Swansea University
Prifysgol Abertawe

School of Sports and Exercise Science
Faculty of Science and Engineering

The effect of post-exercise, lower-limb passive heating on endurance performance, muscle and vascular function

Kevin John

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Abstract

Introduction: Chronic application of passive heating has been shown to enhance factors associated with aerobic metabolism. Likewise, post-exercise passive heating provides an attractive strategy to prolong the exercising stimulus and has shown to improve endurance performance in temperate conditions. It is typical to use hot water immersion, sauna baths, hot-water perfused suits or diathermy to provide the necessary thermal stimulus, thus confining the studies to laboratory settings and may prevent their real-world application. In light of the recent developments in personal heating garments, the current study aimed to develop a mobile, home-based lower-body passive heating system, which was applied in a post-exercise format. The primary aim of the study was to investigate the effect of four-weeks of post-exercise passive heating on markers of endurance performance in recreationally trained adults compared to routine endurance training. **Methods:** The study included 30 recreationally trained individuals, who were randomly allocated to post-exercise passive heating (PH, n = 16) or control group (CON, n = 14). The PH group wore the passive heating system immediately following training for 90-120 min/day, completing a total of 20 heating sessions across the four-weeks. The CON group continued with their normal training without passive heating. To characterise the endurance phenotype, hallmark endurance markers were assessed: maximal oxygen uptake, gas exchange threshold, pulmonary oxygen uptake kinetics and critical torque, with simultaneous near infrared spectroscopy, vascular assessment of brachial artery flow mediated dilation and venous blood sample collection for analysis of heat shock proteins, nitric oxide and vascular endothelial growth factors. All of the above-mentioned characteristics were assessed at three testing blocks; PRE, MID & POST, each separated by two-weeks. Changes between groups from PRE to POST were analysed using analysis of co-variance. **Results:** Resting brachial artery diameter (p = 0.002) and maximal vasodilatory diameter (p < 0.001) was significantly higher in PH compared to CON after four-weeks. There was no significant difference between or within group for markers of endurance performance. Finally, a significant slowing of phase II time constant (p = 0.02) was found in the PH group, whilst the CON group improved their critical torque–deoxygenation (p = 0.03) ratio relative to the PH group. **Conclusion:** The four-week, post-exercise passive heating approach used in the current study did not improve endurance performance. Despite some improvements in the components of systemic resting blood delivery, the nature of the intervention appeared to result in suppression of beneficial adaptations that would otherwise be anticipated with routine endurance training. This study signifies the need for better understanding the dose-response relationship when utilising dual adaptative stimuli (exercise + thermal) for the purpose of enhancing endurance performance among healthy, active individuals. Transference of fundamental training principles to the design of thermal interventions, such as consideration of thermal load and recovery, may help to individualise future approaches, and thereby increase the probability of beneficial heat-mediated adaptations.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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List of abbreviations

5-min AOCT Five-minute all out critical torque test

A Amplitude

A-VO_2 Arteriovenous oxygen difference

ADP Adenosine diphosphate

Akt Protein kinase B

AMP Adenosine monophosphate

AMPK AMP-activated protein kinase

ANCOVA Analysis of co-variance

ATP Adenosine triphosphate

ATPase Adenosine triphosphatase

BLa Blood lactate accumulation

BV Blood volume

Ca^{2+} Calcium ion

CK Creatine kinase

CNS Central nervous system

CO_2 Carbon-dioxide

CON Control group

CP Critical power

$CR_{T/HHb}$ Critical torque/ Deoxygenated haemoglobin ratio

$CR_{CT/TSI}$ Critical torque/Tissue saturation index ratio

CS Citrate synthase

CT Crtitical Torque

D arterial diameter

DO_2 Diffusing capacity for oxygen Deoxygenated haemoglobin

$D_{BASELINE}$ baseline arterial diameter

D_{MAX} maximal arterial diameter

EDV End diastolic volume

END endurance training

eNOS Endothelial nitric oxide synthase

ETC Electron transport chain

$FADH_2$ Flavin adenine dinucleotide

FMD Flow mediated dilation

GE Gross efficiency

GET Gas exchange threshold

h Hour

H^+ hydrogen ion

H_2O_2 hydrogen peroxide

HA Heat acclimation programme

Hb Haemoglobin

HHb Deoxygenated haemoglobin

HIF-1a Hypoxia-inducible factor 1-alpha

HIT High intensity interval training

HSP Heat shock proteins

HWI Hot water immersion

I Total impulse

I' Impulse prime

IL-6 Interleukin 6

JNK c-Jun N-terminal kinases

K^+ Potassium ion

kg Kilograms

kJ kilo Joule

Km Kilometres

L Litres

La^- Acidic lactate concentration

MAP mean arterial pressure

MB Myoglobin

min Minutes

mL Millilitres

mm Millimetres

MPAK Mitogen-activated protein kinase

mRNA messenger Ribonucleic acid

mTOR mammalian target of rapamycin

MVC Maximal voluntary contraction

NADH Nicotinamide adenine dinucleotide

NIRS Near-infrared spectroscopy

NO Nitric oxide

NO_2^- Nitrite

NO_3^- Nitrate

O_2 Oxygen

O_2^- Superoxide

O_2Hb Oxygenated haemoglobin

OXPHOS oxidative phosphorylation

P power output

PCr Phosphocreatine

PGC1-a Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

pH Potential of hydrogen

PH post exercise PH

Pi Inorganic phosphate

PO_2 Oxygen partial pressure

PO_2mv partial pressure of O2 within the vasculature

PO_{GET} Power at gas exchange threshold

PO_{peak} Peak power output

PV Plasma Volume

$\dot{Q}$ Cardiac output

RBC Red blood cell

RER Respiratory exchange ratio

ROS Reactive oxygen species

RPE Rate of perceived exertion

$R_{T/HHb}$ Torque/ Deoxygenated haemoglobin ratio

$R_{T/TSI}$ Torque/Tissue saturation index ratio

s Second

SD Standard deviation

SERCA Sarcoplasmic reticulum calcium -ATPase pump

SR sheer rate

SR_{AUC} Sheer rate area under the curve

SV Stroke volume

t Duration

τ (tau) Time constant

TCA Tricarboxylic cycle

TD Time delay

TL Training load

Tlim time to exhaustion

TSI Tissue saturation index

T_{sk} Skin temperature

UCP3 Uncoupling protein 3

V mean velocity

$\dot{V}CO_2$ Carbon dioxide output

V_E Ventilatory equivalent

VEGF Vascular endothelial growth factor

VL Vasitus Lateralis

$\dot{V}O_2$ Oxygen uptake

$\dot{V}O_2$ kinetics Oxygen uptake kinetics

$\dot{V}O_{2max}$ Maximal oxygen uptake

$\dot{V}O_{2p}$ Pulmonary phase II oxygen kinetics

$\dot{V}O_{2peak}$ Peak oxygen uptake

W Watts

W' Work prime

Chapter 1.0 Introduction

Endurance performance is dependent on the complex interaction between the cardiorespiratory and muscular system to sustain skeletal muscle contractions over prolonged durations (Joyner & Coyle, 2008). Here, the transport of oxygen to the working muscles, combined with its cellular metabolism, determines the duration and intensity that activities, such as running, cycling, swimming, can be performed. These processes underpin the following physiological determinants of endurance performance: maximal oxygen uptake ($\dot{V}O_{2max}$), gas exchange threshold (GET), pulmonary oxygen uptake kinetics ($\dot{V}O_2$ kinetics), exercise economy or gross efficiency (GE) and critical power (Bassett & Howley, 2000; Poole et al., 2021). Tests of these determinants, and knowledge of their underpinning mechanisms, are useful to those participating in endurance exercise, as they provide deeper insight into the factors limiting performance or adaptation to training interventions. For example, it is well established that routine endurance training can improve aerobic capacity, muscle function and overall health status by enhancing various central (SV, $\dot{Q}$, BV) and peripheral (muscle capillarity, mitochondrial density and oxidative enzyme activity) physiological factors (Hawley, 2002; Jones & Carter, 2000; Kumboyono et al., 2022; Midgley, McNaughton, & Jones, 2007a; Rivera-Brown & Frontera, 2012). However, whether it is athletes or recreationally active populations, there are many potential barriers that can limit the time available for exercise (Kirk & Rhodes, 2011; Lambert et al., 2022). Therefore, passive interventions with the potential to augment the physiological response to endurance exercise programmes in a time efficient manner, have developed wider interest (Hyldahl & Peake, 2020; Patterson et al., 2019; Veldman et al., 2016) .

