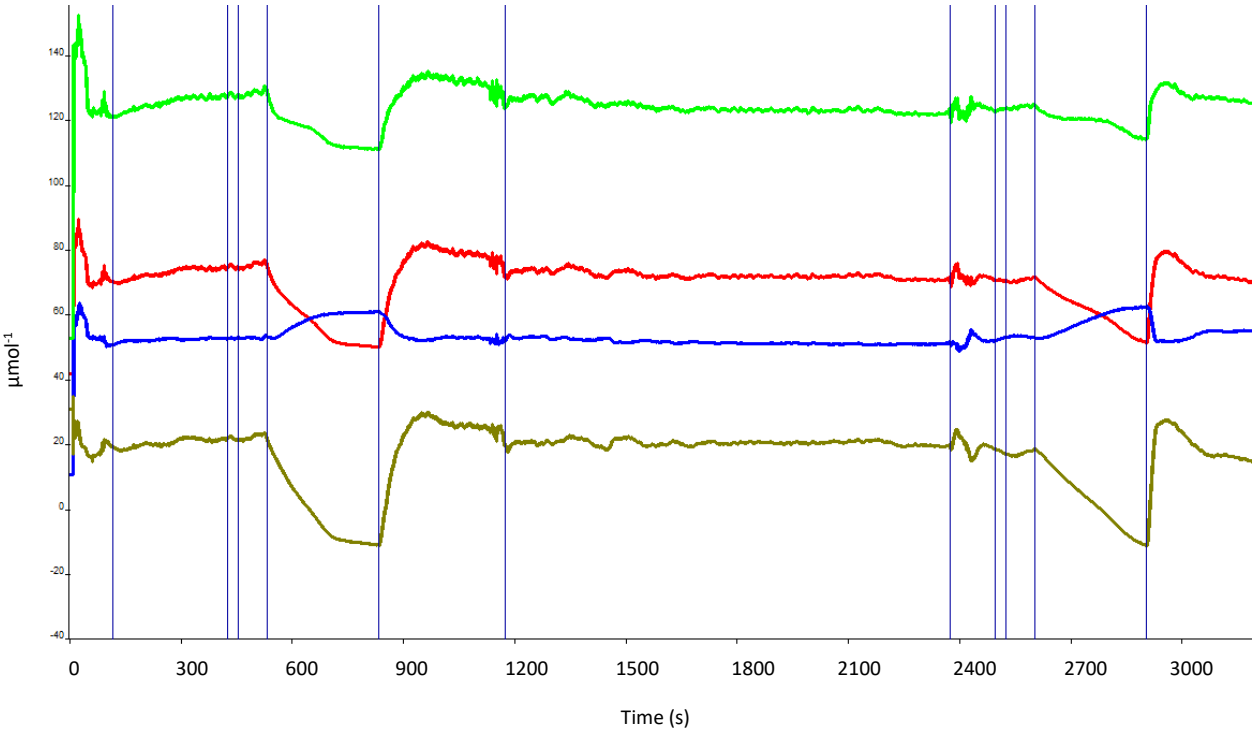
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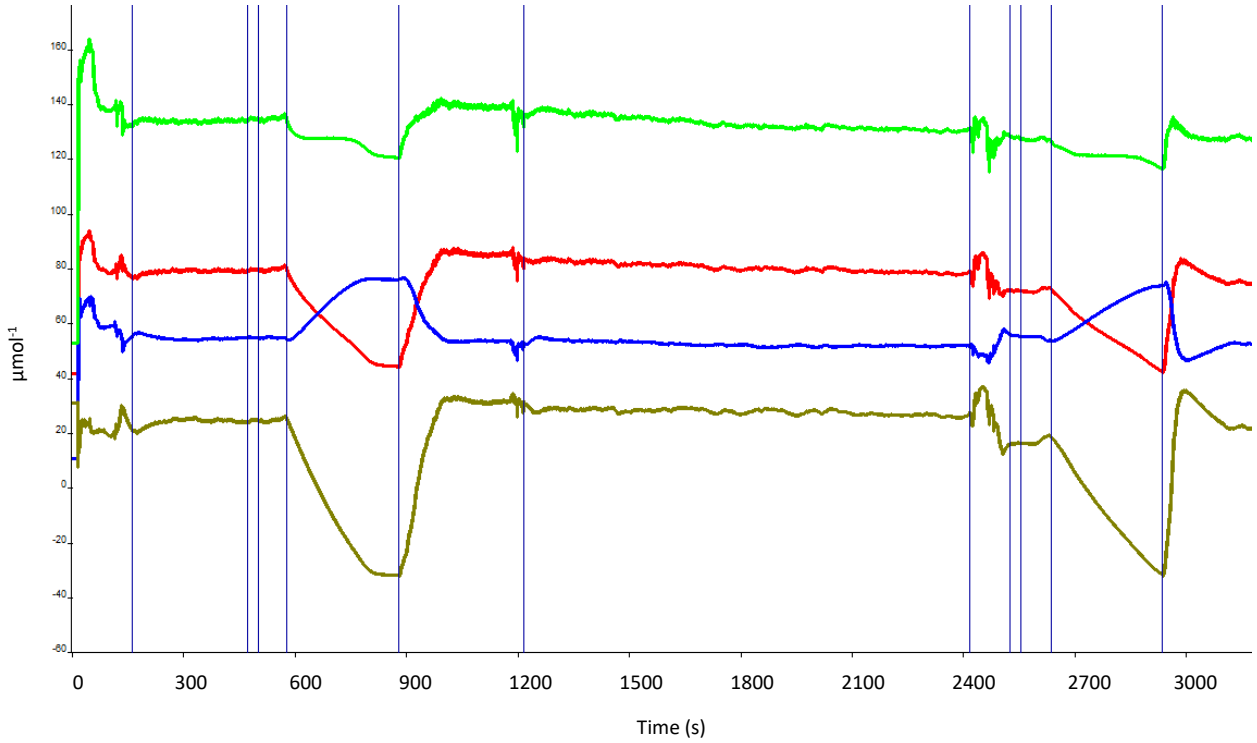
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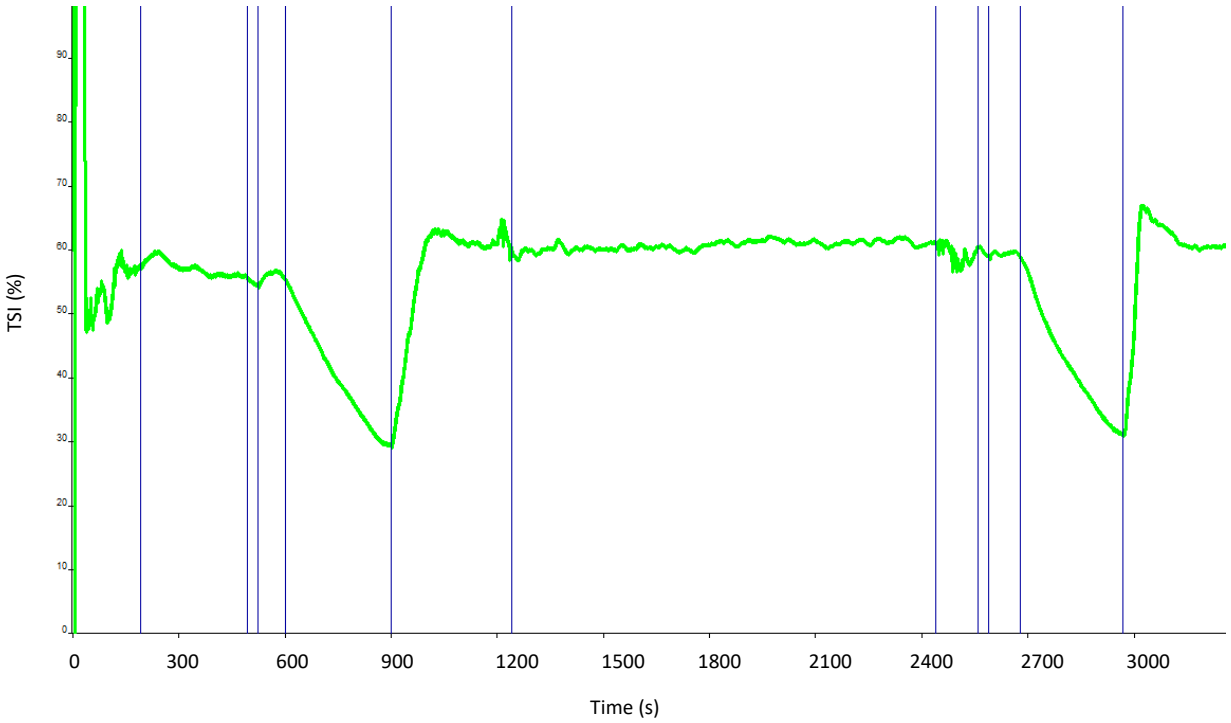
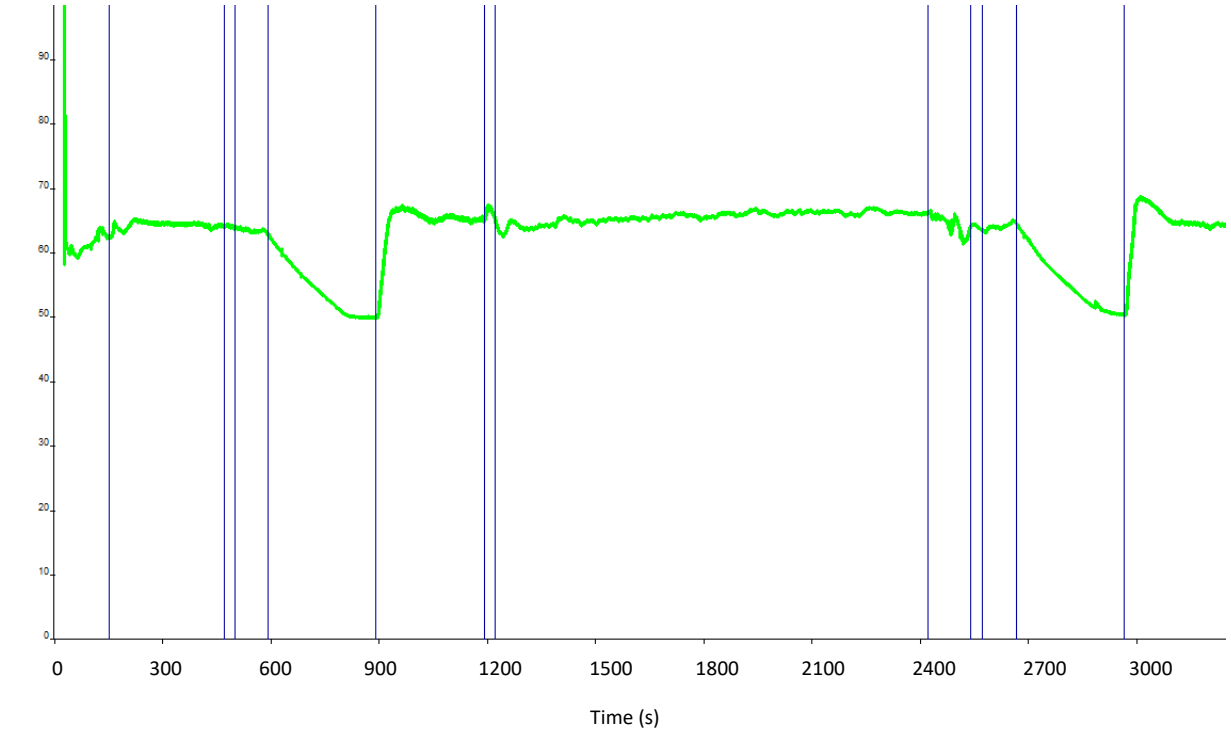
Appendix C: TSI (%) NIRS trace (displayed as green) during experimental protocol for all participants, (15 °C CWI temperature).

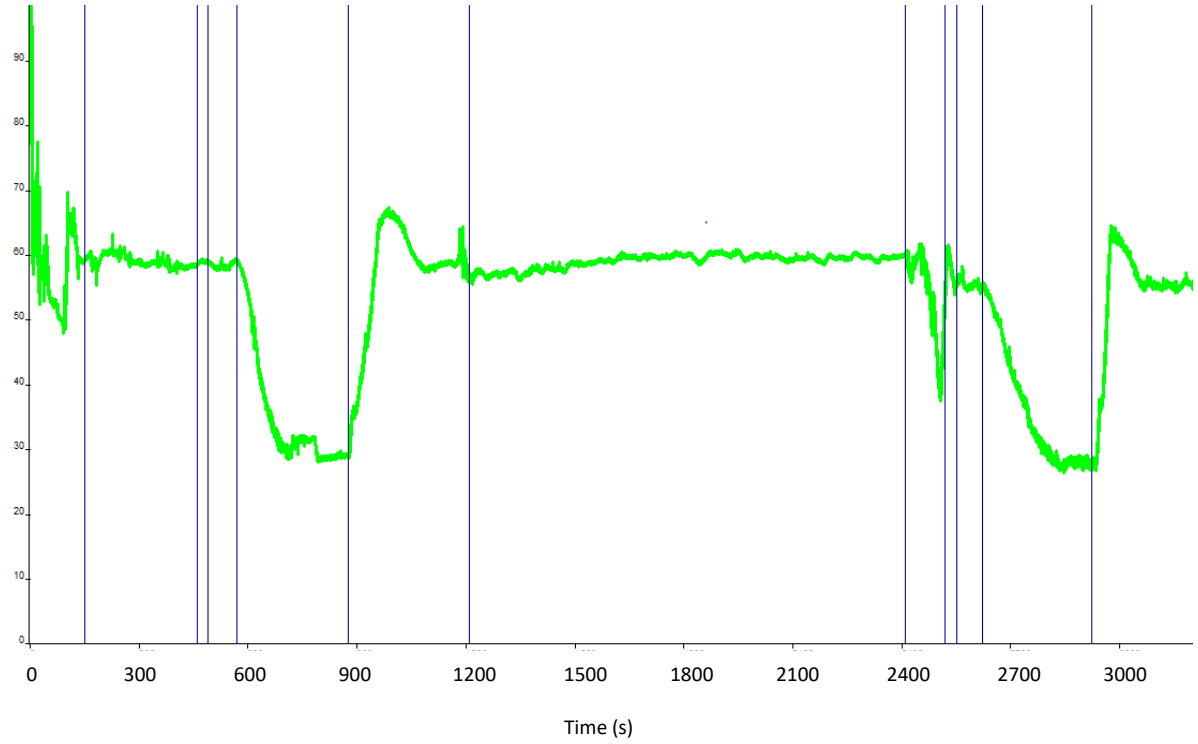
(a)



(b)



(c)