Recently, topical application of heat applied to the skin surface, commonly known as passive heating, has gained attention based on the reported physiological adaptations, which might have relevance for both health and performance (Cullen et al., 2020). For example, Brunt et al. (2016) were the first to show that a passive thermal stimulus applied over eight-weeks (four to five hot water immersions per week at 40.5 °C, with each session lasting ~ 90 min) induced systemic vascular adaptations in sedentary adults, including, improved endothelial functioning (via flow mediated dilation; FMD) and arterial conductance. These changes might be attributed to an increased expression of endothelial nitric oxide synthase (eNOS) (Hesketh et al., 2019; Kim et al., 2020), which mainly synthesises the key vasodilatory molecule nitric oxide (NO), in response to sheer stress and also aid in the release of endothelial growth factors (VEGF) that

facilitate vascular growth (Gustafsson, 2011; Wragg et al., 2014). As reported in acute response to exercise, passive heating can acutely increase blood flow and vascular sheer stress (Chiesa et al., 2016), which indicates a commonality in the potential angiogenic stimuli. Interestingly, functional enhancements of large blood vessels via passive heating were also replicated downstream at the micro-circulatory level i.e., capillaries (Brunt et al., 2016). The improvements in macro- and micro-vasculature noted above were later attributed to increased bioavailability of NO in the serum of individuals who underwent a similar heating intervention (Brunt et al., 2019). Expansion of the vascular network with passive heating is important for the endurance phenotype, as this would indicate the possibility of improved muscle perfusion and oxygen availability, which are mechanistically linked to key endurance performance determinants, such as $\dot{V}O_{2max}$ (Wagner, 2015). Indeed, there is subsequent evidence that six-weeks of whole-body passive heating (50 min resting in heat chamber [40 °C and 40% humidity], three sessions per week) increased capillary density of the vastus lateralis by 21 %, which occurred alongside 8 % increases in eNOS expression (Hesketh et al., 2019). Thus, passive heating appears to elicit adaptation of the vasculature, which might also translate to other peripheral tissues

Owing to the pro-angiogenic stimulus provided by passive heating, it is feasible that adaptations within the skeletal myocyte are also promoted. In this regard, using a cell cultured mice myoblast model, Liu and Brooks (2012) reported that passive thermal stress applied over five days upregulated activity of AMP activated protein kinase (AMPK) and peroxisome proliferator-activated receptor γ-coactivator 1α (PGC1α). Both AMPK and PGC1α are important transcription factors that regulate mitochondrial biogenesis within the muscle tissue (Hood, 2009). Recently, Hafen et al. (2018) showed similar increases in expression of AMPK and PGC1α in human skeletal muscle biopsies following six consecutive days of passive heating. Upregulation of these specific factors improved mitochondrial respiratory capacity within individual muscle fibres (Hafen et al., 2018). Here, proliferation of mitochondria within the myocyte could potentially increase the proportion of energy (ATP) generated from oxidative processes. Additionally, an increase in either subsarcolemmal or intermyofibrillar mitochondria would further enhance oxidative phosphorylation potential, whilst reducing the diffusion distance of O_2 after it exits the capillaries (Clanton, 2018; Poole, Musch, et al., 2022). This would permit greater O_2 extraction, which is commonly expressed among endurance trained individuals (Degens et al., 2019; Skattebo et al., 2020).

Another important by-product of thermal stress is the heat shock response, whereby heat shock proteins (HSPs) act as molecular chaperones that facilitate protein synthesis, maintain muscle function and regulate cellular energy metabolism (Liu et al., 2006; Morimoto, 1998). Passive heating was reported to reduce muscle atrophy and prevent loss of oxidative potential due to limb immobilisation (Hafen et al., 2019). Heat-mediated protection of muscle structure against disuse, was mainly attributed to elevations in HSP70 and HSP90 following the heating intervention (Hafen et al., 2019). These changes could partly explain the improvements in torque production during maximal voluntary contractions (MVC) of the lower-limbs reported after 11-weeks of passive heating (Racinais et al., 2017). Indeed, a feature of longer passive heating protocols (10-weeks) appears to be improved muscle function, with improved isometric knee strength and increased cross-sectional area of the quadriceps demonstrated in sedentary adults (Goto et al., 2011). Whether similar HSP-dependent pathways are associated with a range of performance tests in humans requires further investigation.

The above findings collectively demonstrate that the delivery and metabolism of O_2 can be enhanced with passive heating, which are hallmark phenotypic expressions of endurance-trained individuals (Lundby & Robach, 2015). This could explain why chronic passive heating alone improved cardio-respiratory fitness to a similar extent as time-matched moderate-intensity exercise (Bailey et al., 2016b; Hesketh et al., 2019; Wagenmakers et al., 2019). However, because these studies have investigated passive heating among clinical or sedentary populations, it is unclear whether healthy, young individuals partaking in regular endurance training might adapt similarly to chronic passive heating. It is reasonable to suggest that this could occur, since training in hot environments can improve aerobic capacity in the heat (>37 °C) (Périard et al., 2016) or possibly cool environments (Périard et al., 2015; Tyler et al., 2016; Waldron et al., 2021). These adaptations have largely been attributed to systemic adaptations, such as expansion of plasma volume (PV), greater stroke volume (SV) (Périard et al., 2015), yet changes in the peripheral musculature might also be implicated (Sawka et al., 2011; Waldron et al., 2020). However, the notion that passive heating strategies can elicit similar adaptations, or perhaps enhance the endurance-trained phenotype, has more recently emerged (Minson & Cotter, 2016).

Post-exercise passive heating presents a practical, adjunct strategy for individuals residing in cool climates to heighten the overall thermal stimulus for adaptation (Akerman et al., 2016;

Regan et al., 1996). For example, 12 sauna sessions (90 °C for 30 min) completed immediately after training improved running time to exhaustion by 32 % in temperate conditions (Scoon et al., 2007). This was equivalent to a ~ 2 % reduction in 5 km time-trial and was attributed to increases in plasma volume (Scoon et al., 2007). In contrast, no ergogenic benefit was reported for a similar 10-day post-exercise sauna intervention (Stanley et al., 2015), which might be explained by two-week interval between cessation of the intervention and post-testing. Others have reported that completing six post-exercise hot water baths improved 5 km time trial by 5 % in hot but not in cool conditions (Zurawlew et al., 2016). These data might indicate a minimum dose requirement of passive heating for achieving successful transfer to cool conditions.

Recently, Dalleck et al. (2019) showed that three-weeks of post-exercise passive heating (three days/week) significantly improved $\dot{V}O_{2max}$ (~ 3 mL/kg/min) lactate threshold (~14 %) and exercise economy (~8 %). Interestingly, supplementary addition of heat stimulus was far superior in bringing about changes in endurance-related markers compared to moderate-intensity exercise alone (Dalleck et al., 2019). Similarly, in trained middle-distance runners, post-exercise sauna bathing over three weeks increased $\dot{V}O_{2max}$ by 8% when measured in temperate conditions (Kirby et al., 2021). Likewise, these findings indicate the potential of post-exercise passive heating in complementing the physiological stimulus created by traditional endurance training. Indeed, greater improvements reported in the heating as opposed to control group (Dalleck et al., 2019; Kirby et al., 2021), support the ergogenic value of passive heating.

A major limitation of passive heating studies, to date, is the dependence upon laboratory visitation or equipment, such as heat chambers (Racinais et al., 2017), saunas (Scoon et al., 2007), hot water baths (Brunt et al., 2018), diathermy (Hafen et al., 2019) and water or steam based circulatory suits (Goto et al., 2011). As a result, all passive heating studies have been confined to laboratory settings and, therefore, less accessible to recreationally active or trained populations, who could benefit from these interventions. With the recent advances in clothing technology (Fang et al., 2020), it has been possible to integrate electrically-heated elements into textile fabric. Previously, electrically-heated garments have been used in sporting events that face significant heat-loss windows (Wilkins & Havenith, 2017), providing a practical solution to preserve acute temperature-related neuromuscular enhancements (Racinais & Oksa,

2010). To our knowledge, no study exists to have used electrically heated garments as tool for long-term passive heating. Therefore, the aims of the study were two-fold: (i) to compare the effects of 20 consecutive post-exercise passive heating sessions using electrically heated garments (PH) *vs.* exercise-only controls (CON) on $\dot{V}O_{2max}$, $\dot{V}O_2$ kinetics, GE and critical torque (CT), among recreationally trained individuals in thermoneutral conditions (ii) to understand systemic adaptations associated with chronic lower-body thermogenic stimulus by monitoring changes in brachial artery vascular function (FMD) and expression of key angiogenic markers (NO, VEGF, HSP, eNOS). It was hypothesised that supplementing training with a home-based PH intervention would improve endurance performance to a greater extent compared to exercise alone.

Chapter 2.0 Review of the Literature

2.1 The endurance phenotype

2.1.1 Maximal oxygen uptake

Maximal oxygen uptake ($\dot{V}O_{2max}$) can be defined as the highest amount of oxygen (O_2) that can be utilised by the body during maximal intensity exercise (Hill & Lupton, 1923). As described by Hill et al. (1924), O_2 consumption increases almost linearly with exercise intensity, ultimately plateauing despite further increases in exercise intensity. When a plateau in $\dot{V}O_2$ is not observed (Rivera-Brown et al., 2016; Wood et al., 2010), secondary criteria (Midgley et al., 2009; Midgley et al., 2007b) or a subsequent supramaximal verification test (Poole & Jones, 2017) can be used to establish the attainment of $\dot{V}O_{2max}$. In this case, the response is more appropriately termed as peak oxygen uptake ($\dot{V}O_{2peak}$) . The $\dot{V}O_{2max}$ is one of the most widely studied performance variables, which is frequently used as an indicator of cardiorespiratory fitness, health status (Aspenes et al., 2011; Snell et al., 2007), or to determine the effectiveness of training programmes for enhancing endurance performance (Funch et al., 2017; Ovretveit, 2019; Smith et al., 2013). For instance, elite endurance-trained athletes achieve $\dot{V}O_{2max}$ values of 70-80 mL/kg/min, which is almost double that of recreationally-active individuals, therefore making it an important phenotypic expression of aerobic performance.

To better understand how $\dot{V}O_{2max}$ adapts to physical training, the underlying factors that limit $\dot{V}O_{2max}$ must be considered. For example, the Fick equation describes $\dot{V}O_{2max}$ as the product of cardiac output ($\dot{Q}$) and arterial-venous O_2 content difference (A-VO_2) (Sutton, 1992), where $\dot{Q}$ determines the cardiovascular 'supply' of oxygenated blood and A-VO_2 determines the 'extraction' at the tissue level (i.e. skeletal muscle). Thus, physiological limitations theoretically occur somewhere along the pathway, which is ultimately responsible for transfer of O_2 from the atmosphere to the mitochondria within the contracting muscle (Hoppeler & Weibel, 2000). More specifically, the left-hand side of the equation is limited by: (i) the diffusion of O_2 into the pulmonary system of the lungs (ii) the heart's ability to pump the required volume of blood, to meet the demands of intense exercise and (iii) increased volume and O_2 carrying capacity blood (Bassett & Howley, 1997). The right-hand side of the equation is limited by: (i) the number of capillaries per unit area of the contracting muscle fibre (ii) the diffusion gradient (pressure) for O_2 between the red blood cell (RBC) and the sarcolemma of the contracting muscle (Wagner, 1996) and (iii) the number of mitochondrial cells and the enzyme levels present in those cells responsible for metabolising O_2 to energy (ATP) for muscular contraction (Schwerzmann et al., 1989).

There has been a long-standing debate over whether $\dot{V}O_{2max}$ is limited by central (left-hand side of Fick equation) or peripheral (right-hand side of Fick equation) factors during maximal exercise. Using mathematical modelling, di Prampero (1985) came to the conclusion that during two-legged whole-body exercise, 75% of $\dot{V}O_{2max}$ is limited by central factors and the remaining 25% is limited by peripheral factors. With the above mathematical interpretation, it is reasonable to assume that improvements in $\dot{V}O_{2max}$ reported after a period of whole-body exercise (recruiting large muscle groups) might be due to significant central adaptations. Using a six-week high-intensity interval cycling programme, Astorino et al. (2017) reported a ~ 9% increase in $\dot{V}O_{2max}$ compared to baseline. The authors also noted parallel increases in $\dot{Q}$ and stroke volume (SV) across the six-week training period (Astorino et al., 2017). Here, increases in $\dot{V}O_{2max}$ were evidently attributed to the improvements in O_2 delivery brought about by central adaptation. Similar increases in $\dot{Q}$ and SV in response to various endurance training regimes have been reported previously (Bonne et al., 2014; Daussin et al., 2007; Montero et al., 2015; Wang et al., 2014; Wilmore et al., 2001). The increase in pumping capacity of the heart could be a phenotypic response to training, whereby endurance-trained athletes are shown to have altered left ventricular dimensions with increased mass and wall thickness, allowing them to

pump a greater a volume of blood (Morganroth et al., 1975; Pelliccia & Thompson, 2006). However, the magnitude of cardiac remodelling has shown to be highly variable between individuals and, therefore, should not be considered as the sole driver of an increased $\dot{Q}$ following endurance training (Arbab-Zadeh et al., 2014; Spence et al., 2011).

Another major central adaptation reported in endurance trained athletes is an expansion in blood volume (BV), commonly termed 'hypervolemia' (Convertino, 1991; Hagberg et al., 1998). Previous studies (Kanstrup, 1998; Warburton et al., 1999), have reported a strong relationship between BV and cardiac function, therefore making it a potential contributor to the endurance performance phenotype. Warburton et al. (2004) showed that 12-weeks of continuous endurance training (cycling at 65% $\dot{V}O_{2max}$ for 30-40 min, three days/week) significantly increased BV, the increase in BV also accounted for 40% of the increase in $\dot{V}O_{2max}$ compared to baseline. Interestingly, increases in BV were accompanied by increases in $\dot{Q}$ and SV, thereby demonstrating that vascular expansion can directly enhance cardiac functioning. The increases in $\dot{Q}$ and SV when BV is augmented could be explained by Frank-Starling law (Gledhill et al., 1999) , where vascular expansion results in a greater ventricular pre-load (i.e. greater end diastolic volume (EDV) causing SV and $\dot{Q}$ to increase and ultimately influencing $\dot{V}O_{2max}$.

Finally, the first point of entry of O_2 in the human vessels takes places at the lungs (pulmonary system). As O_2 cannot be stored, it is the mitochondria that sets the demand for pulmonary circulation to eliminate carbon dioxide and re-oxygenate the incoming blood previously deoxygenated by the working muscles (Hoppeler & Weibel, 2000; Lahiri et al., 2006). Here, the importance of the lungs in aerobic metabolism was eloquently shown by Powers et al. (1989), where breathing hyperoxic air (26% O_2) increased $\dot{V}O_{2max}$ by ~5 mL/kg/min in highly trained athletes. Conversely, breathing hypoxic air reduced $\dot{V}O_{2max}$ by ~26 %, further showing that the site if gaseous exchange i.e. lungs, might be an important limiting factor (Martin et al., 1993). Therefore, optimising the diffusion capacity of the lungs might result in greater $\dot{V}O_{2max}$ (Wu et al., 1996). However, it is still not clear weather endurance athletes display a greater lung diffusion capacity as a response to training (Dempsey et al., 1990) or a genetic predisposition/self-selection bias (Cerny et al., 1973).

With the major central adaptions mentioned above, one might tend to overlook the importance/contributions of the peripheral factors. Studies (Hardman & Williams, 1984; Saltin et al., 1976) that have conducted single-leg endurance training help to highlight the importance of peripheral systems in the O_2 uptake chain. For example, single-leg training over four-weeks resulted in a 24% increase in $\dot{V}O_{2max}$ in the trained leg as opposed to only 6% increase in the untrained leg (Saltin et al., 1976). Similar improvements in the trained leg were reported by Hardman and Williams (1984), which were later explained by changes in the ability of the skeletal muscle to metabolise O_2 in response to the training stimulus. Furthermore, peripheral adaptations, such as enhanced mitochondrial biogenesis (Hood, 2009; Zoladz et al., 2022) and proliferation of the capillary network (Charifi et al., 2004; Liu et al., 2022), have the potential to increase the O_2 transit time between the blood and the contracting muscle. Together, these adaptations are in parallel with the suggestions of Wagner (1996), where $\dot{V}O_{2max}$ is described as a complex integrated measure, comprising an O_2 transport cascade from the mouth to the muscle, the capacity of which will determine $\dot{V}O_{2max}$. Despite this logic, it should be recognised that isolated muscle exercise, as opposed to whole-body, diverts a greater proportion of $\dot{Q}$ to the local area (Saltin, 1985). More importantly, Rud et al. (2012) showed that seven-weeks of single leg training increased O_2 uptake in the trained leg by 21% and this increases was supported by a 16% increase in blood flow compared to the untrained leg. Interestingly, the above single leg improvements did not positively alter $\dot{V}O_{2max}$ during double leg cycling (Rud et al., 2012), which indicates that the metabolic demand and the ensuing hyperaemic effect of the exercising skeletal muscle, directs blood flow at a capacity above that of the pumping capacity of the heart alone. Therefore, during whole-body exercise that activates a larger muscle mass, blood delivery as indicated by $\dot{Q}$ seems to be the major limiting factor for $\dot{V}O_{2max}$. In addition, Boushel et al. (2011) demonstrated that mitochondrial respiratory capacity measured *ex-vivo* exceeded the O_2 delivery capacity of the femoral artery during *in-vivo* whole-body exercise by 50%. The mismatch in delivery and consumption further pointing to central factors (i.e. blood delivery) as major rate-limiting step for $\dot{V}O_{2max}$.

To summarise, $\dot{V}O_{2max}$ sets the upper-limit of exercise capacity. A high $\dot{V}O_{2max}$ implies that an individual can sustain a high rate of energy (ATP) generation. However, while the $\dot{V}O_{2max}$ represents an unsustainable maxima, it is also important to investigate the percentage (%) or fractional utilisation of $\dot{V}O_{2max}$ that can be sustained during prolonged efforts, as seen in endurance events. Therefore, while $\dot{V}O_{2max}$ sets the upper-most ceiling (maximal intensity) of

O_2 utilisation, it is the sub-maximal characteristics and thresholds therein that contribute to endurance capability.

2.1.2 Gas exchange threshold

During incremental exercise, there is a point at which the contribution of energy from anaerobic metabolism increases to meet the progressive mechanical (external) demands. The transport and buffering of H^+ from the muscle tissue to the blood results in excess generation of carbon dioxide (CO_2) (Anderson & Rhodes, 1991; Wasserman et al., 1986). The exercise intensity or $\dot{V}O_2$ at which this excess CO_2 (i.e. non-metabolic CO_2 in addition to the CO_2 generated via aerobic metabolism) is first generated, as detected by an increase in respiratory CO_2 output ($\dot{V}CO_2$) at the mouth, is termed as gas exchange threshold (GET) (Wasserman et al., 1986). The physiological response at the GET often includes simultaneous reduction of muscle and blood bicarbonate concentration (Beaver et al., 1985), as a result of H^+ buffering to maintain metabolic homeostasis and occurs in the absence of hyperventilation.