90
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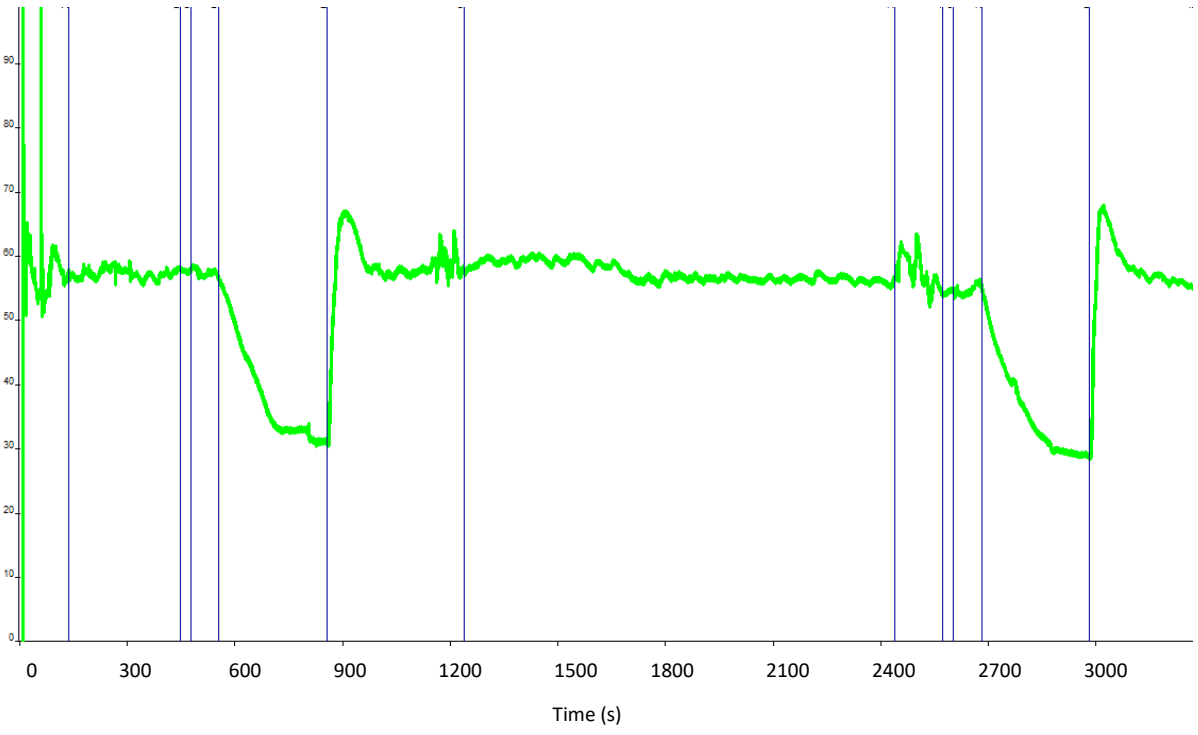
Time (s)

(d)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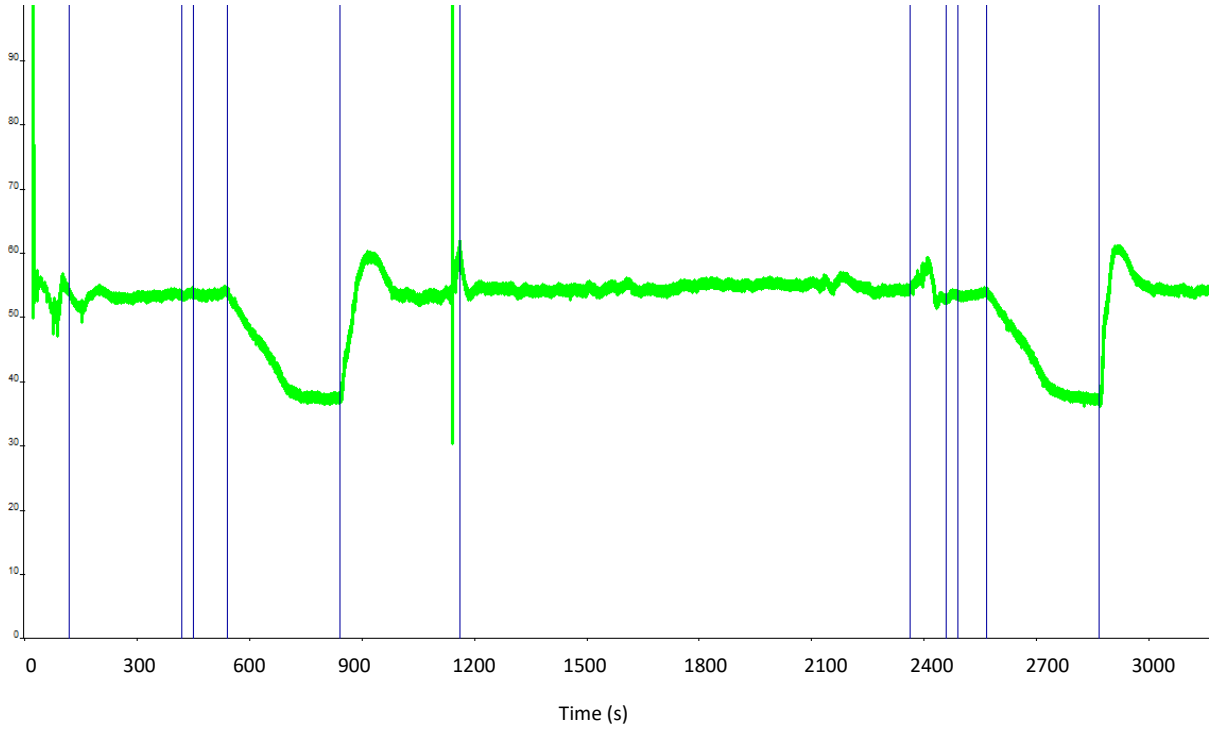
Time (s)

(e)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2700
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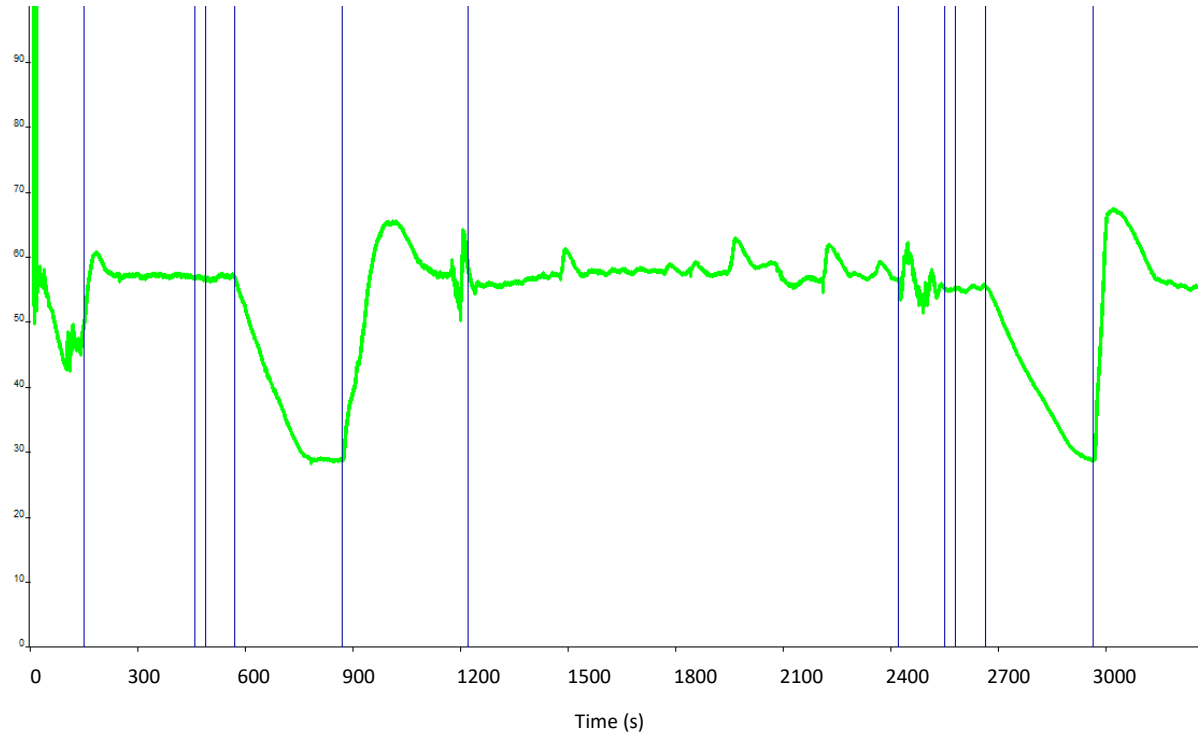
Time (s)

(f)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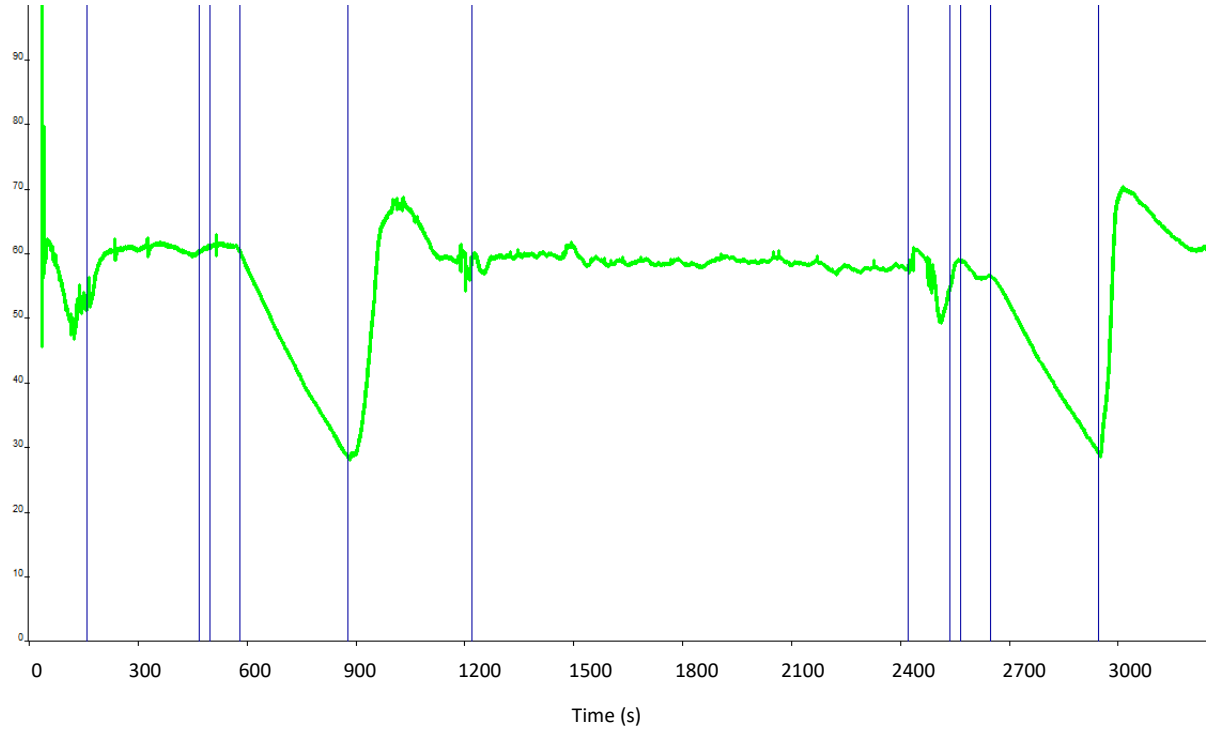
Time (s)

(g)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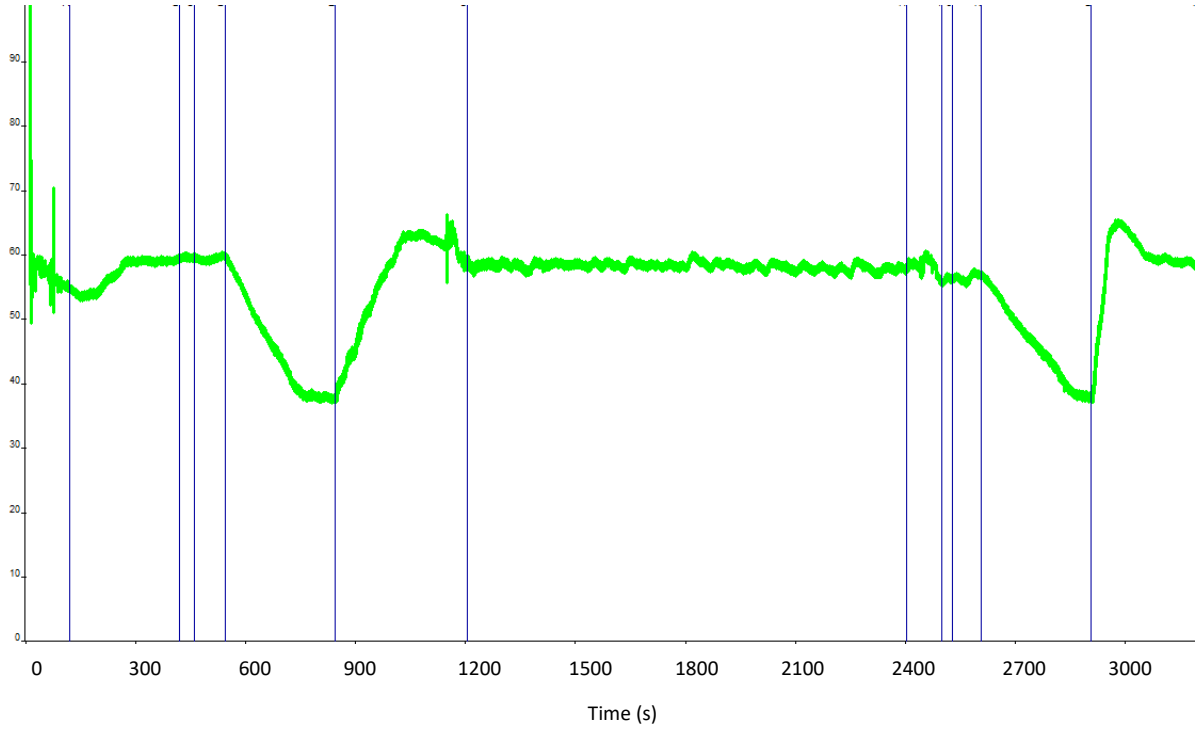
Time (s)

(h)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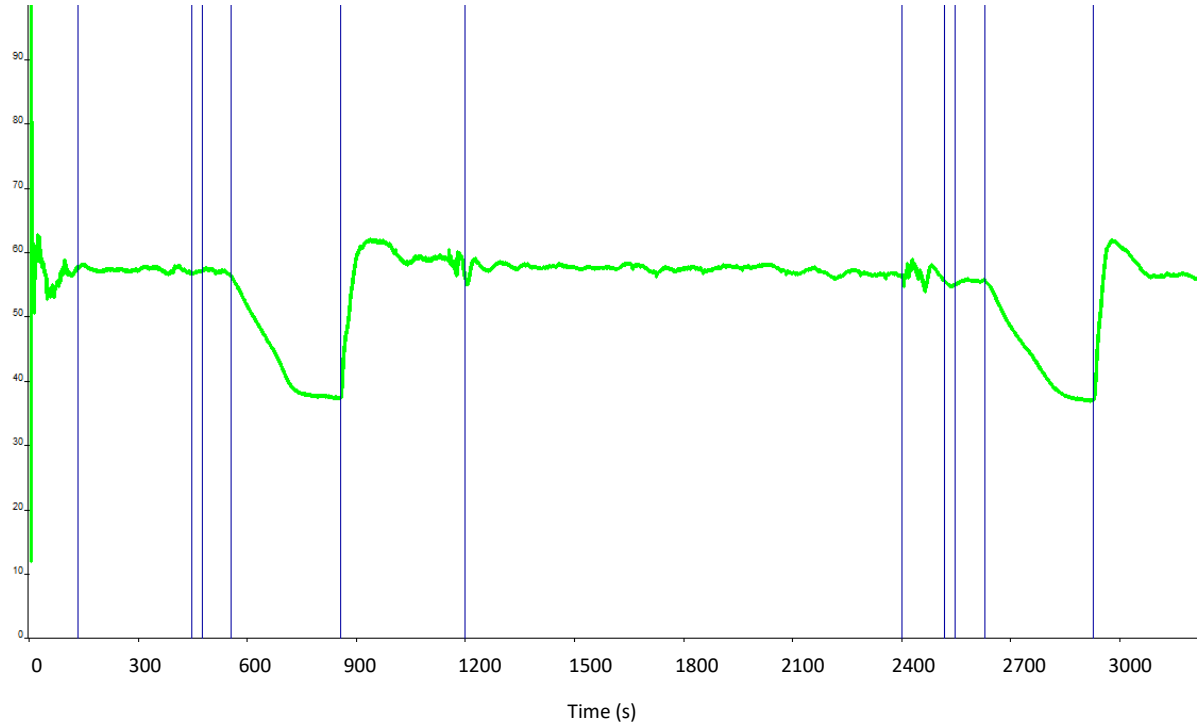
Time (s)