An incremental ramp cycling protocol, with recorded breath-by-breath changes in minute ventilation (V_E), $\dot{V}CO_2$ and $\dot{V}O_2$ is regarded as the 'gold standard' for non-invasive detection of GET (Kelly, 2001; Wasserman et al., 1973, 1986). Valid identification of GET also depends on the exercise stimulus, where the ramp rate protocol must be sufficient to distinguish whether excess $\dot{V}CO_2$ is a result of metabolic buffering or mechanical hyperventilation to decrease arterial pressure of CO_2 (Boone & Bourgois, 2012; Buchfuhrer et al., 1983). For this purpose, studies have usually employed a ramp rate of 20-25 Watts/min (W/min) for healthy and endurance trained individuals (Hug et al., 2003; Whipp et al., 1981). After retrieving the gas data for V_E, $\dot{V}CO_2$ and $\dot{V}O_2$, the GET is determined graphically using the V-slope method as proposed by Beaver et al. (1986). The breakpoint in $\dot{V}CO_2$ - $\dot{V}O_2$ relationship can be identified by visual inspection or by dividing the $\dot{V}CO_2$ vs.$\dot{V}O_2$ data into two segments (i.e., lower segment and upper segment) and applying statistical regression to find the point corresponding to the smallest difference (> 0.1) between the slope of the line describing the lower segment and upper segment respectively. Another common visual method to identify GET is to find the first point at which ventilatory equivalent of O_2 ($V_E/\dot{V}O_2$) begins to rise while the ventilatory equivalent of CO_2 ($V_E/\dot{V}CO_2$) remains constant or flat (Wasserman, 1984). However, the later method is recommended only to be used as a secondary criteria to cross-validate the GET

determined from the V-slope method (Beaver et al., 1986). This is because chemoreceptors responsible for ventilatory control are sensitive to changes in partial pressure of CO_2 (Myers & Ashley, 1997), thus adding a source of variance when using ventilatory data to determine GET.

From an endurance standpoint, GET is an important threshold as it establishes the boundary between moderate and heavy-intensity domains of exercise (Poole & Jones, 2012). A GET that occurs at a higher percentage (%) of an individual's $\dot{V}O_{2max}$ indicates a superior integration of O_2 supply together with aerobic metabolism, therefore, setting the upper-limit of exercise intensity or workload that can be sustained by an individual for an extended period of time without the accumulation of lactate. Historically, the above concept has been termed as fractional utilisation of $\dot{V}O_{2max}$ and has been closely linked to the lactate threshold (Bassett & Howley, 1997). Despite conceptual differences between the two thresholds, specifically linking accumulation of blood lactate accumulation and ventilation (Brooks, 1985; Davis, 1985), the GET detected by v-slope method seems to have some associations with lactate threshold (Beaver et al., 2016; Henson et al., 1989). Taking this into consideration, GET can explain variance in performance between athletes of similar fitness levels. For instance, in two athletes with similar $\dot{V}O_{2max}$ values but differing in GET, the athlete with a GET occurring at a higher % of $\dot{V}O_{2max}$ would be able to sustain a greater work rate or running speed compared to the other. This was demonstrated by Coyle et al. (1988), who showed that elite cyclists with similar $\dot{V}O_{2max}$ (4.8 ± 0.04 L/min) could be differentiated into groups based on their blood lactate threshold in response to incremental exercise - a more invasive measure of determining GET using capillary blood samples. The superior group displayed GET at 80% of $\dot{V}O_{2max}$ compared to 60% for the inferior group, and as a consequence, the former were able to sustain a higher workload (cycling >88% of $\dot{V}O_{2max}$) for a longer duration (60.8 ± 3.1 min vs. 29.1 ± 5.0 min, $p<0.05$) compared to the latter (Coyle et al., 1988).

Endurance training can increase the exercise intensity at which GET occurs, in absence of any changes in $\dot{V}O_{2max}$ (Denis et al., 1982; Henritze et al., 1985). For example, 12 weeks of continuous endurance training improved GET by 8% in healthy adults; however, the improvements were only noted in the group that trained above their GET (Henritze et al., 1985). The above results indicate that the improvements reported in GET are dependent on the exercise intensity. Similar intensity-dependant improvements in GET were reported by Burke et al. (1994), who showed that seven-weeks of high intensity-interval training (HIT) cycling

completed at 90-95% $\dot{V}O_{2max}$ (1:1 work: rest ratio) significantly increased GET by ~19%. Interestingly, intermittent type exercise activities commonly undertaken in team sports (soccer, basketball etc.) have also shown to improve GET (Edwards et al., 2003; Laplaud et al., 2004). Therefore, it is apparent that the GET is adaptable through various training modes, primarily via physiological pathways that are responsible for BLa generation and clearance (Wasserman et al., 1986).

The fundamental link between GET and bicarbonate buffering (Wasserman et al., 1967; Beaver et al., 1985) indicate that peripheral adaptations at the muscular level logically contribute to improvements in submaximal exercise performance. Increases in mitochondrial volume (Meinild-Lundby et al., 2018), oxidative enzyme activity (Fritzen et al., 2020), greater recruitment of type I/IIa muscle fibers (Luden et al., 2012) and increased capillarisation (Laughlin & Roseguini, 2008) as a result of endurance training, allow for a phenotypic expression that favours greater oxidative metabolism. The above peripheral adaptations, coupled together, not only allow an individual to buffer BLa more rapidly (Sahlin & Henriksson, 1984), but also reduce the total BLa generated due to an increased oxidative capacity. This could possibly explain why the accumulation of BLa and the resulting GET occurs at a higher workload or % $\dot{V}O_{2max}$ in endurance trained athletes (Farrel et al., 1993; Lucia et al., 2001). Therefore, future research of interventions that promote peripheral adaptations similar to those mentioned above is required.

2.1.3 The torque- and power-time relationship

The curvilinear relationship between power output (P) and the duration (t) for which it can be sustained in a sporting event was first noted by Hill (1925). The curvilinear nature of the P-t curve is described by the asymptote termed as critical power (CP; measured in watts [W]) and the curvature constant is denoted by W' (measured in kilojoules [kJ]) (Pooles et al., 1988). Here, CP represents the highest sustainable steady-state work rate whereas W' is the finite amount of work that can be done above CP (Monod & Scherrer, 1965). Knowledge of an individual's CP and W' allows prediction of the finite duration (t_{lim}) for sustaining an intolerable exercise intensity that proceeds to failure or stoppage of exercise. For instance, exercise done above the GET but below CP can be sustained for up to ~3-h, whereas exercise above CP can be sustained for less than ~30-min (Burnley & Jones, 2018; Jones et al., 2008). This implies that, for exercise below CP, the dependance on the finite W' for energy production

will be less, causing t_{lim} to be longer, while the opposite would be true for exercise above CP. The integrative mechanism between the parameters of the P-t relationship and its impact on t_{lim} can therefore be explained mathematically by; $t_{lim} = W'/(P-CP)$ (Monod & Scherrer, 1965), where P is the power-output (i.e. intensity) at which the exercise is being performed. Finally, the above equation highlights the significance of CP, as it directly defines the boundary between the heavy and severe-domain of exercise intensity. With the majority of endurance events performed in the heavy-intensity (marathon: 42 km) and severe-intensity domain (800-10,000 m; Jones & Vanhatalo, 2017), CP provides an important concept to better understand the physiological underpinnings of prolonged high-intensity efforts.

The power-time relationship holds true for various locomotor or exercise modes such as, running (Bulbulian et al., 1986), cycling (Wright et al., 2017), swimming (di Prampero et al., 2008), rowing (Cheng et al., 2012) and has recently been applied to intermittent-team sports (Vassallo et al., 2020). Traditionally, CP is determined by completing three-to-eight exhaustive tests of various durations, performed on separate days (Carnevale & Gaesser, 1991; Housh et al., 1989; Laughlin & Roseguini, 2008). The previous time-consuming procedure was later replaced by a 3-min all out test, based on the concept that W' is a finite source that can be depleted and has subsequently shown to reliably estimate CP (Burnley et al., 2006; Vanhatalo et al., 2007, 2008). More importantly, the P-t parameters can also be derived from isometric muscle contractions (Burnley, 2009), where critical torque (CT) and impulse prime (I') are analogous to CP and W'. This is advantageous, as it helps to better understand limitations in isolated conditions i.e., fatigue at the muscular level, as opposed to whole-body dynamic exercise. Here, peripheral fatigue quantified by a reduction in the force or torque output can be narrowed down to two potential sites, (i) local-distal to the neuromuscular junction (i.e., within the muscle fibers itself) and (ii) central-proximal to the neuromuscular junction (i.e., neural network linking the central nervous system [CNS] to the muscle) (Bigland-Ritchie et al., 1986; Place et al., 2009). Previous research indicates (Smith et al., 2007; Søgaard et al., 2006), that for low-intensity contractions, central mechanisms explain the majority of performance decrement, whereas high-intensity contractions are limited predominantly by local mechanisms. The relationship between contributors of peripheral fatigue mechanism (local *vs.* central) and exercise intensity was further investigated using the CT concept by (Burnley et al., 2012). Interestingly, both local and central fatigue occurred during contractions below the CT, but more importantly for contractions above CT, local and central fatigue developed four-to-five times faster when compared to the former condition (Burnely et al., 2012). These data

imply the absence of a linear relationship between fatigue and contractile force demands but, rather, a sudden progressive development in peripheral fatigue when exercising above CT (i.e., when I' is being utilised).

The distinct fatigue profile when exercising above CT can be attributed to metabolic disturbances as a result of high-intensity contractions (Kent et al., 2016). The increased energy demand placed with high-intensity contraction results in the accumulation of inorganic phosphate (Pi) and hydrogen ion (H^+), which are known to contribute towards contractile failure (Westerblad et al., 1991). Indeed, Jones et al. (2008) were the first to show that exercising above CP resulted in a rapid degradation of phosphocreatine (PCr) stores and a simultaneous accumulation of intramuscular Pi and H^+. The progressive recruitment of high-order type II muscle fibers for exercise above CP, increases reliance upon on anaerobic metabolism to generate ATP, the resultant metabolites associated with this particular metabolism could be an important contributor to local fatigue. Finally, similar profiles for the neuro-muscular (Burnley et al., 2012) and bioenergetic response (Jones et al., 2008) to exercise above CT/ CP, further establishes the components of the P-t relationship as the 'gold standard measure' of fatigue, whereby, exercising above CP results in significant perturbation of systemic homeostasis, leading to exhaustion or exercise failure (Poole et al., 2021).

It is well established that CP denotes the highest effort that can be sustained via oxidative pathways and, therefore, has a bias towards endurance phenotypes (Greco et al., 2012; Moritani et al., 1981). Previous work (Barker et al., 2006) showed that cross-country runners had higher CP values compared to sprinters. It was also noted that the runners expressed higher O_2 uptake at CP, indicative of a superior oxidative capacity (i.e., greater O_2 metabolism) compared to the sprinters (Barker et al., 2006). The above findings interlay well with recent marathon race analysis, with the current world record holder Eliud Kipchoge running the Boston and London marathon at ~ 94% of his critical speed (CS). Though, historically, the parameters of the P-t curve are derived from repeated trials that result to exhaustion in 2-15 min (Poole et al., 1988), CP in particular is able to closely predict performance of longer events (>2-h). This indicates that the majority of the physiological adaptations associated with endurance training allow for a higher oxidative potential and hence a greater CP.

Differences in CP between groups of cross-country runners and sprinters (Barker et al., 2006) could potentially be attributed to the differences in demands placed by the respective sports.

Here, endurance athletes are known to possess highly oxidative type I muscle fiber (Myburgh & Weston, 1998; Schiaffino & Reggiani, 2011) and achieve higher $\dot{V}O_{2max}$ values (Mitchell et al., 2018), which might cause CP to occur at a higher % of $\dot{V}O_{2max}$ (Poole et al., 2016). Indeed, a strong correlation was reported between type 1 muscle fibers and CP, estimated either from a three-min all out test (3-AOT) (Vanhatalo et al., 2016) or the traditional multi-day test (Mitchell et al., 2018). Furthermore, a steady muscle metabolic state as demonstrated by muscle pH, PCr and [BLa] when exercising 5% below but not above CP (Vanhatalo et al., 2016), suggests that energy metabolism at and below CP are predominantly supported by oxidative metabolism and to a lesser extent by PCr hydrolysis. The bigger oxidative machinery, represented by a greater mitochondrial size, density and oxidative enzyme content in type I fibres (Dubowitz & Pearse, 1960; Schiaffino & Reggiani, 2011), provide additional morphological explanations that are congruent with the above findings.

Resistance of skeletal muscle fatigue during heavy-intensity exercise can be related to the tight coupling of O_2 demand and delivery, facilitated by muscle capillary networks (Joyner & Coyle, 2008). A dense capillary network allows greater proportion of RBC's to be in 'contact' with the muscle sarcolemma (Wagner, 2000). Assuming that continuous RBC flow is present in almost all capillaries at rest or exercise (Poole, 2019), a greater number of capillaries in contact with the myocyte would result in better muscle O_2 diffusion capacity. This is brought about by the pressure gradients for O_2 (PO_2) between the capillary, interstitial fluid and myocyte (Hirai et al., 2018). The above explanation would logically implicate muscle capillarisation as an important determinant for sustaining a high oxidative metabolism, and hence CP. Unsurprisingly, a strong positive relationship has been reported between the number of capillary contacts and CP (Mitchell et al., 2018). The inverse relationship has been reported previously between CP and time to steady state (τ) for $\dot{V}O_2$ kinetics measured during severe-intensity exercise (Murgatroyd et al., 2011), which could indicate better demand-delivery matching in individuals expressing a high CP. The various associations of CP noted above, makes it an endurance marker that is sensitive to both, changes in local delivery as well as adaptations in cellular respiratory function.

Various endurance training protocols can be adopted to increase skeletal muscle capillarity (Hoier et al., 2020; Perez-Gomez et al., 2021), as well as oxidative capacity (Burgomaster et al., 2008; Islam et al., 2021). Burgomaster et al. (2008) showed that six-weeks of endurance

training increased citrate synthase (CS) activity by 25% independent of training type (HIT *vs.* END). A previous study reported similar increases in CS activity with a shorter training duration of two-weeks (Gibala et al., 2006). These findings could indicate that adaptations in oxidative capacity occurs in the early phases of training. Simultaneous increases in mitochondrial volume reported during similar time frames (six-weeks) for endurance training (Montero e al., 2015, Meinild-Lundby et al., 2018) can explain improvements in oxidative metabolism post-training, as a greater mitochondrial volume allows for greater enzyme content and activity (Lundby & Jacobs, 2016).

Similarly, as mentioned earlier, capillaries surrounding the muscle not only represent the final component of blood delivery but also aid in the diffusion of O_2 into the mitochondria. Previous studies (Hoier et al., 2012; Hoier & Hellsten, 2014; Jensen et al., 2004), using various endurance training protocols ranging from 4-24 weeks, have reported increased capillary density and capillary to fibre ratio by 20 and 40% respectively. Here, it is vascular endothelial growth factors (VEGF) that are deemed to be the most important signalling molecules for expansion of the capillary network (Egginton, 2009). VEGF also regulates endothelial nitric oxide synthase (eNOS) that is responsible for NO generation within the arterial wall (Gentile et al., 2013). NO stimulates vasodilation in the endothelial layer of arterial wall, ensuring increased blood perfusion around the myocytes (Tousoulis et al., 2011).

To conclude, integration of the mechanism regulating O_2 delivery and metabolism would allow for greater expression of oxidative metabolism and could potentially explain higher CP values possessed by endurance-trained phenotypes. Therefore, interventions that have the potential to stimulate similar physiological adaptations as seen with endurance training could be of great value.

2.1.4 Oxygen uptake kinetics

Oxygen uptake kinetics ($\dot{V}O_2$ kinetics), helps to quantify or describe the rate at which the oxidative machinery can adjust whilst transitioning from a low-energy state to a high-energy state (Poole & Jones, 2012; Whipp, 1971). More importantly, it reflects the integration of the cardiovascular, pulmonary and neuromuscular systems to effectively generate energy (ATP) from oxidative phosphorylation (OXPHOS), which is the preferred metabolic pathway for sustained efforts (Grassi, 2003). Given the above, $\dot{V}O_2$ kinetics would directly influence all the

hallmark endurance parameters mentioned in this chapter and ultimately determine an individual's exercise tolerance and capability (Burnley & Jones, 2007; Jones & Burnley, 2009).

Historically, $\dot{V}O_2$ kinetics has been understood by tracking the time-course of O_2 uptake ($\dot{V}O_2$) in response to a sustained-exercise stimulus beginning from rest (Whipp & Wasserman, 1972). In this instance, the $\dot{V}O_2$ response at the muscle will depend on the amount of O_2 transported to it (central factors) and the amount of O_2 extracted (peripheral factors) by the myocyte. Keeping this concept in mind, breath-by-breath pulmonary $\dot{V}O_2$ is commonly used as a non-invasive and indirect measure of muscle $\dot{V}O_2$ (Macfarlane, 2017).

The response pattern of O_2 by the muscles depend on the intensity at which exercise is being performed (Özyener et al., 2001). For moderate-intensity exercise below the GET, the initial transition from rest-to-exercise results in a sudden increase in $\dot{V}O_2$. This initial response can be attributed to a sudden increase in venous return due the recruitment of a larger muscle area, leading to an enhanced muscle pump, which causes an overshoot in $\dot{Q}$. The heightened response in $\dot{V}O_2$ at exercise onset has been termed as phase I or the cardio-dynamic phase (Barstow et al., 1990; Linnarsson, 1974; Whipp & Wasserman, 1972). Here, the pulmonary $\dot{V}O_2$ measured at the mouth does not reflect that of the muscle, as the rise occurs in the absence of an increased O_2 extraction at the muscle (Murias, et al., 2011b). As moderate-intensity exercise continues, $\dot{Q}$ increases further, with a greater percentage of venous blood being deoxygenated, indicating an elevated O_2 extraction from the muscle. This second rise in $\dot{V}O_2$ represents phase II of oxygen kinetics, referred to as 'primary' or the 'fast' component (Whipp et al., 1982). Further exercise at moderate-intensity drives phase II to a visible steady state (Phase III), which represents the time at which the demand and supply of energy are matched for the given exercise intensity (< GET). Usually, steady-state is achieved within 120-180 s after exercise onset in healthy adults when exercising below GET (Özyener et al., 2001; Whipp & Ward, 1990).

This triphasic nature of $\dot{V}O_2$ kinetics during moderate-intensity exercise highlights the importance of the phase II response, as it describes how an individual achieves a new steady-state. The phase II response leading to steady phase III of $\dot{V}O_2$ kinetics has been quantitively described using a mono-exponential equation, where the time constant (τ) of the phase II represents the time taken to attain 63 % of the final steady state amplitude (A), achieved in

phase III (Koga et al., 1999; Linnarsson, 1974; Rossiter et al., 1999; Whipp & Ward, 1990). Here, the amplitude represents the absolute difference between resting $\dot{V}O_2$ and the new steady-state $\dot{V}O_2$ achieved following rest-to-exercise transition during moderate intensity exercise (< GET). The *A* and τ are useful in explaining the accumulated O_2 deficit when transitioning from different metabolically demanding activities. More importantly, those with quicker phase II response or lesser τ values would be less reliant on energy from anaerobic processes i.e. glycolysis/glycogenolysis, when transitioning from rest to exercise (Jones & Burnley, 2009). This reduces the magnitude of O_2 deficit and prevents depletion of the finite anaerobic energy stores in the muscle (muscle glycogen and PCr) (Rossiter et al., 1999), which together increase exercise tolerance by delaying the accumulation of metabolites associated with anaerobic metabolism that ultimately leads to fatigue (Fitts, 1994; Westerblad et al., 1991).