(i)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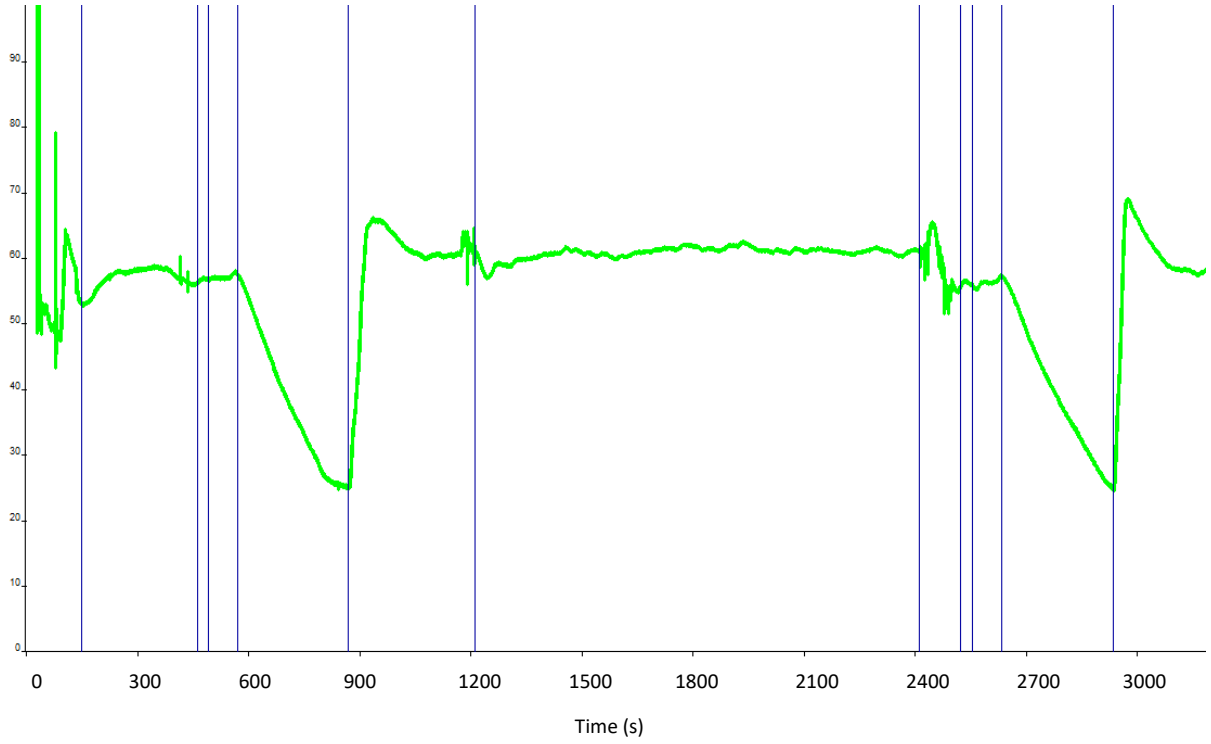
Time (s)

(j)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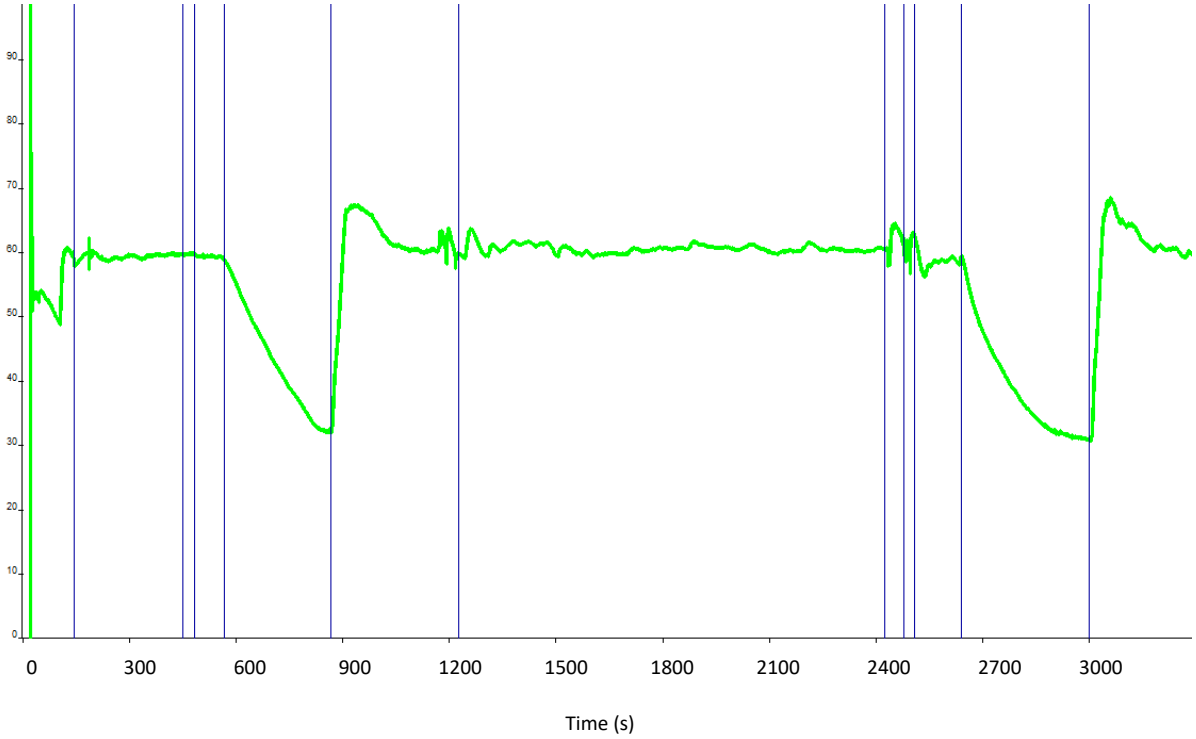
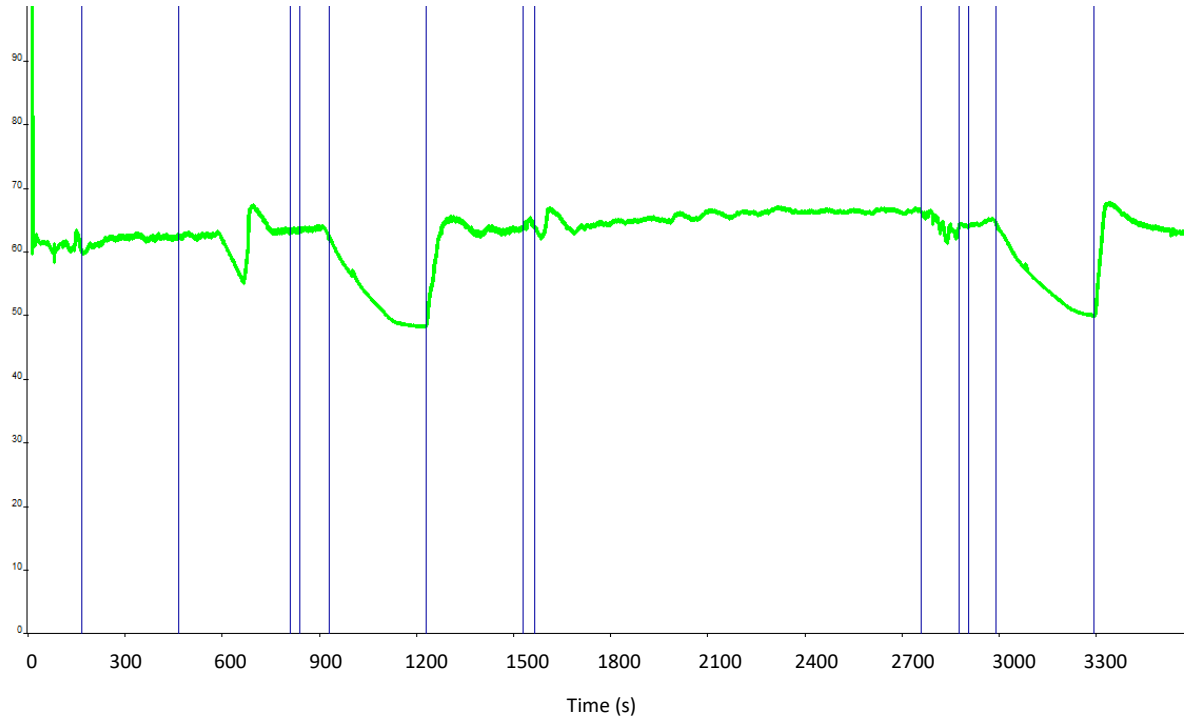
Time (s)

Appendix D: TSI (%) NIRS trace (displayed as green) during experimental protocol for all participants, (10 °C CWI temperature)

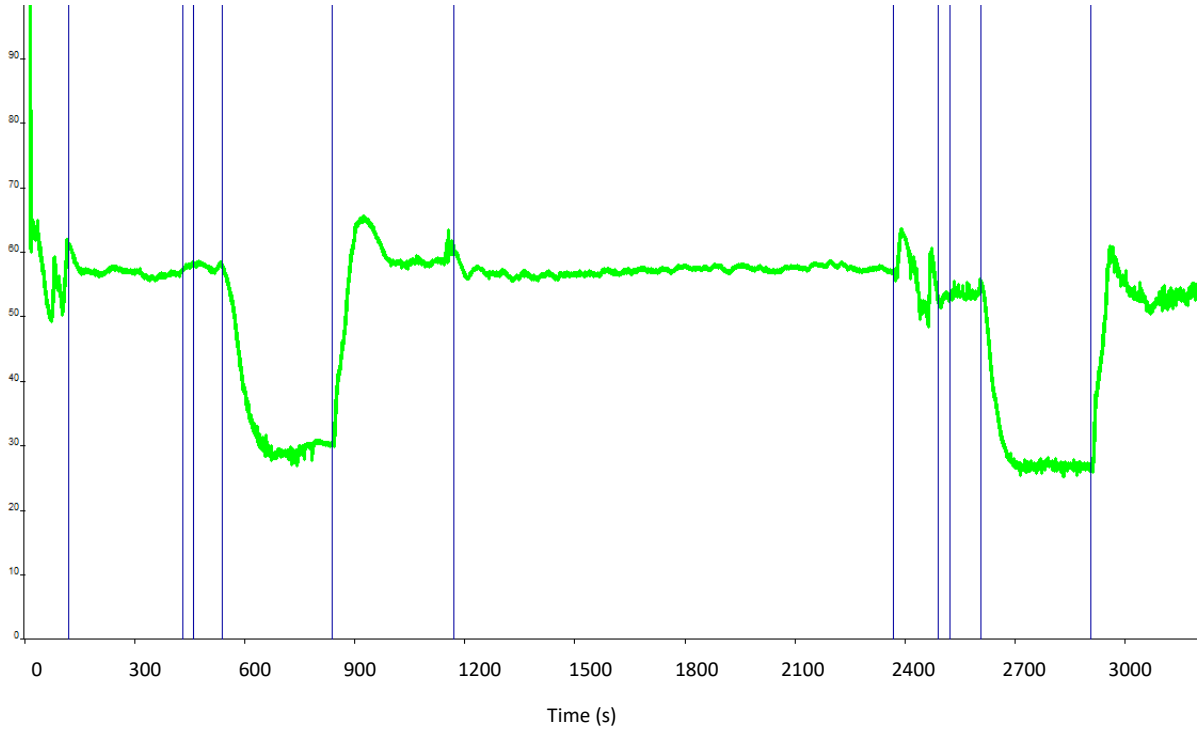
(a)



(b)



(c)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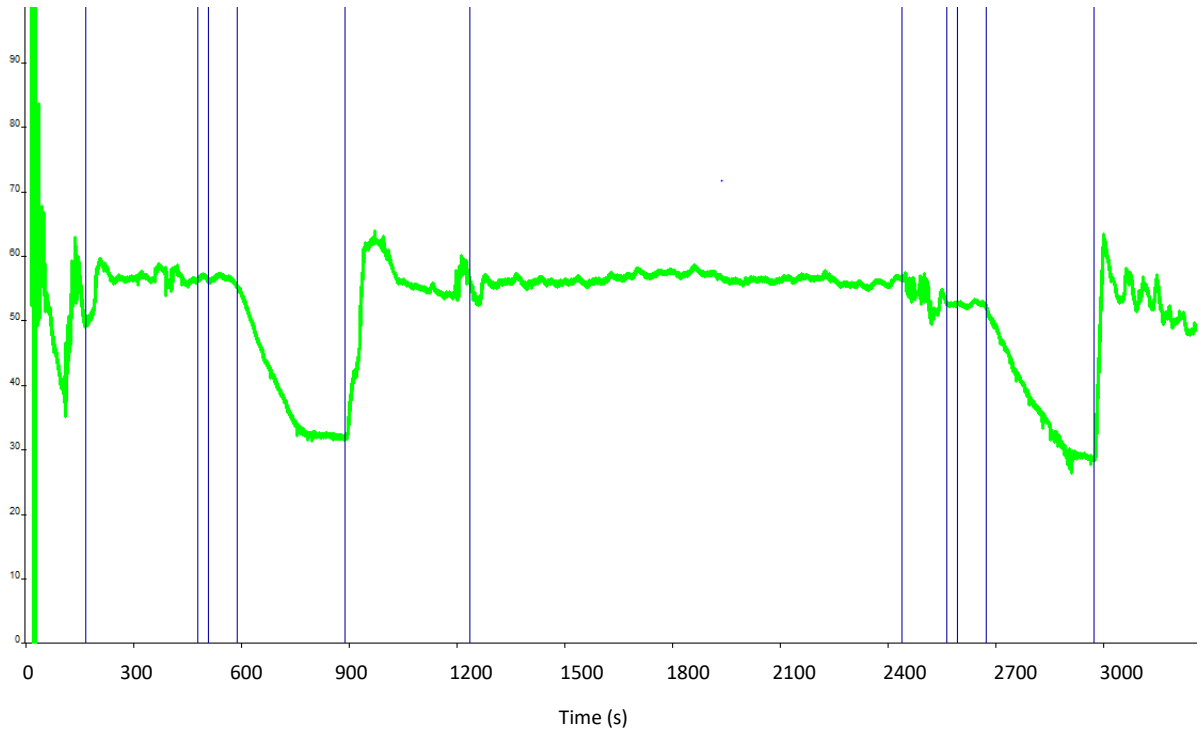
Time (s)

(d)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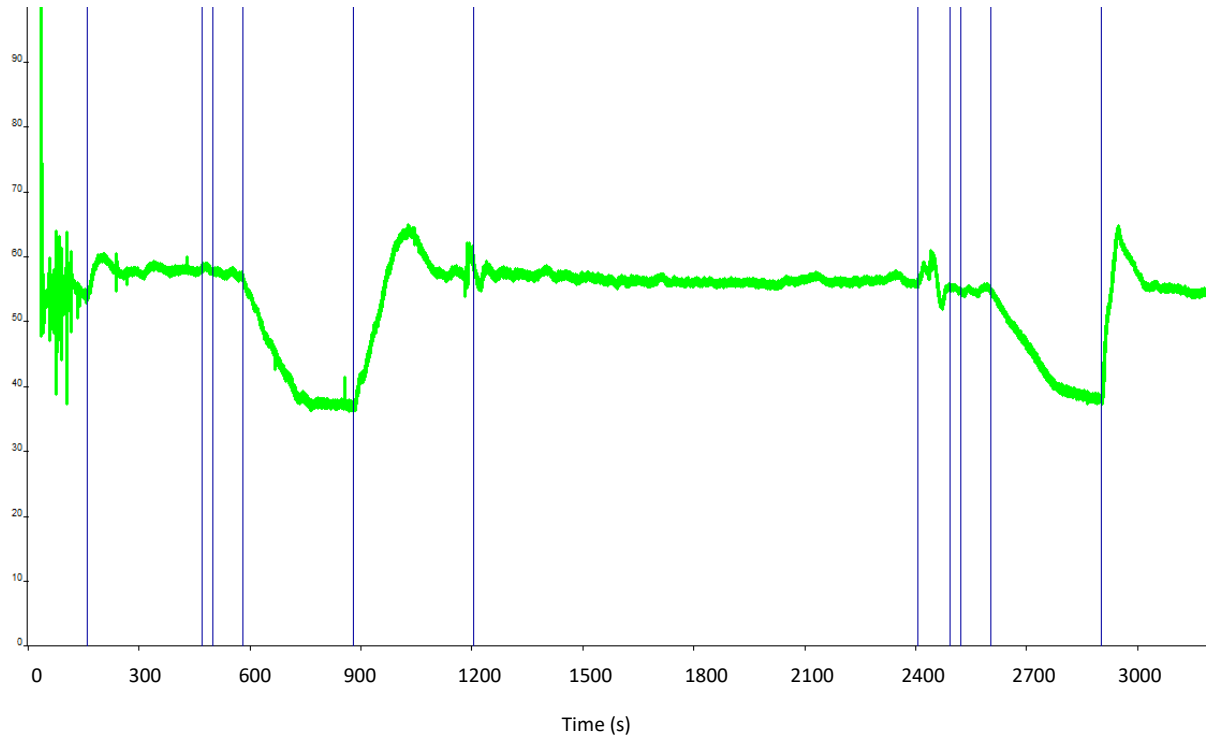
Time (s)