Endurance training administered with the appropriate intensity and duration can influence pulmonary $\dot{V}O_2$ kinetics For instance, 4-12 weeks of endurance training at 60-70% $\dot{V}O_{2max}$ for 30-120 min can significantly reduce τ during transitions to moderate-intensity exercise in untrained healthy young adults (Murias, Kowalchuk, & Paterson, 2010b; Phillips et al., 1995). A similar endurance training protocol administered over eight-weeks (Norris & Petersen, 1998), resulted in faster phase II $\dot{V}O_2$ kinetics i.e. reduced τ values, in highly trained cyclists, where significant reductions in τ (PRE: 29.2 ± 6.6 s *vs.* week 4: 24.4 ± 5.1 s *vs.* week 8: 21.9 ± 5.5 s, $p < 0.05$) was detected at week four and eight, thereby demonstrating that $\dot{V}O_2$ kinetics is also adaptable in highly-trained phenotypes. To summarise, following the start of endurance training, acute changes in $\dot{V}O_2$ kinetics can occur within two to four training sessions (McKay et al., 2009; Phillips et al., 1995) and on continuation further reduction τ have been noticed by week three (Murias et al., 2010b) and six (Berger et al., 2005), with no further improvement reported thereafter (Fukuoka et al., 2002; Murias et al., 2010b). These studies provide evidence that τ is very sensitive to training stimulus and is characterised by having a large adaptation window, ranging from ~2-40 days in healthy young adults.

Due to the large time-frame (2-40 days) associated with the speeding of $\dot{V}O_2$ kinetics in response to training, it is difficult to identify a single variable responsible for the adaptative phenomenon. However, the variables can be narrowed down to two broader factors: (i) increased O_2 availability to the working muscles and (ii) quicker onset of OXPHOS for ATP production. The availability of O_2 for working muscles is dependent on blood flow (delivery)

and, therefore, has been considered as a possible site of adaptation that can enhance $\dot{V}O2$ kinetics (Hughson et al., 2001). In a seminal study conducted by Bell et al. (2001), five old adults underwent single-leg endurance training for nine-weeks. Interestingly, while femoral-artery blood-flow kinetics measured via doppler-ultrasound remained unchanged in the trained and untrained leg, faster $\dot{V}O_2$ kinetics were reported in the trained leg. In addition, blood velocity in the femoral- artery was greater than $\dot{V}O_2$ kinetics in both legs, pre and post intervention, thus indicating that bulk O_2 delivery to the working muscle might not be the most significant variable that causes faster $\dot{V}O_2$ kinetics following a training intervention. However, the study by Bell et al. (2001) cannot completely eliminate O_2-transport as an import factor for faster $\dot{V}O_2$ kinetics, as reducing O_2 availability via inspiring hypoxic gas (Hughson & Kowalchuk, 1995), reducing blood flow using tourniquets (Knight et al., 2004) and hindering $\dot{Q}$ by ingesting beta blockers (Hughson, 1984), have resulted in slowing of phase II $\dot{V}O_2$ kinetics, while transitioning from rest to moderate-intensity exercise. Here, it could be considered that bulk O_2 delivery does not completely represent the availability of O_2 to exercising muscle fibre. Instead, the mechanical muscle pump (Sheriff, 2005) and vasoactive metabolites (Hester & Choi, 2002) that influence microvascular properties of vessels (arterioles and venules) might be of greater importance for muscular perfusion, thereby influencing $\dot{V}O_2$ kinetics.

In this regard, recent advances in near-infrared spectroscopy (NIRS) has allowed for non-invasive measurement of oxy- and deoxy-haemoglobin at the peripheral level (muscle tissue) helping to better understand muscular $\dot{V}O_2$ kinetics. More importantly, the deoxy-haemoglobin (HHb) signal tightly reflects deoxygenation and therefore O_2 consumption at the muscle (muscle $\dot{V}O_2$), which would reasonably be more sensitive than pulmonary $\dot{V}O_2$ when considering rest to exercise transitions. Likewise, DeLorey et al. (2003) showed that τ for deoxygenation index (Δ HHb) derived from NIRS units was faster than pulmonary phase II ($\dot{V}O_{2p}$) τ measured simultaneously during rest to moderate-intensity exercise transition. Importantly, the difference between the two time constants (τ Δ HHb *vs.* τ $\dot{V}O_{2p}$) was greater in older adults, indicating the greater muscle O_2 extraction at the rest to exercise transition (represented by Δ HHb/ Δ $\dot{V}O_{2p}$) for a relative workload (< GET) in older adults could be related to reduced muscular blood perfusion resulting in slower onset of OXPHOS. This might explain the slower $\dot{V}O_2$ kinetics seen in older adults compared to younger individuals (DeLorey et al., 2004; Padilla et al., 2008). The mismatch in muscle O_2 delivery and utilisation, as

represented by Δ HHb/ Δ $\dot{V}O_{2p}$, has also been reported by Murias et al. (2010b), where the authors identified an overshoot of Δ HHb/ Δ $\dot{V}O_{2p}$ (ratio > 1) during transition from rest to exercise. Interestingly, six-weeks of endurance training (45-min of cycling at 70% $\dot{V}O_{2max}$) was able to completely attenuate the overshoot Δ HHb/ Δ $\dot{V}O_{2p}$ in healthy young adults but not in older adults. However, Murias and co. also noted that overshoot of Δ HHb/ Δ $\dot{V}O_{2p}$ was reduced among older adults post three-weeks of training and both groups had faster τ $\dot{V}O_{2p}$ post six-weeks of training. A strong association (young: r = 0.98, old: r = 0.93; $p < 0.05$) was also reported between lowering of Δ HHb/ Δ $\dot{V}O_{2p}$ and improvement in τ $\dot{V}O_{2p}$ for both age groups (Murias et al., 2010b). The above findings clearly indicate the ability of the micro-circulation to alter $\dot{V}O_2$ kinetics at the peripheral and, ultimately, systemic level. Likewise, an increased capillarisation would facilitate superior distribution of O_2 across the activated musculature (Poole, Musch, et al., 2022), ensuring accurate matching of O_2 demand with delivery. Additionally, in accordance with the ascending vasodilation concept (Murrant et al., 2021), a denser capillary network would subsequently allow a greater transfer of vasodilatory signals from the activated skeletal muscles to the upstream feed arterioles and arteries, promoting selective distribution of $\dot{Q}$ to capillary beds of recruited motor units.

The attenuation of metabolic 'sluggishness' or faster onset of OXPHOS when transitioning from a state of low-energy demand to high-energy demand, can also be considered as an important adaptation for endurance training (Grassi, 2006; Jones et al., 2021). Likewise, previous (Freyssenet et al., 1996) and recent (Bonafiglia et al., 2021; Meinild et al., 2018) studies have reported mitochondrial biogenesis and increased oxidative enzyme activity following endurance training. In the study of Bell et al. (2001), previously discussed, training an individual limb can increase citrate synthase (CS) (a marker of mitochondrial biogenesis) activity by 70% compared to the control leg. This, coupled with a lack of change in blood flow kinetics, means that faster $\dot{V}O_2$ kinetics were achieved via enhanced mitochondrial functioning. This is consistent with the notion that faster activation of oxidative enzymes when transitioning from rest to exercise would reduce the dependence on substrate-level phosphorylation (glycogen and PCr), thus allowing quicker achievement of steady–state, while incurring lower O_2 deficit/cost. Indeed, LeBlanc et al. (2004) showed that seven-weeks of endurance training increased CS activity but decreased pyruvate dehydrogenase activity during sub-maximal exercise, which indicates a higher involvement of the tricarboxylic cycle (TCA) and electron transport chain (ETC) in the production of energy during sub-maximal exercise, adding support

to the above notion. This adaptation would also result in sparing of glycogen and PCr, which has been common adaptation reported following endurance training interventions (Chesley et al., 1996). In addition, speeding of OXPHOS rate depends upon adenosine di-phosphate (ADP) and inorganic phosphate molecule [Pi] availability in the mitochondria (Poole et al., 2008); however, hydrolysis of PCr via creatine kinase (CK) competes with mitochondrial respiration to use ADP (reactant) for anaerobic energy production i.e., ATP (product). Interestingly, Kindig et al. (2005) showed that inhibition of CK in isolated frog muscle cells resulted in faster intracellular O_2 kinetics following onset of exercise, thus highlighting the importance of ADP buffering for mitochondrial respiration. Therefore, increased oxidative potential of mitochondria post-training could result in quicker matching of ATP supply and demand aerobically, as opposed to anaerobically via PCr and glycogen hydrolysis, simultaneously improving $\dot{V}O_2$ kinetics and exercise tolerance.

Finally, the evidence from the literature mentioned above indicates the difficulty in establishing a singular adaptation responsible for faster $\dot{V}O_2$ kinetics following endurance training. However, it appears that a combination of increased muscle blood perfusion and enhanced mitochondrial functioning integrate to allow for faster phase II $\dot{V}O_2$ kinetic response (Poole & Jones, 2012). Although, the degree to which these factors adapt to a training stimulus will depend on the individual's age, training status and baseline physiology.