(e)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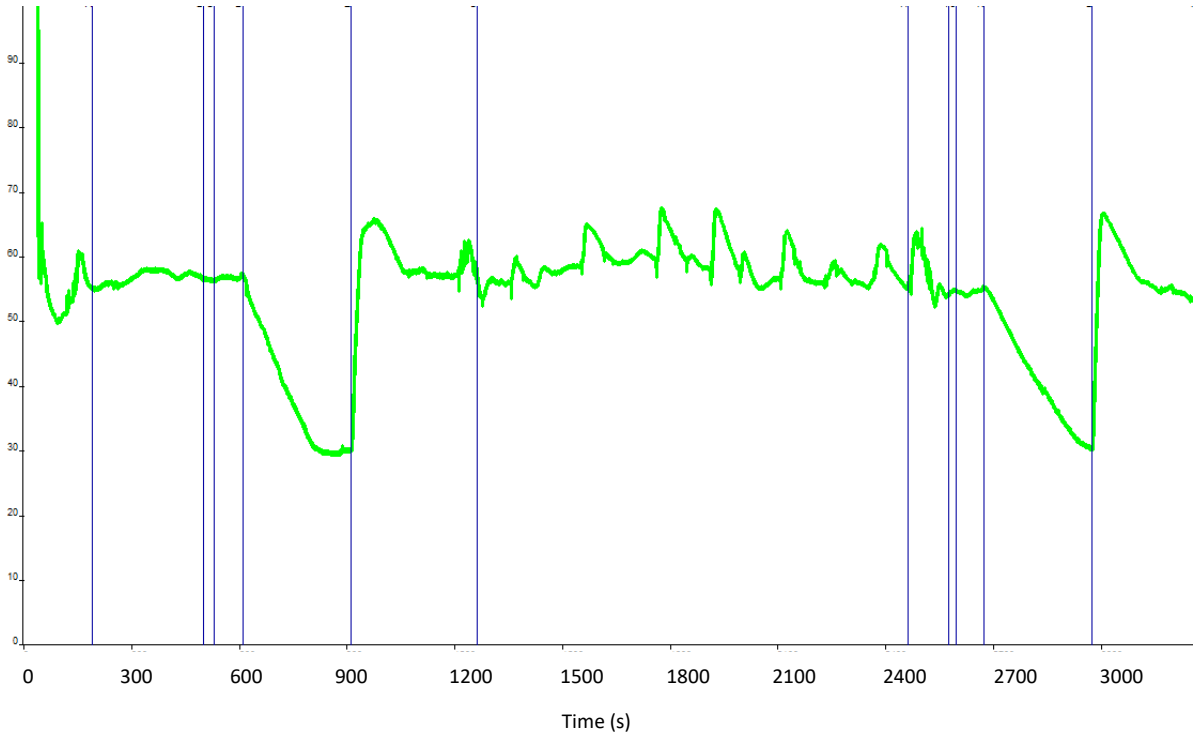
Time (s)

(f)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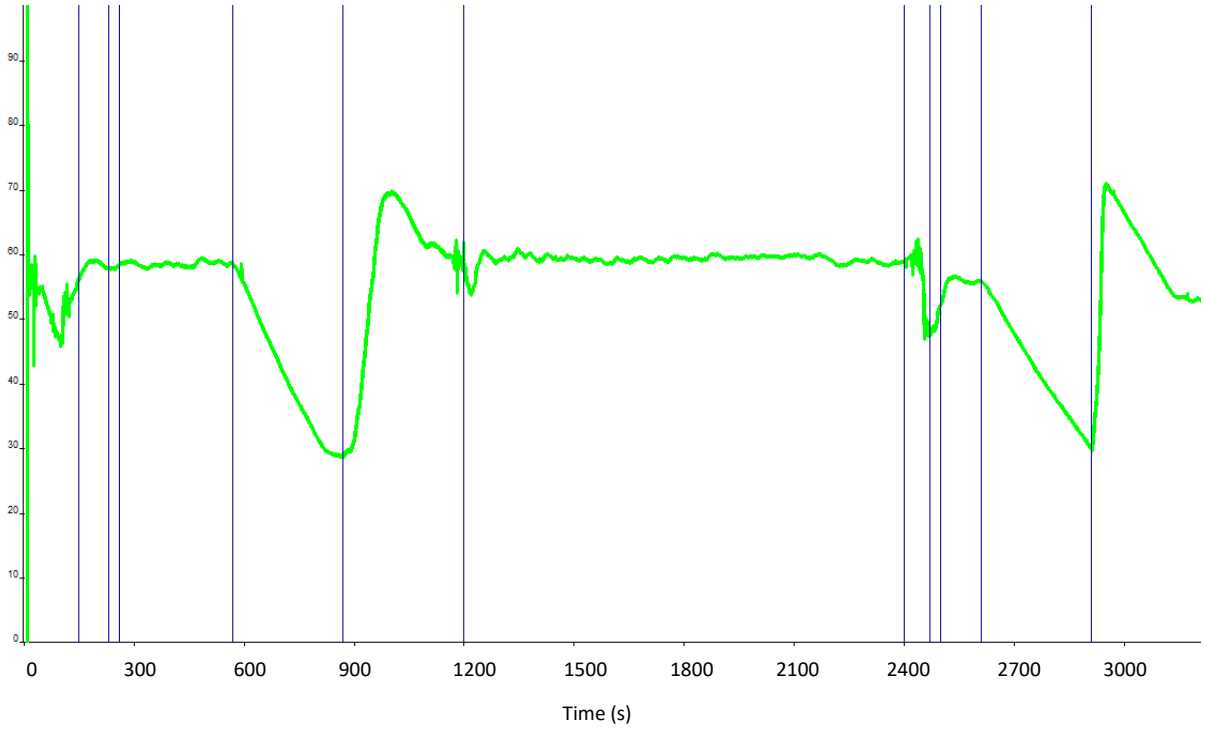
Time (s)

(g)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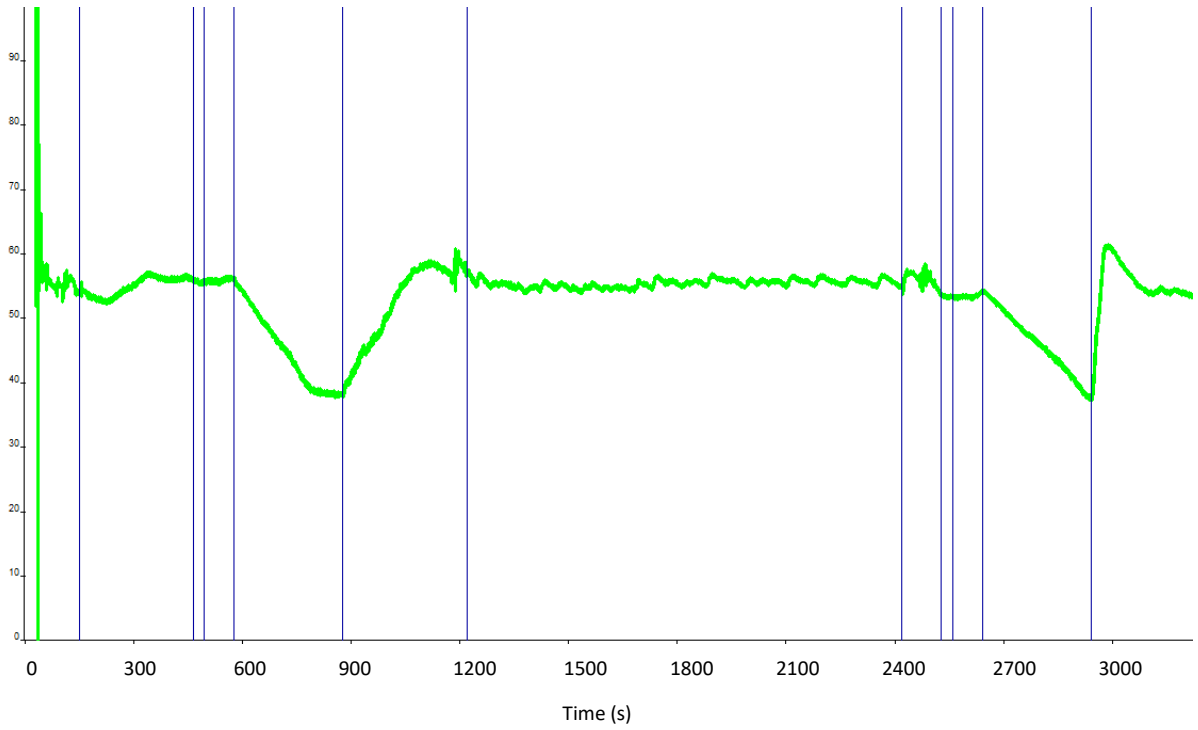
Time (s)

(h)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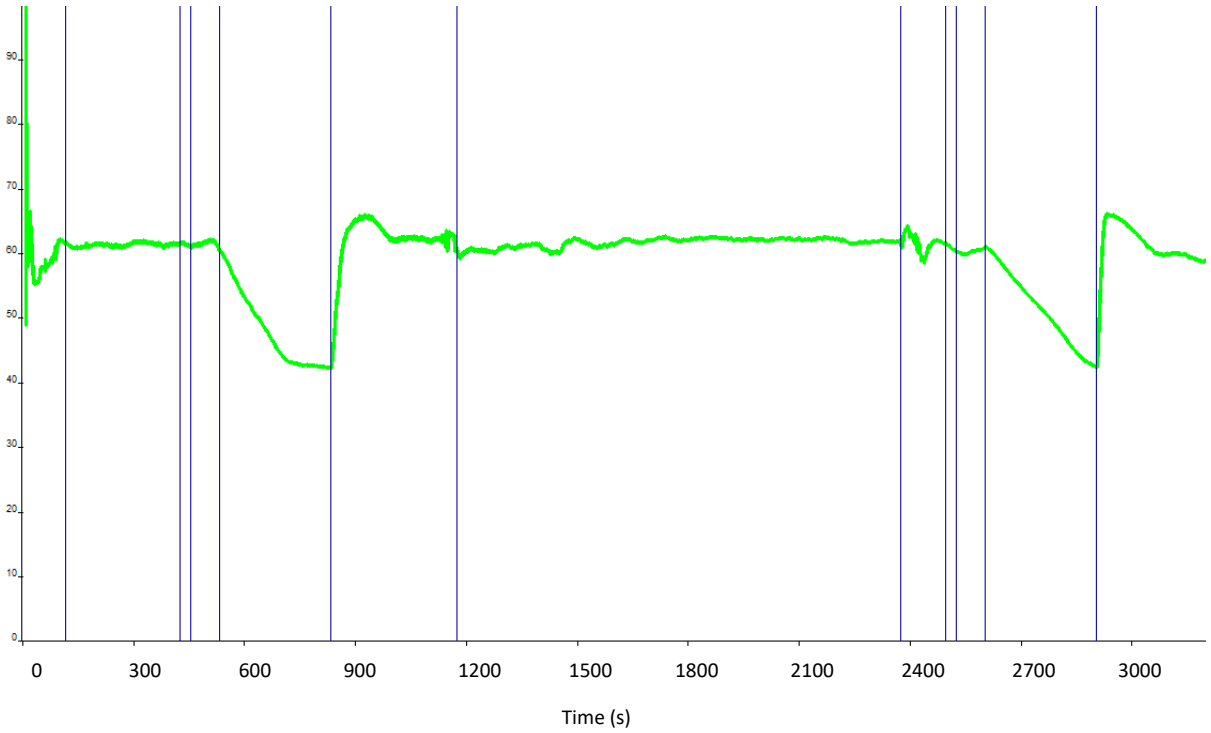
2700
3000
Time (s)

(i)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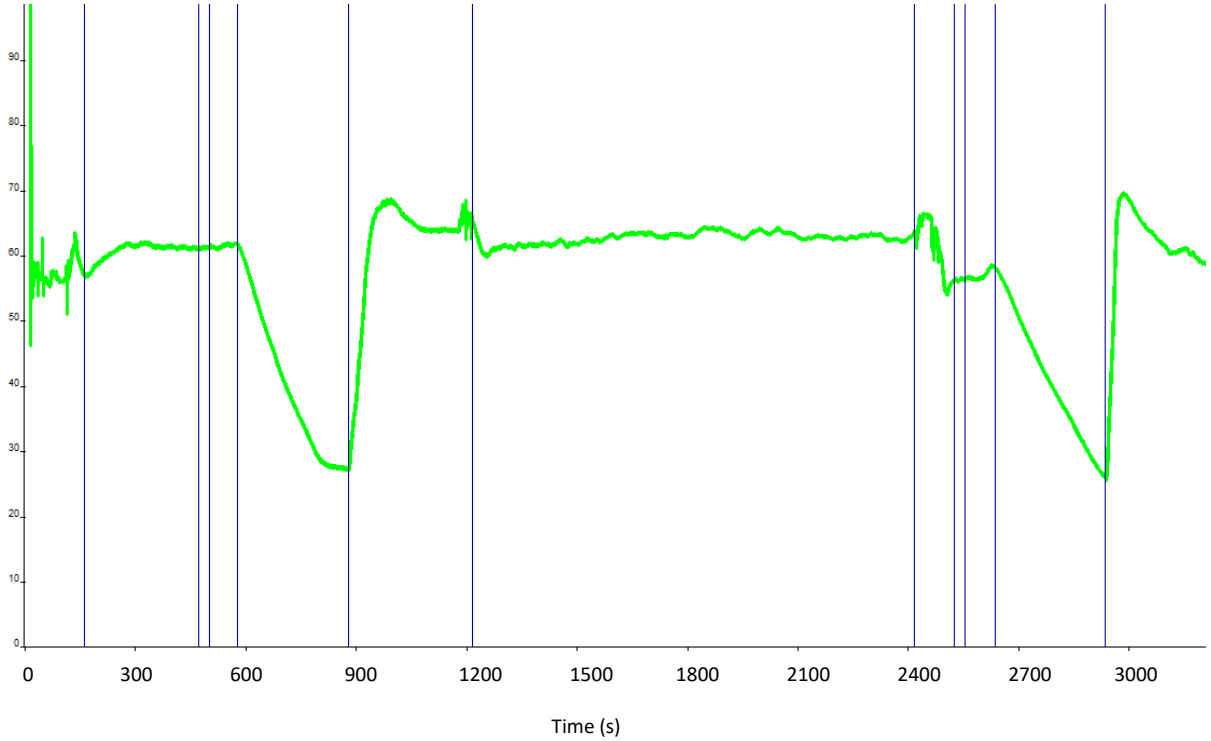
2700
3000
Time (s)