2.1.5 Gross efficiency

Gross efficiency (GE) can be defined as the ratio of mechanical work done and the energy expended to achieve it. In simpler terms, it explains the effectiveness of the human body to metabolise O_2 for a desired locomotory outcome (Coyle, 1999; Coyle et al., 1992; Passfield & Doust, 2000). Usually, GE is determined using moderate-intensity exercise (exercise performed below GET) (Jeffries et al., 2019; Waldron, 2014; Waldron et al., 2016), which is characterised by a sudden exponential increase in pulmonary O_2 uptake ($\dot{V}O_2$) at exercise onset and on continuation plateaus within 2-3 min in healthy individuals (Whipp & Wasserman, 1972). The plateau, also known as steady state, represents the dynamic balance between ATP production and consumption achieved solely through OXPHOS (Rossiter, 2011; Whipp, 1996).

Gross efficiency has been widely recognised as an important determinant of endurance performance (Joyner & Coyle, 2008). Typically, endurance-trained cyclists demonstrate GE

values ranging from 18.5 to 22.6 % when cycling at 80 revolution per minute (rev/min) (Coyle et al., 1992). This is important, as individuals with a higher GE are more efficient in converting chemical energy to mechanical work compared to their lower counterparts. In agreement with the above, (Jansson & Kaijser, 1987) showed that trained cyclists were ~3% more efficient than the untrained group and were able to sustain a higher load (83 W) when cycling at similar relative intensity (65% $\dot{V}O_{2max}$). Likewise, within a group possessing similar $\dot{V}O_{2max}$ values, a 1.8% difference in efficiency led to a significant increase in maximal power output (9 %) that could be sustained for extended durations (Horowitz et al., 1994). Though the difference might seem minimal in absolute terms, mathematical modelling predicts that increasing GE by only 1% can result in a 48 s improvement in 40 km time-trial performance (Jeukendrup et al., 2000). These findings imply that marginal improvements in GE can have a major impact on endurance performance.

Various internal (i.e. within an individual) and external factors (i.e. sporting equipment) seem to influence GE (Jeukendrup & Martin, 2001; Saunders et al., 2004); however, this review will focus mainly on the physiological determinants of GE and how they are adapted with training. Historically, a strong correlation has been reported between type I muscle fibres and GE (Coyle et al., 1992; Horowitz et al., 1994). Horowitz et al. (1994) reported that cyclists with higher GE displayed a greater percentage of type I fibres (70% *vs.* 40%) compared to the group with lower GE. The improved efficiency found among athletes with greater type I fibre distribution might be attributed to a higher contractile efficiency, where the ATP cost of each contraction cycle is reduced when type I fibres are predominantly activated for locomotion (Stienen et al., 1996). However, *in-vitro* observation by He et al. (2000) indicates that thermodynamic efficiency during sarcomere shortening are similar for type I and II fibres, although the latter required a higher contraction velocity to achieve maximum efficiency. This has led to the popular suggestion that contraction velocities during cycling favour maximum efficiency of type I fibres, with optimal cadence reported to fall between 60-120 rev/min (Hansen et al., 2002; Horowitz et al., 1994). Numerous animal studies have reported fast-to-slow fibre type transition (type IIX→ IIA → I) following long-term endurance training (Demirel et al., 1999; Serrano et al., 2000). Similar longitudinal (> 13 weeks) investigations in humans have provided some initial evidence in favour of type I fibre shifts with training (Luden et al., 2012; Trappe et al., 2006). Additionally, cross-sectional studies have indicated a significantly higher expression of type I fibres in trained compared to untrained or sedentary groups (Costill et al., 1976; Thayer

et al., 2000); however, due to the cross-sectional nature of the studies, it is difficult to conclude whether fibre transitions are a result of training or a genetic-predisposition. Furthermore, improvements in GE with training have been reported within shorter time-frames (Hintzy et al., 2005), which might not be long enough to allow fibre-specific transitions. Therefore, other adaptations more sensitive to training might account for improvements in GE.

Adaptation within the muscle cells could provide an alternative explanation for improvements seen in GE with training. Endurance training has shown to increase mitochondrial volume and enhance oxidative enzyme activity. These adaptations together promote a metabolic shift from glycolytic to oxidative processes, thereby reducing the accumulation of metabolites that negatively impact contractile efficiency of skeletal muscles. In agreement, Sahlin et al. (2005) showed that elevated concentrations of lactate in the blood and muscle prior to completing a sub-maximal cycling test, increased the $\dot{V}O_2$ asymptote and subsequently decreased GE. On the other hand, ATP cost of contraction depends on the interaction between actin-myosin cross-bridge formation (myosin-ATPase) and the resultant calcium (Ca^{2+}) cycling by the sarcoplasmic reticulum Ca^{2+}-ATPase pump (SERCA) (Periasamy et al., 2017). Likewise, SERCA pumps alone can consume 30-40% of ATP during contractions making them energetically costly (Walsh et al., 2006; Barclay et al., 2007). Interestingly, endurance training has shown to lower the expression of SERCA pumps in humans (Majerczak et al., 2008, 2012). For instance, five-weeks of endurance training downregulated SERCA pumps and concomitantly improved GE during moderate-intensity cycling (Majerczak et al., 2008). Here, the suppression of SERCA pumps could have reduced ATP demand of Ca^{2+}cycling and might explain the observed decrease in O_2 cost for sub-maximal exercise post training. Although, it must be noted that SERCA are differentiated into various isoforms that are tissue-specific (Brandl et al., 1987; Periasamy & Kalyanasundaram, 2007). Likewise, in skeletal muscles, SERCA1 and SERCA2 are expressed in type II and type I muscle fibers, respectively (Wuytack et al., 2002). Interestingly, in the previous study by Majerczak et al. (2008), training resulted in a suppression of SERCA2 expression without any changes in type 1 fiber content. The above results indicate that alterations in SERCA expression are more sensitive to training compared to fiber-specific transitions (Short et al., 2005), and therefore might explain improved efficiency in pre-existing fibers within shorter-time frames (Majerczak et al., 2008, 2012; Zoladz et al., 2013).

Finally, mitochondrial efficiency (ATP to O_2 ratio) can be compromised by the back leak of protons through the inner mitochondrial membrane, resulting in an uncoupling between ATP synthesis and OXPHOS (Jastroch et al., 2010). In skeletal muscles, uncoupling protein 3 (UCP3) has been linked as a mediator for proton leak, and therefore might play an important role in increasing the O_2 cost of aerobic metabolism (Vidal-Puig et al., 1997). In theory, higher expression of UCP3 would reduce GE during exercise. Endurance-trained athletes have shown to express lower UCP3 mRNA and protein content as opposed to untrained controls (Schrauwen et al., 1999). Additionally, the content of UCP3 is fibre-specific, with it being expressed the least in type I fibres (Russell, Wadley, et al., 2003). Furthermore, only six weeks of endurance training can reduce UCP3 content in human muscles (Fernström et al., 2004; Russell, Somm, et al., 2003), and has been inversely correlated to GE in moderately-trained individuals (Schrauwen et al., 1999). These findings were later extended in a heterogenous cohort of cyclist (Mogensen et al., 2006), wherein a similar negative relationship between UCP3 and cycling efficiency was noted. Taken together, lower UCP3 in working muscles might improve efficiency of OXPHOS, increasing whole-body GE. To conclude, interventions that can reduce the O_2 cost of ATP generation might be valuable, allowing individuals to sustain a greater work rate for a given $\dot{V}O_2$. The cite of these adaptations appears to occur peripherally in the skeletal muscle during exercise.

2.2 Vascular and blood marker responses in endurance trained individuals

2.2.1 Flow mediated Dilation

Flow-mediated dilatation (FMD), was first introduced by Celermajer et al. (1992), where ultrasound imaging was used to measure conduit artery dilation in response to a period of ischemia, developed by supra-systolic occlusion. Following removal of the ischemic stimulus, the ensued hyperemic response imparts a heightened shear stress on the endothelial layer of tunica interna i.e innermost arterial layer in contact with blood. Which commences the endothelial release of NO and ultimately leads to vasodilation, in order to accommodate the increased blood flow, while simultaneously reducing the shear stress (Palmer, Ashton, et al., 1988). Indeed, the mechanistic link between NO and FMD was reported in a meta-analysis (Green et al., 2014), which revealed that NO contributed to ~70 % of the dilatory response in healthy young individuals. As impairment in endothelial function can lead to progressive vascular disease (Roquer et al., 2009; Vanhoutte et al., 2017), FMD been widely used as non-

invasive tool for assessing vascular dysfunction and cardiovascular disease risk (Anderson et al., 1995; Inaba et al., 2010; Schroeder et al., 1999), though the clinical efficacy has been questioned (Atkinson & Batterham, 2015). Likewise, conduit vessel functioning is crucial for endurance endeavors, as they solely dictate the upstream influx of blood flow from the heart to the microvasculature bed of working muscles.