(j)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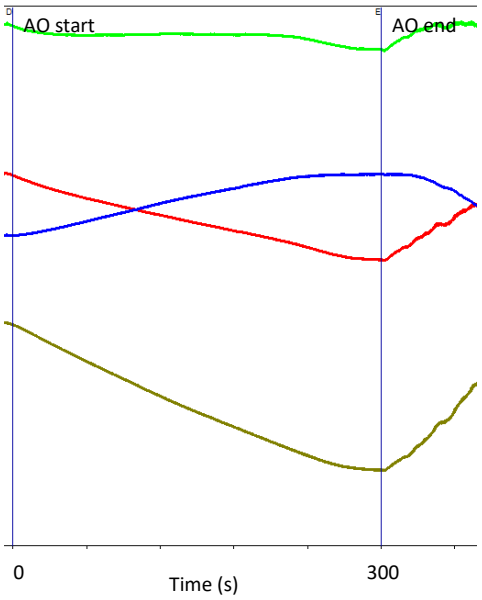
Time (s)

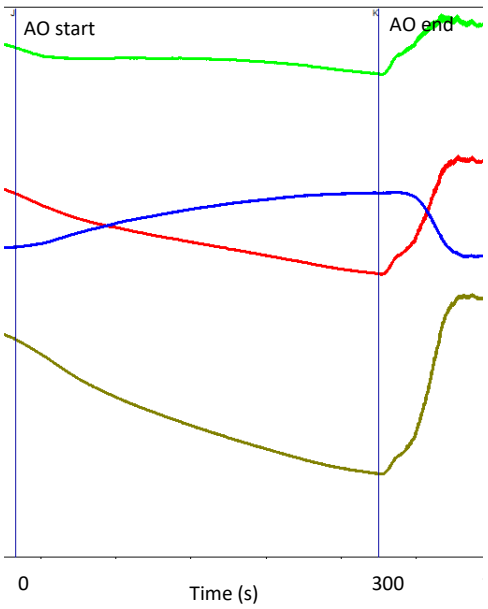
Appendix E: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during arterial occlusion pre- and post-CWI for all participants, (15 °C CWI temperature)

(a)

Pre-CWI

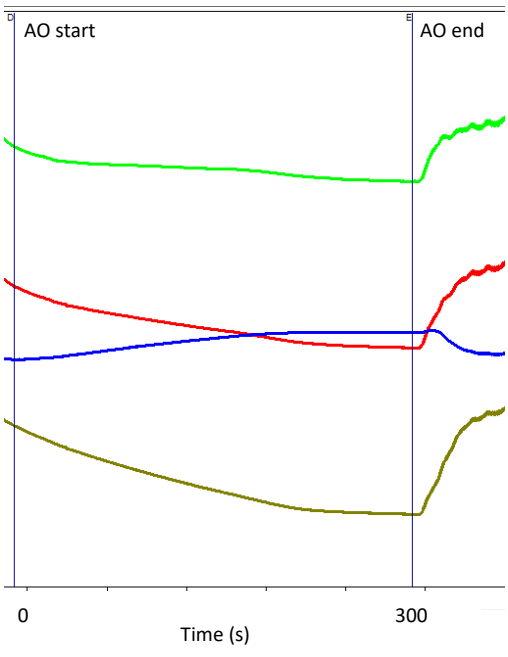


Post-CWI

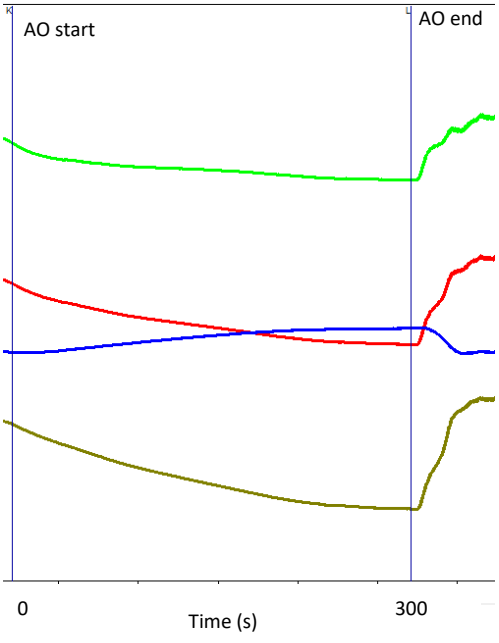


(b)

Pre-CWI

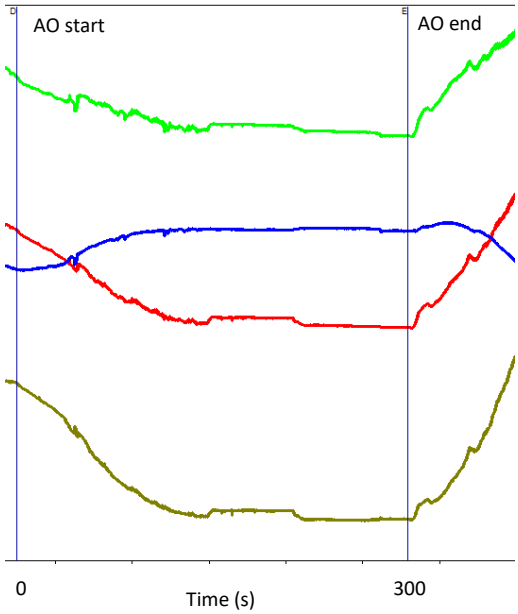


Post-CWI

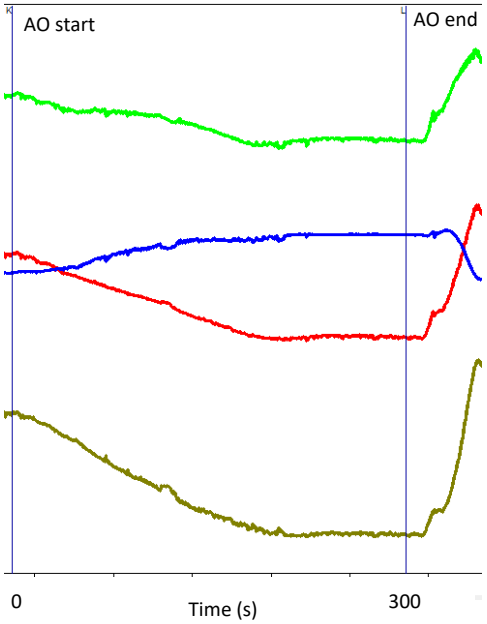


(c)

Pre-CWI

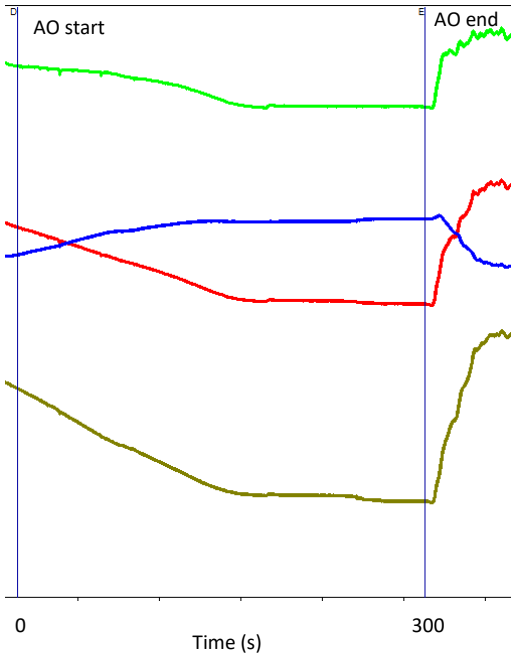


Post-CWI

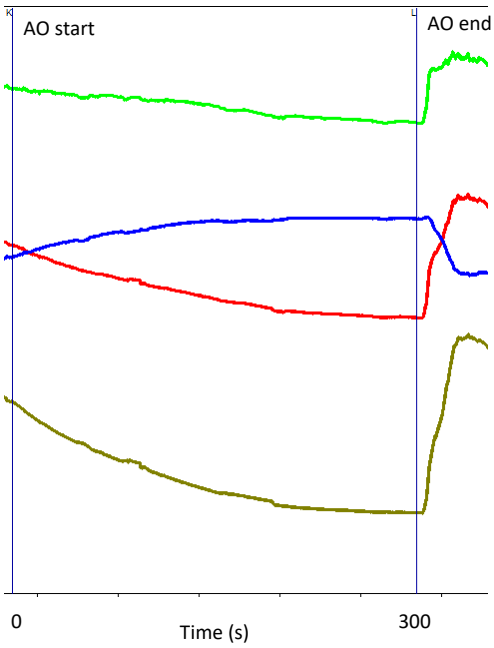


(d)

Pre-CWI

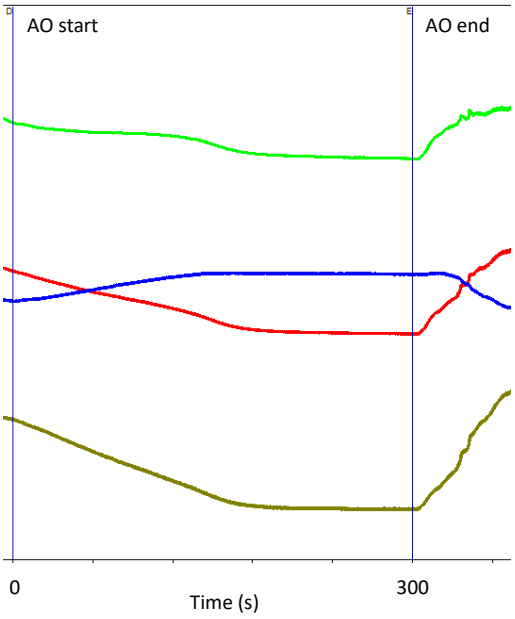


Post-CWI

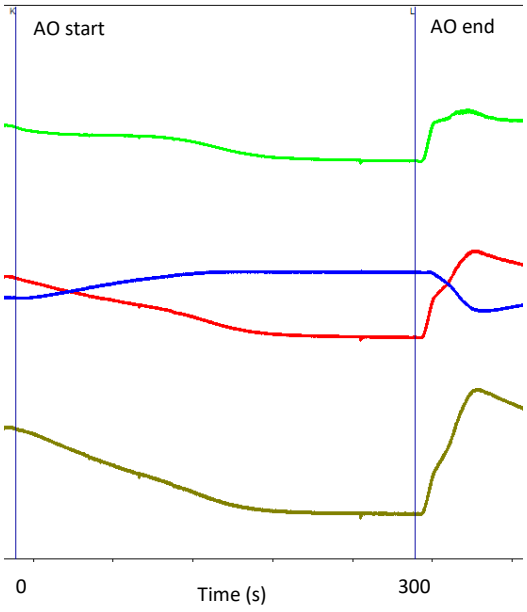


(e)

Pre-CWI

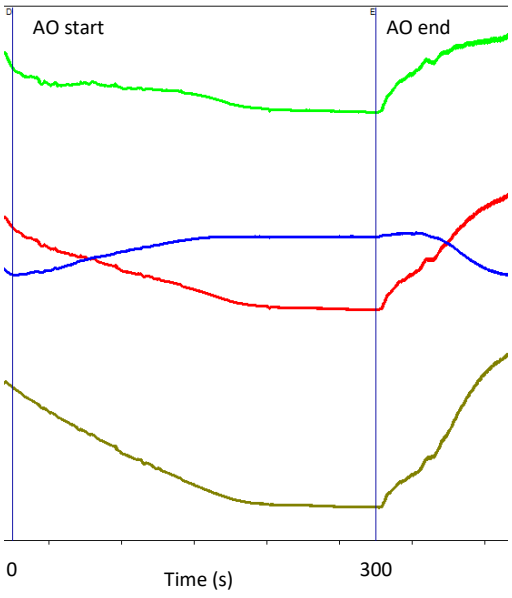


Post-CWI

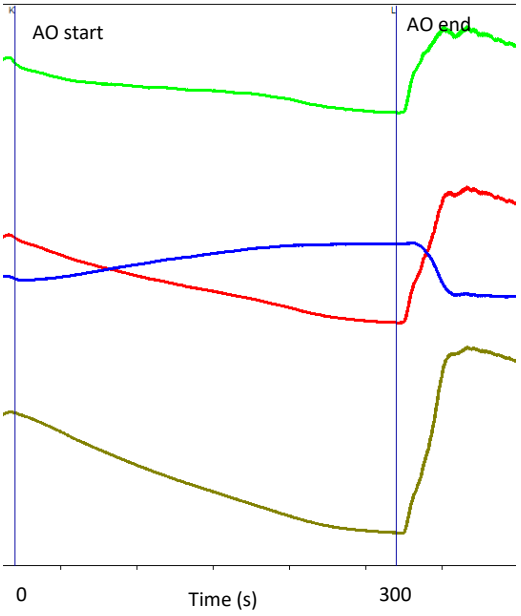


(f)

Pre-CWI

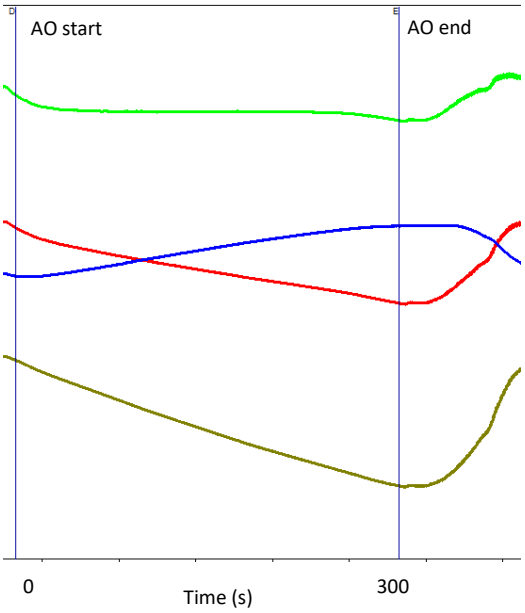


Post-CWI

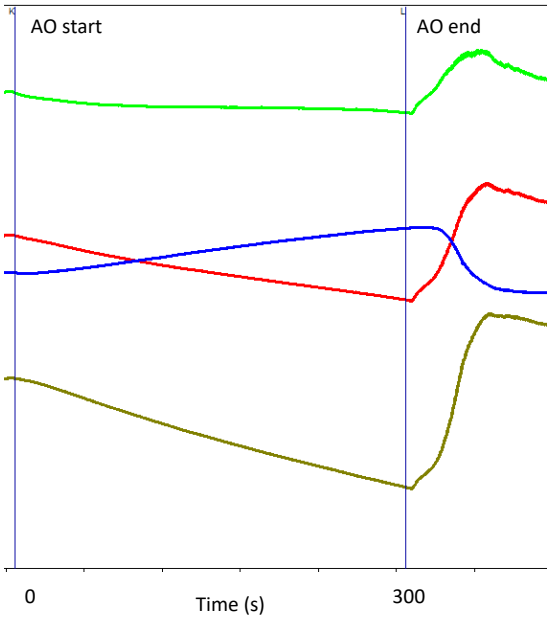


(g)

Pre-CWI

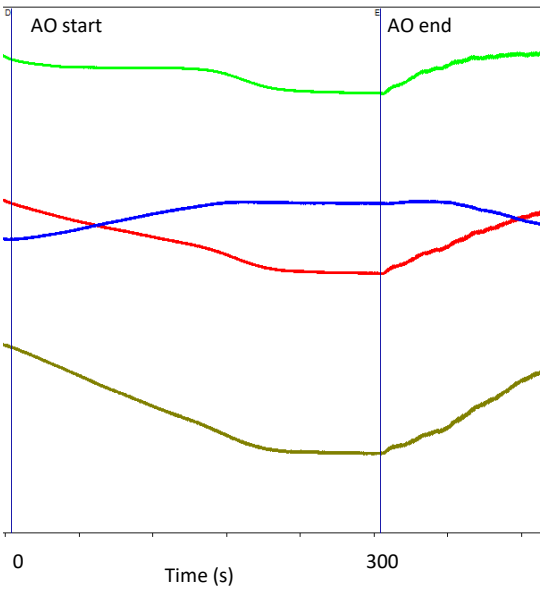


Post-CWI

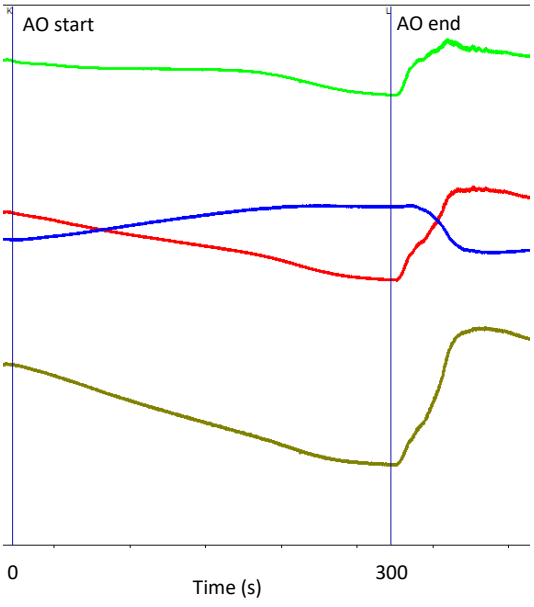


(h)

Pre-CWI

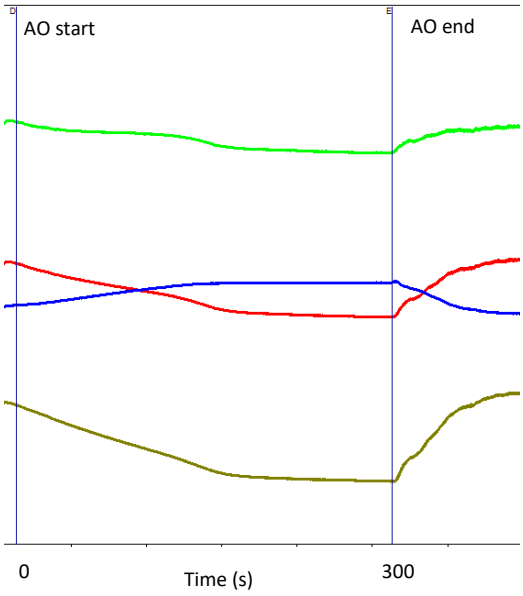


Post-CWI

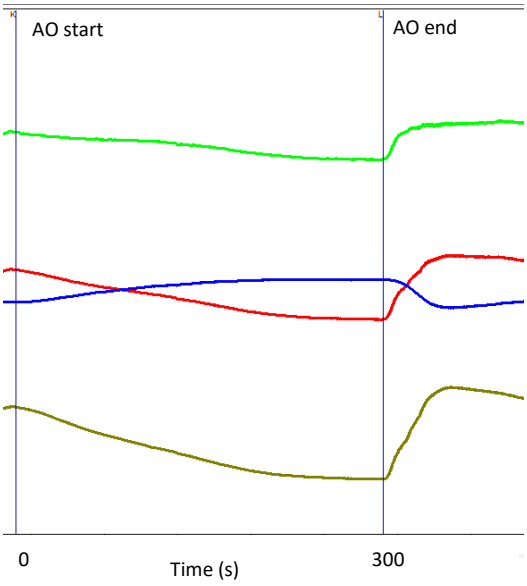


(i)

Pre-CWI

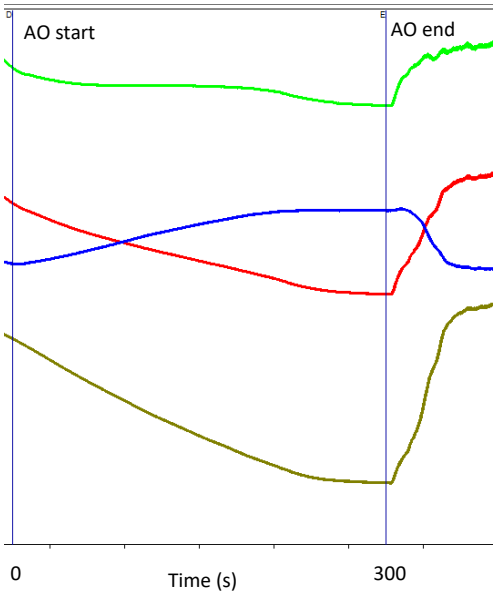


Post-CWI

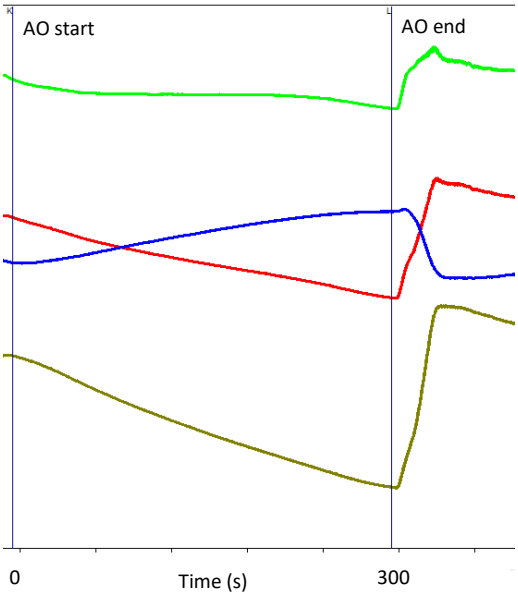


(j)

Pre-CWI



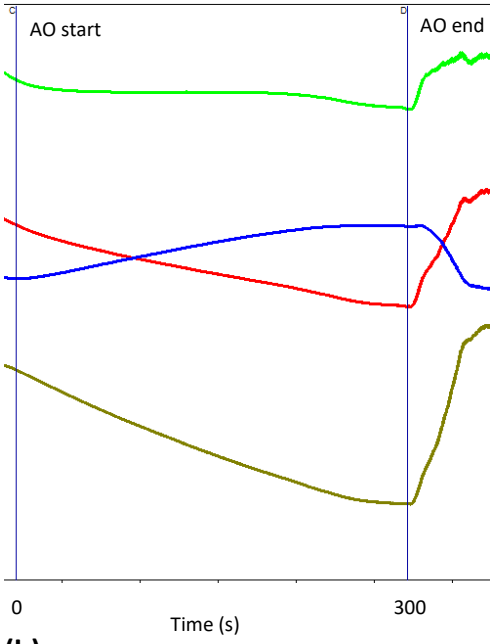
Post-CWI



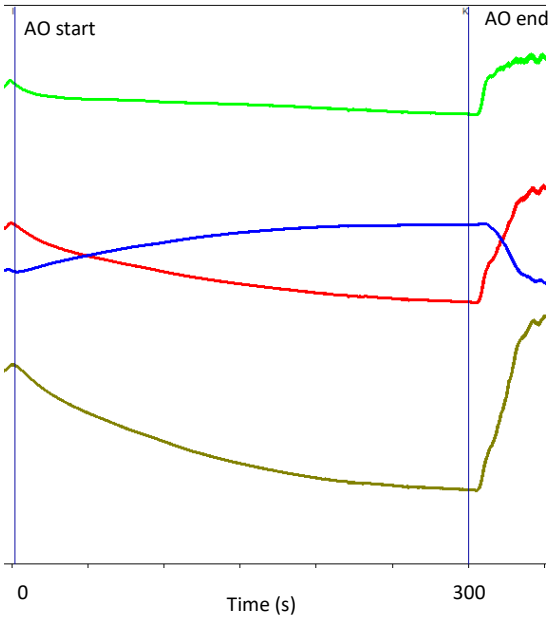
Appendix F: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during arterial occlusion pre- and post-CWI for all participants, (10 °C CWI temperature)

(a)

Pre-CWI

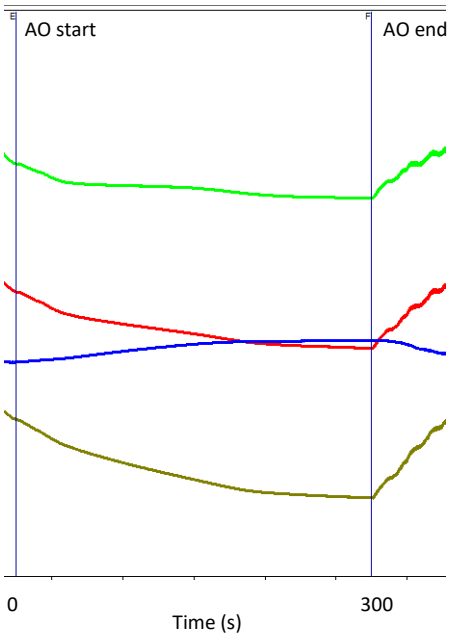


Post-CWI

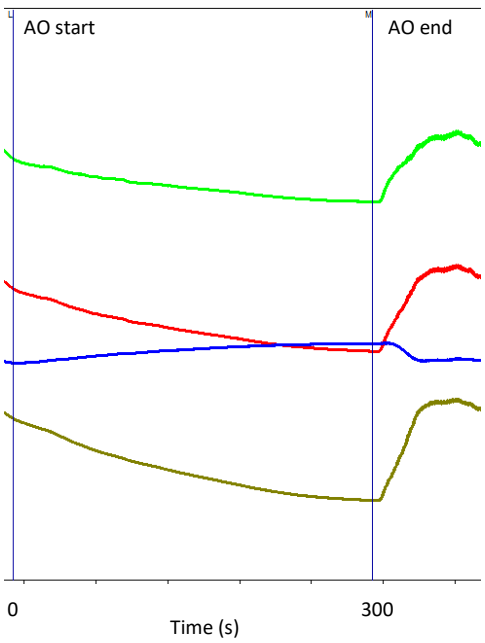


(b)

Pre-CWI

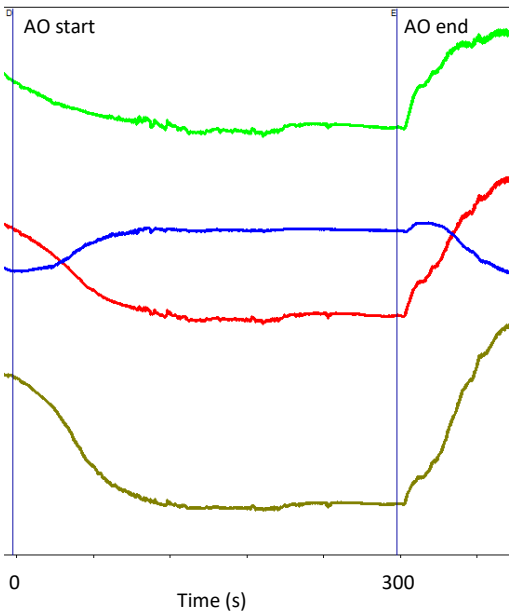


Post-CWI

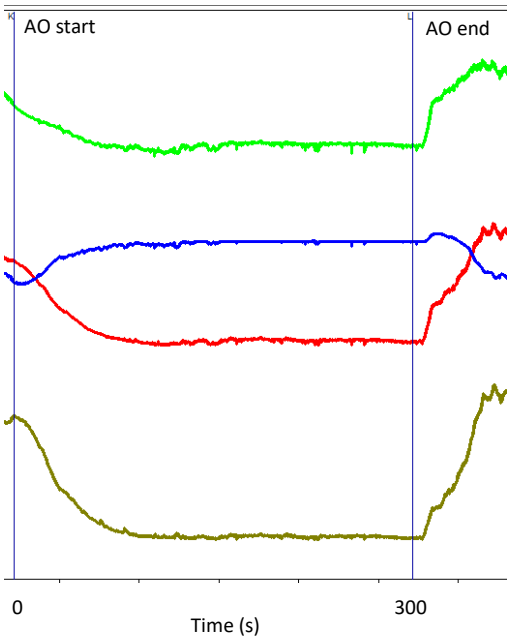


(c)

Pre-CWI



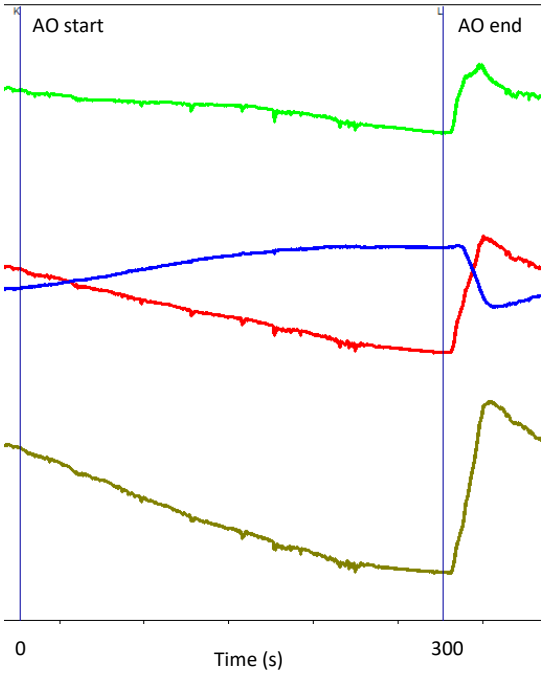
Post-CWI



(d)

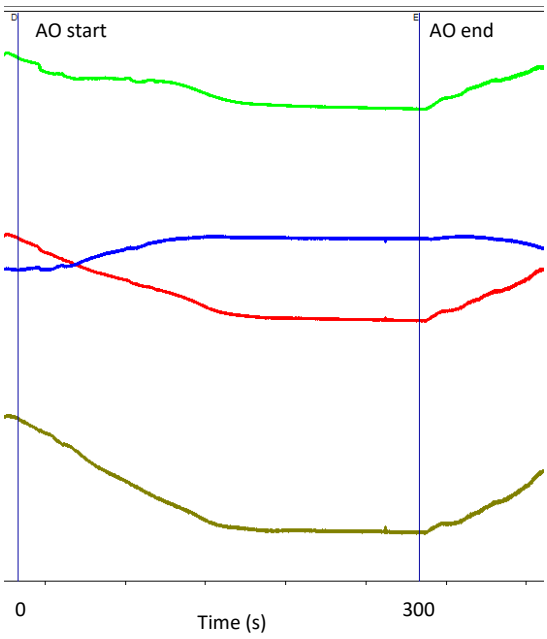
Pre-CWI

Post-CWI

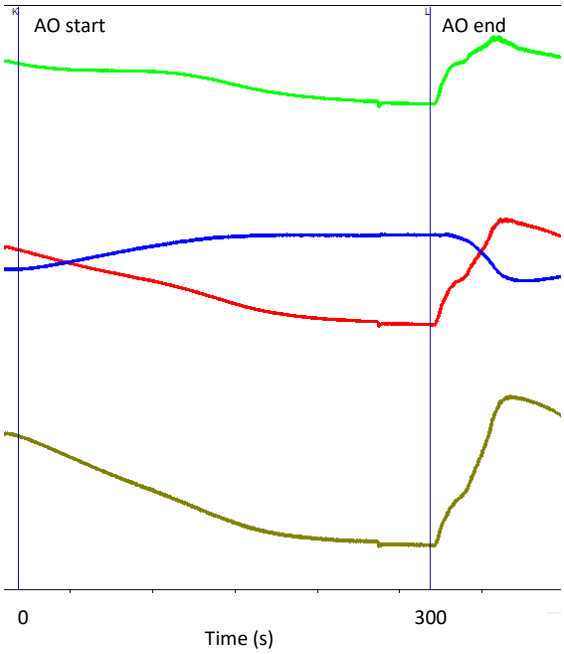


(e)

Pre-CWI

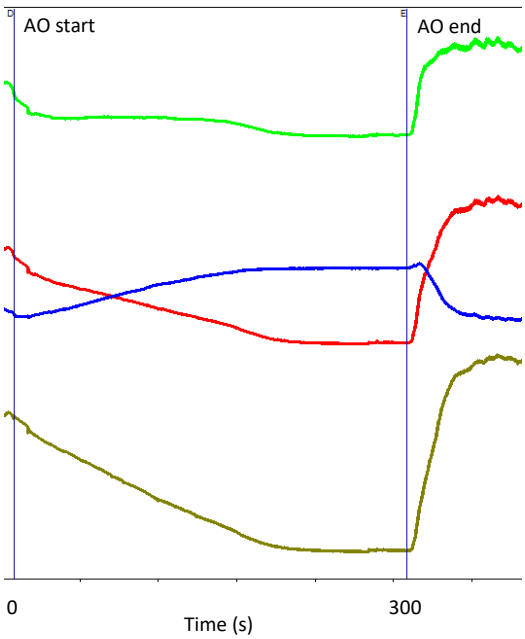


Post-CWI

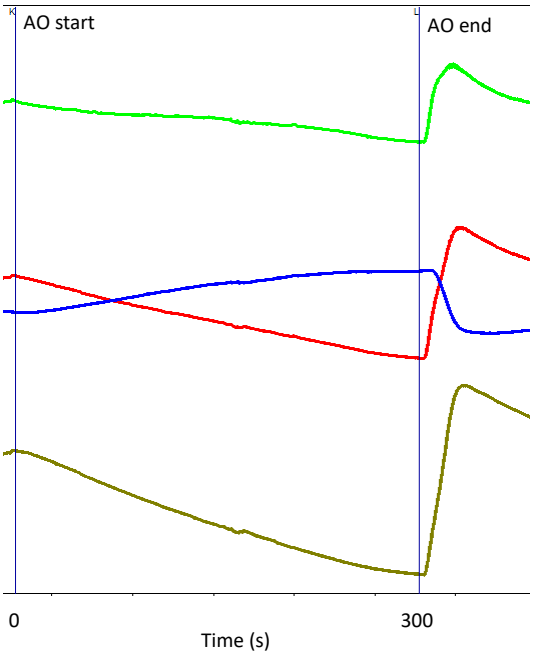


(f)

Pre-CWI

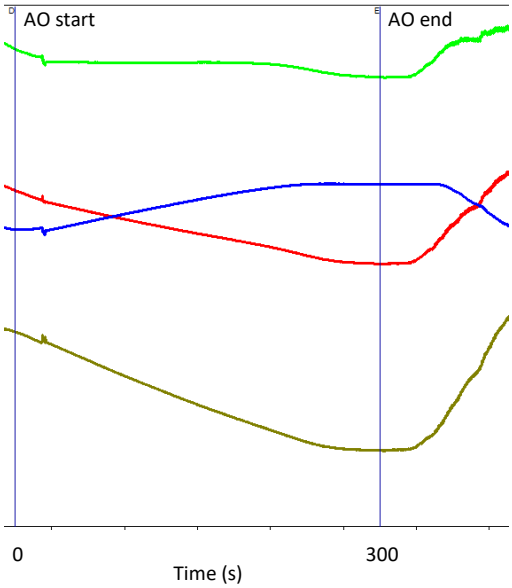


Post-CWI

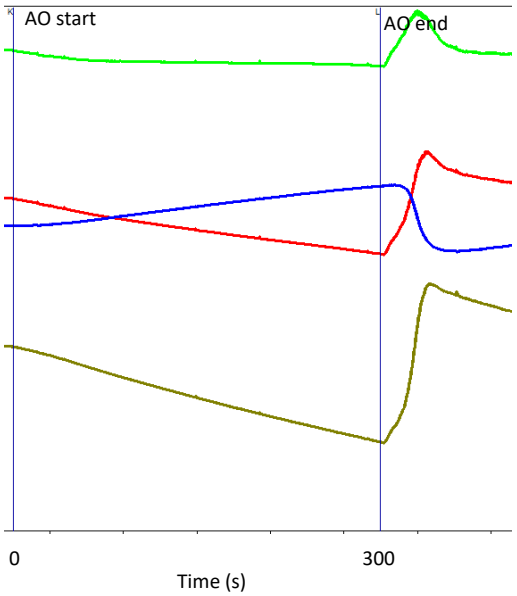


(g)

Pre-CWI

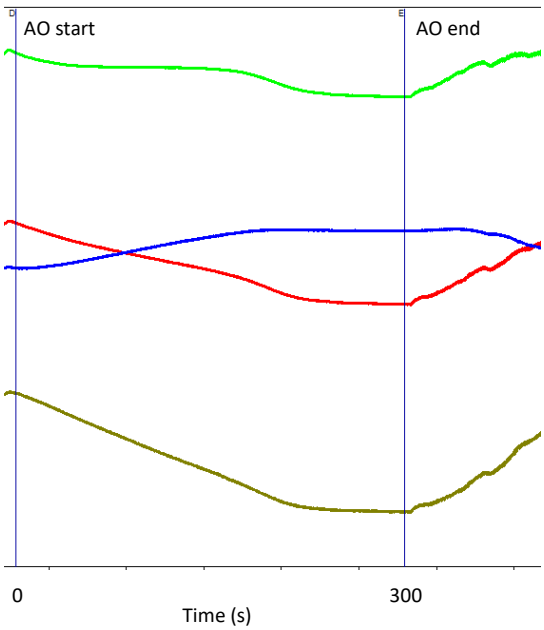


Post-CWI

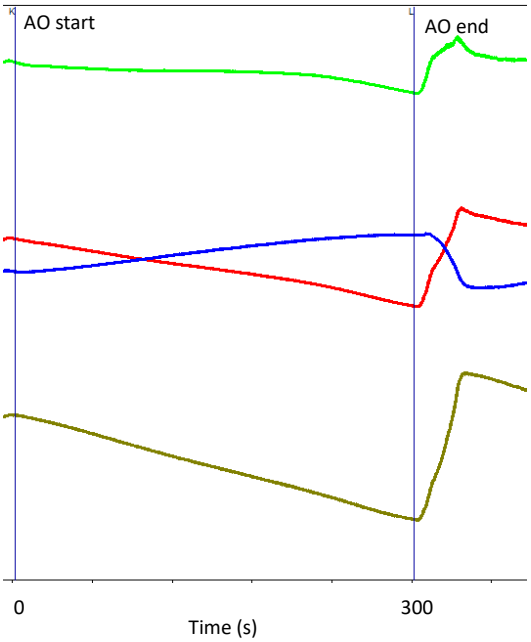


(h)

Pre-CWI

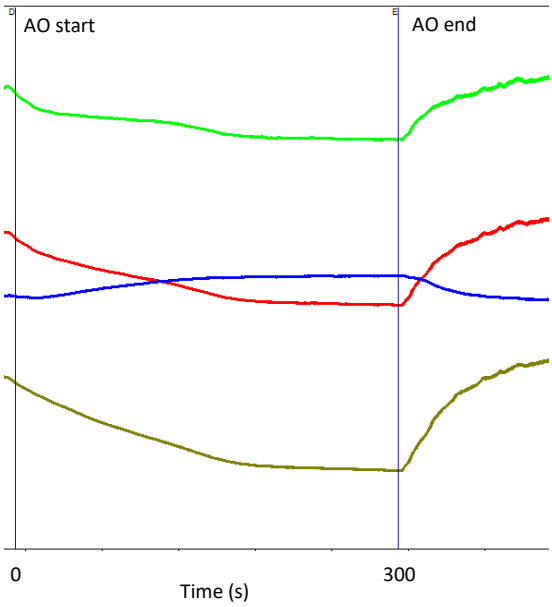


Post-CWI

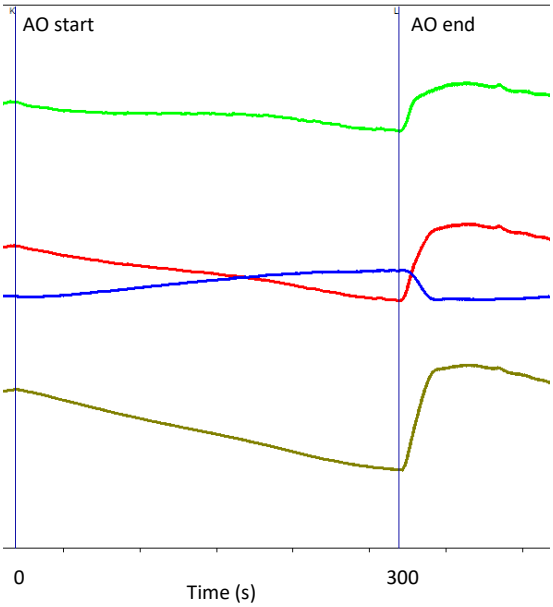


(i)

Pre-CWI

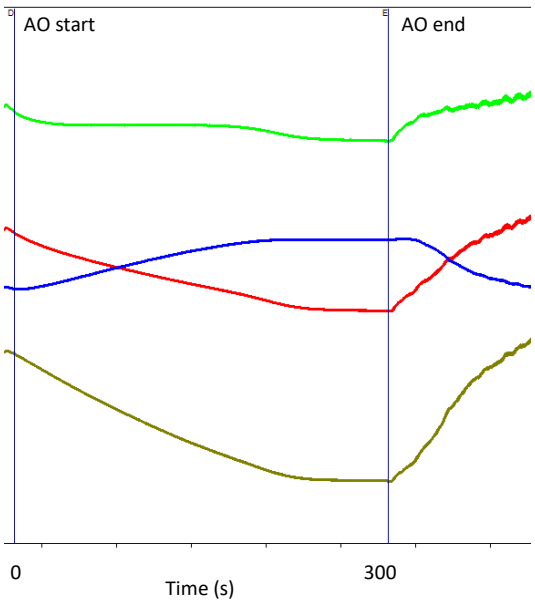


Post-CWI

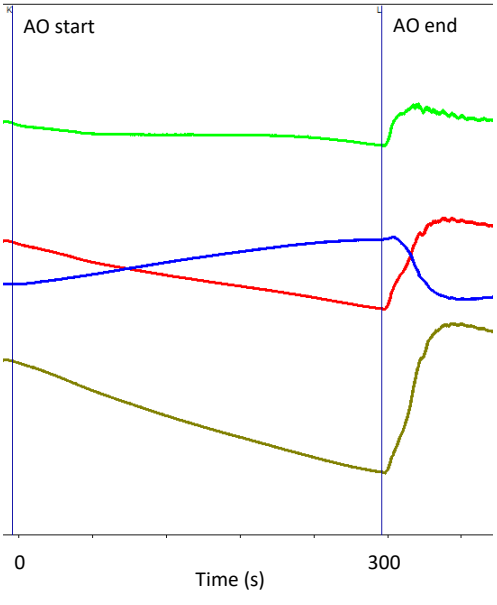


(j)

Pre-CWI



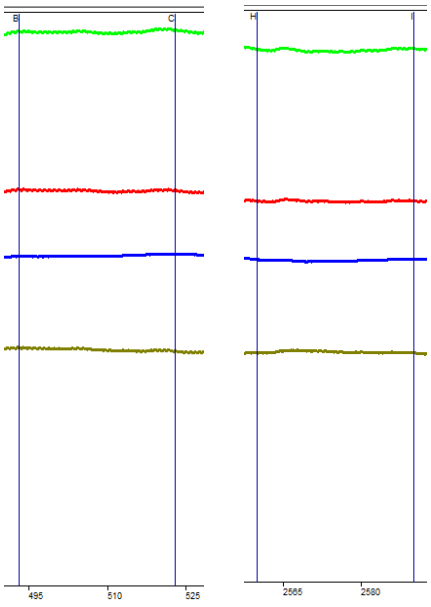
Post-CWI



Appendix G: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during venous occlusion pre- and post-CWI for all participants, (15 °C CWI temperature)

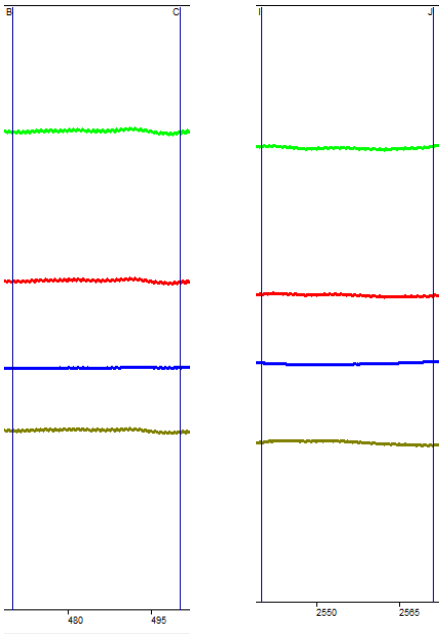
(a)

Pre-CWI **Post-CWI**



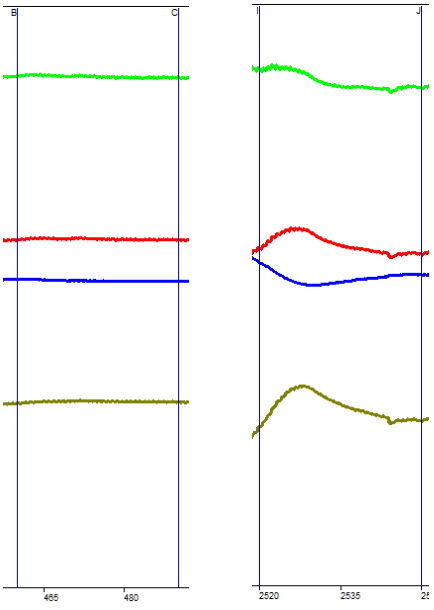
(b)

Pre-CWI **Post-CWI**



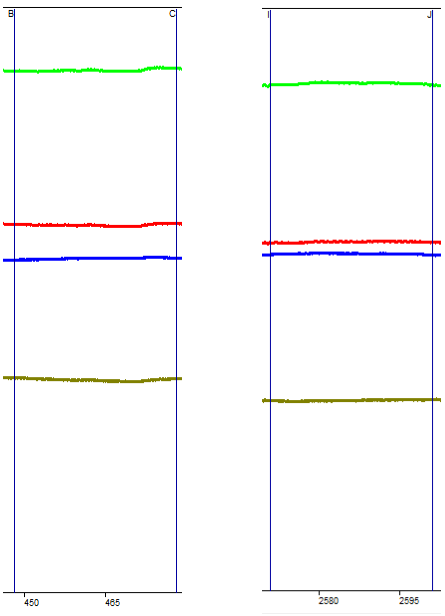
(c)

Pre-CWI **Post-CWI**



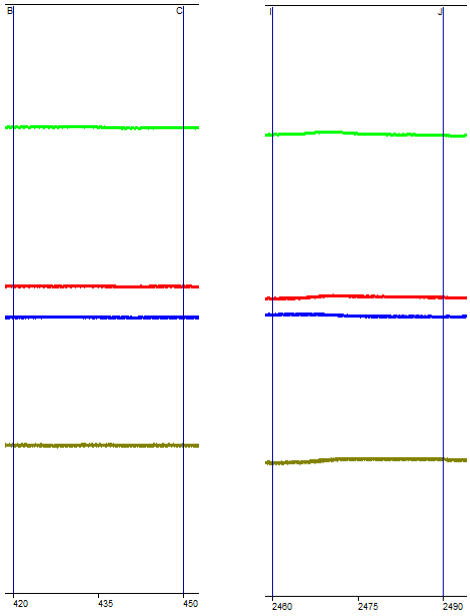
(d)

Pre-CWI **Post-CWI**



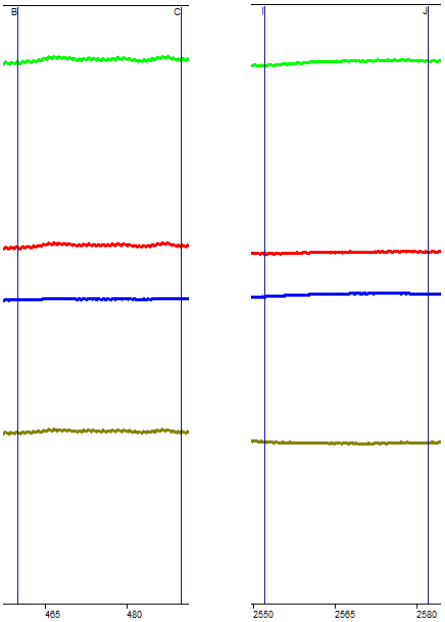
(e)

Pre-CWI **Post-CWI**



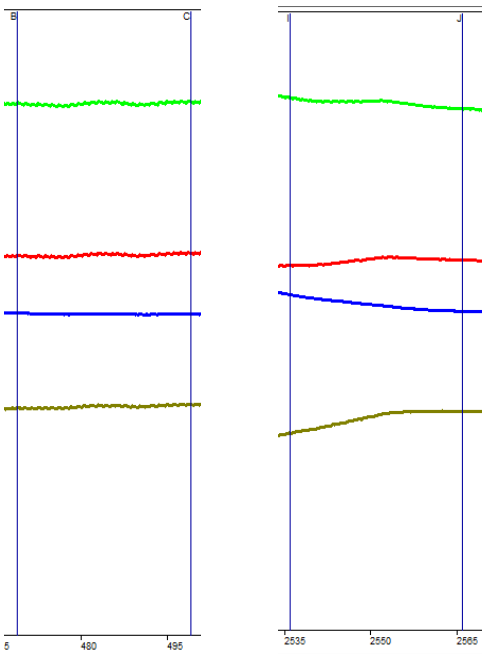
(f)

Pre-CWI **Post-CWI**



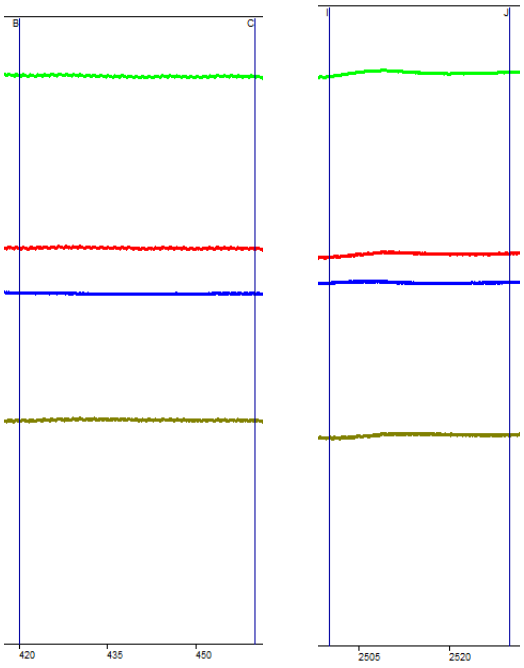
(g)

Pre-CWI **Post-CWI**



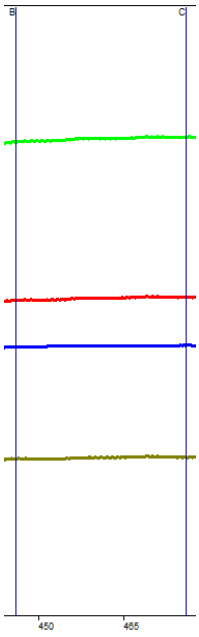
(h)

Pre-CWI **Post-CWI**

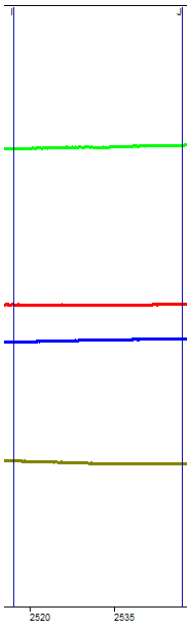


(i)

Pre-CWI

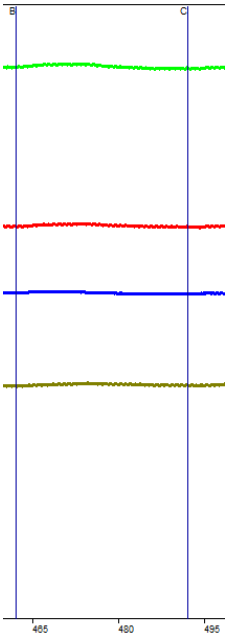


Post-CWI

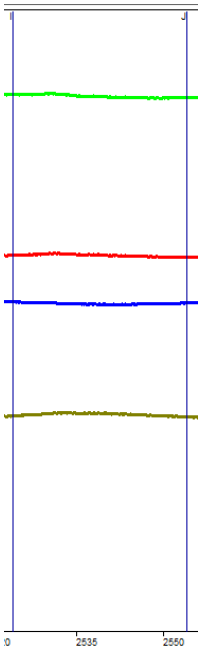


(j)

Pre-CWI



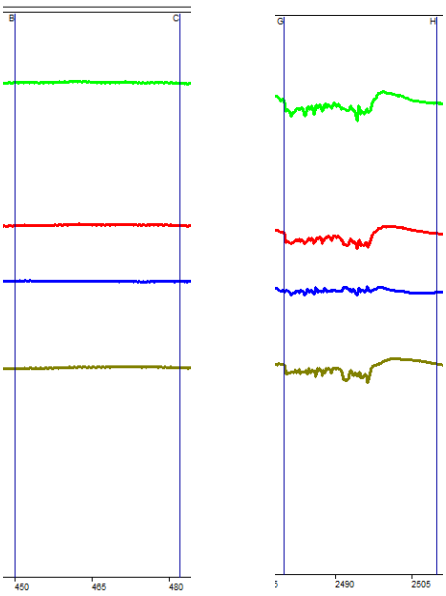
Post-CWI



Appendix H: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during venous occlusion pre- and post-CWI for all participants, (10 °C CWI temperature).

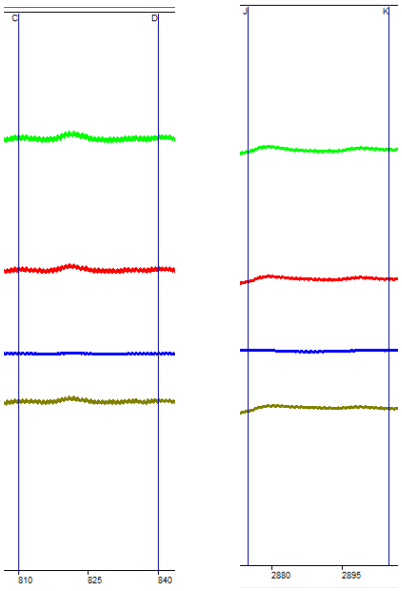
(a)

Pre-CWI **Post-CWI**



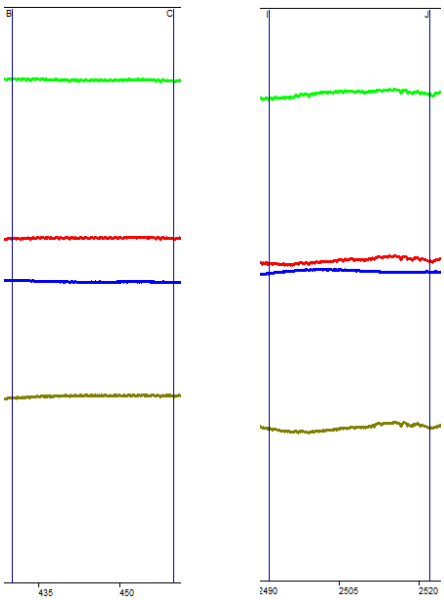
(b)

Pre-CWI **Post-CWI**



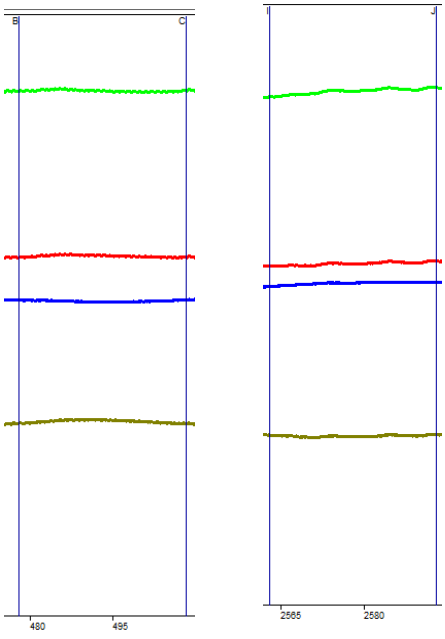
(c)

Pre-CWI **Post-CWI**



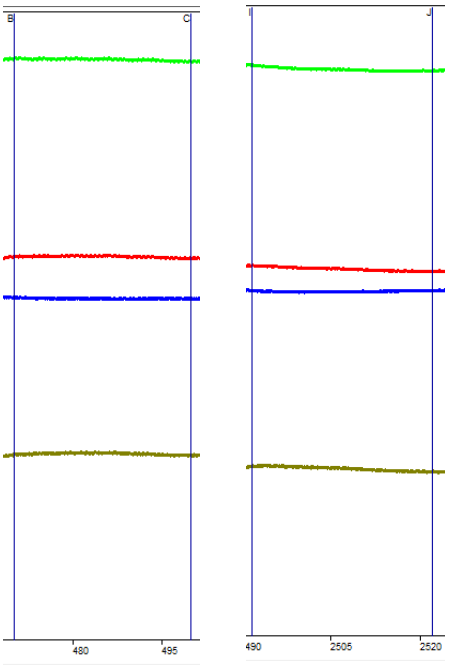
(d)

Pre-CWI **Post-CWI**



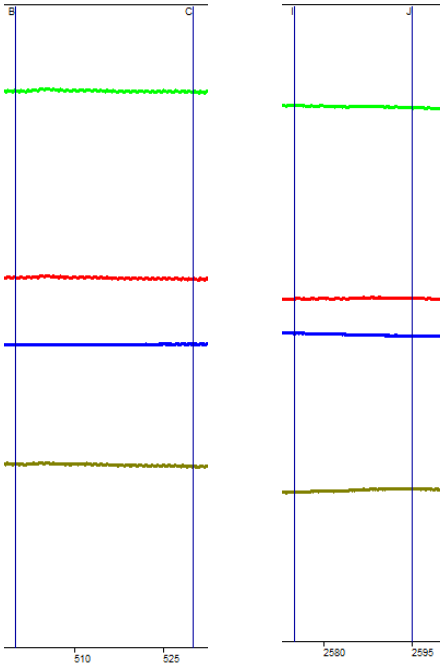
(e)

Pre-CWI **Post-CWI**



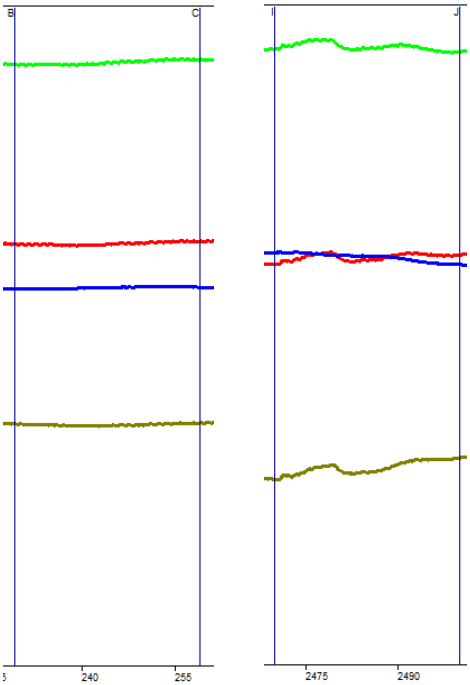
(f)

Pre-CWI **Post-CWI**



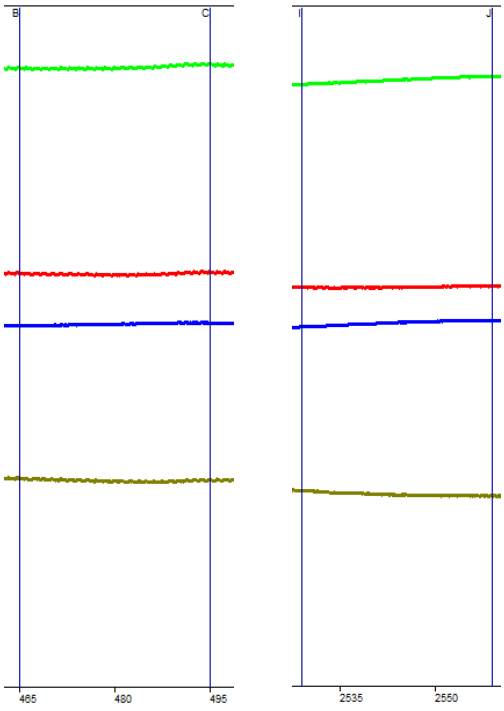
(g)

Pre-CWI **Post-CWI**



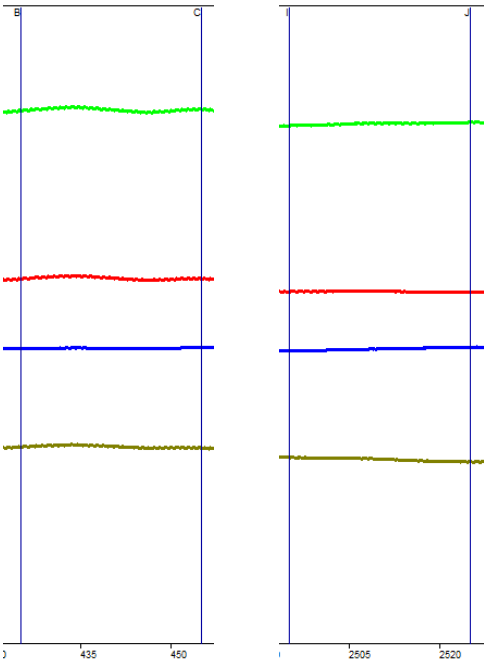
(h)

Pre-CWI **Post-CWI**



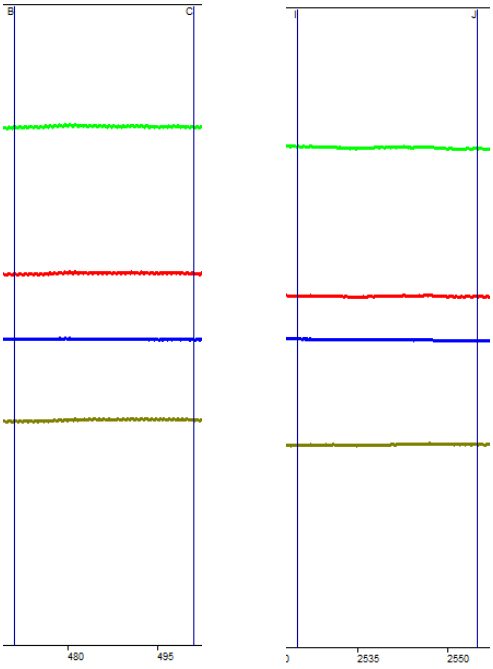
(i)

Pre-CWI **Post-CWI**



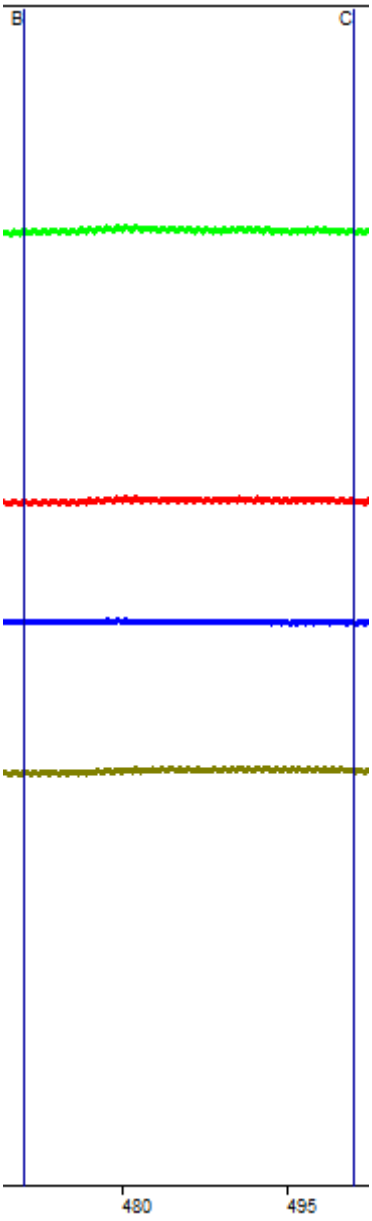
(j)

Pre-CWI **Post-CWI**



Appendix I: NIRS traces: tHb (green), O_2Hb (red), HHb (blue), Hb_{diff} (brown) during venous occlusion from a representative participant vs. expected trace.

Representative participant



Vs.

Expected NIRS trace (from Van Beekvelt (155))