Acutely, endurance exercise can affect FMD in a biphasic manner. Initially FMD is reduced immediately (< 30 min) post-exercise (Brick et al., 2014; Johnson et al., 2012), but as the resting duration extends (> 60 min) a super-compensatory effect is noticed (Cosio-Lima et al., 2006; Goel et al., 2007; Harris et al., 2008; Johnson et al., 2012), that ultimately reduces to baseline values within 24-48 h (Gonzales 2011). More importantly, the initial FMD reduction is dependent on exercise intensity and duration (Brick et al., 2014; Johnson et al., 2012), whereas factors such as oxidative stress, shear stimulus, baseline diameter, blood pressure and sympathetic neural activity are proposed to dictate the biphasic pattern seen post-exercise (Dawson, Green & Thijssen, 2013). Subsequently, routine endurance training has the ability to positively influence arterial function. For instance, 60-min running bouts (70% $\dot{V}O_{2peak}$) repeated over ten-days, improved FMD by 6% in sedentary adults (Landers-Ramos et al., 2016). Similarly, in recreationally active individuals, FMD improved by 2% following a mixed-intensity training programme (Clarkson et al., 1999). However, this change was noticed only after a longer (10 week) training block. Furthermore, a recent meta-analysis by Early et al. (2017) showed that improvements in FMD were dependent on both, training intensity and duration.

Physiologically, improvements in FMD have been related to a greater production and release of endothelial derived vasodilatory factors (Mitchell et al., 2008). Here, repeated exposures to increased sheer stress as seen with endurance training, result in an upregulation of eNOS and leads to greater NO available for vasodilation (Green et al., 2004). Additionally, Walther et al. (2008) reported sport-specific improvements in FMD wherein swimmers had larger brachial artery FMD (14 %) and cyclist had larger femoral artery FMD (8 %), further indicating the important role of sheer stress for favourable vasodilatory outcomes, as the working limbs would have experienced greater hyperaemic blood flow. Although, irrespective of sporting type, endurance trained individuals are reported to have greater systemic expressions of NO (Rognmo et al., 2008; Yol et al., 2020), which might explain the larger brachial artery FMD reported in trained compared to sedentary counterparts (Libonati, 2007), despite the former

group habitually taking part in lower body exercise. Likewise, FMD has been associated with $\dot{V}O_{2peak}$ (Kasikcioglu et al., 2005) and is therefore a valuable assessment tool to include in interventions that have the potential to increase NO bioavailability or impart vascular sheer stress by augmenting conduit vessel blood flow.

2.2.2 Nitric oxide

Nitric oxide (NO) is a free-radical gaseous molecule that has been widely recogniszed for its vasodilatory effect on blood vessels (Furchgott & Zawadzki, 1980; Palmer et al., 1987) and also plays a fundamental role in regulating vascular tone and blood flow (Vallance et al., 1989). Increased pulsatile blood flow at the onset exercise, imparts a sheer like stimulus on the vascular walls and is a potent stimulator of NO production. In response to the sheer stress, endothelial nitric oxide synthase (eNOS), present within the endothelial layer, converts L-arginine to L-citrulline and NO in the presence of O_2 (Palmer, Rees, et al., 1988). NO then diffuses within the vascular smooth muscles where it binds to guanylate cyclase to produce cyclic guanosine monophosphate which ultimately induces Ca^{2+} dependent vascular muscle relaxation. The above signaling cascade of NO production and the subsequent vasodilation of blood vessels allows for a greater muscle perfusion seamlessly matching the O_2 demand during exercise.

Regular endurance training has the potential to increase the activity of eNOS and likewise bioavailability of NO. A detailed summary by (Maiorana et al., 2003) based on animal investigations indicate a time-dependent nature of NO adaptation, wherein short-term training (< four-weeks) enhances endothelial NO production and longer training regimes (> eight-weeks) facilitate NO-mediated structural adaptations, such as increase in vessel diameter. In humans, longer training regimes (>1-month), activating large muscle groups, have been shown to systemically increase nitrite/nitrate (NO_2^-/NO_3^-) expression in plasma (Maeda et al., 2004; Maeda et al., 2001). Here, NO_2^- and NO_3^- are inert oxidized products of NO and are indirectly used to reflect endothelial NO synthesis (Grau et al., 2007). The improvements in NO bioavailability are important from a peripheral delivery standpoint, as it increases the partial pressure of O_2 within the microvasculature (PO_2mv) (Hirai et al., 2010). Maintaining a high PO_2mv through vasodilation of feed arteries and arterioles is crucial for the forward flux of O_2 from the capillaries into the myocyte (Wagner, 2017). A higher microvascular O_2 conductance mediated by NO would aid in sustaining a greater OXPHOS, reduce intracellular metabolite

formation and possibly speed muscle $\dot{V}O_2$ kinetics (Kindig, Walsh, et al., 2005; Poole, Ferguson, et al., 2022) . Furthermore, as multiple capillaries supply O_2 to resting muscle fibres, and presuming all other factors are equal (blood pressure and concentration of metabolites) any increase in blood flow upstream would increase the RBC velocity within the capillaries (Poole, 2019). This would promote a higher number of RBCs in contact with the myocyte, increasing the diffusive capacity of O_2, ultimately leading to a greater extraction capacity (Roca et al., 1992).

Additionally, endogenous heat production is a major by-product (80%) of repeated muscular contractions, as reported during whole-body aerobic exercise, and the skin is the main site for thermoregulation. Likewise, cutaneous (i.e., skin) blood flow is the primary mode of heat dissipation and is significantly increased by vasodilatory mechanisms, mostly controlled by sympathetic neural activity (Charkoudian, 2003). However, NO-mediated cutaneous vasodilation also plays an important albeit modest role (~30%) in increasing blood flow to the skin during exercise (Mcnamara et al., 2014). Interestingly, endurance trained individuals display a greater NO-dependent microvascular reactivity at the cutaneous level (Boegli et al., 2003), and could possibly account for the higher skin blood flow reported in trained compared to untrained counterparts when exercising at the same relative intensity (Fritzsche & Coyle, 2000). Enhanced heat dissipation through a higher cutaneous circulation might allow individuals to comfortably perform at a higher metabolic rate despite the greater heat production.

Similar to exercise, passive heating has equally shown to improve NO-mediated vascular function. Hesketh et al. (2019) reported an increased expression of eNOS in skeletal muscles following six weeks of PH and this was simultaneously accompanied by an increase in capillarisation. Together, these adaptations would favour better O_2 delivery and extraction at the muscular level and might have contributed to the improvements noted in aerobic capacity post-intervention. Acute passive limb heating studies support the above notion as seen by a significant increase in skeletal muscle blood flow (Heinonen et al., 2011) and muscle oxygenation (Pearson et al., 2011) in response to elevated muscle temperature. Although the degree to which NO increases skeletal muscle microvascular conductance during passive heating is yet to be elucidated.

Majority of the passive heating modalities require direct application of heat over the skin, logically making it the primary stimulated site. Comprehensive work carried out by Brunt et al. (2016) showed that eight-weeks of passive heating improved NO-mediated cutaneous circulation. Using NO blockers (L-NAME), (Del Pozzi et al., 2013) found that NO contributed towards 28% of the overall cutaneous microvascular conductance rate following the application of local skin heating. Providing further evidence of an existing similarity between passive heating and dynamic exercise with respect to NO-mediated vasodilatory expression. The improvements in NO-derived vascular function can be attributed to two main reasons. First, just like exercise, passive heating causes an overall increase in blood flow to the peripheral regions of the body, which mechanically imparts sheer stress on vascular walls. Elegant contralateral experiments (cuffed *vs.* uncuffed arm) conducted by (Green et al., 2010) showed that passive heating (eight-weeks) improved NO-mediated vascular conductance only in the arm (uncuffed) in which skin blood flow was allowed to increase. These studies provide strong evidence that sheer stress is physiologically important for vascular adaptations and does so by increasing eNOS phosphorylation (Iring et al., 2019), resulting in greater NO release. Second, increasing the temperature of muscular tissue and skin could possibly alter the expression of molecular regulators specifically heat shock proteins, which could potentially augment eNOS activity (Sun & Liao, 2004) and NO production (Grau et al., 2007).

2.2.3 Vascular endothelial growth factor

Vascular endothelial growth factors (VEGF) are most widely recognized as endothelial specific mitogens responsible for the growth and development of the circulatory system (Ferrara et al., 2003). The regulation of angiogenesis by VEGF ensures the appropriate formation of new blood vessels to satisfy various physiological demands such as; wound healing, regulating blood flow and maintaining tissue oxygenation (Chintalgattu et al., 2003; Roy et al., 2006; Tammela et al., 2005). However, dysregulation of VEGF can lead to pathologies associated to ischemia (insufficient angiogenesis) or tumor growth (excessive angiogenesis) (Dumont et al., 1998; Ferrara, 2000; Sherwood et al., 1971).

Proliferation of the capillary network surrounding skeletal muscles is one of the most prominent hallmark adaptations associated with regular endurance training (Klausen et al., 1981; Murias et al., 2011). Invasive investigations have revealed that VEGF is present in endothelial cells, pericytes and interstitial space surrounding the myocytes and more

importantly within myocytes itself (Gustafsson et al., 2002; Wagner, 2011b). Making it a key angiogenic factor for capillarisation at the peripheral end. Seminal work by Olfert et al. (2009) showed that mice purposely bred with VEGF deficiency expressed a 40 % reduction in muscle capillarity and this translated to an 80 % reduction in endurance capacity compared to healthy controls. Using a similar transgenic mice model, the same group reported no changes in capillarity and endurance capacity following six-weeks of endurance training (Olfert et al., 2010). Interestingly, training was able to increase oxidative enzyme activity in VEGF deficient mice to a similar extent as controls (Olfert et al., 2010), the fact that this had no effect on endurance performance provides unequivocal evidence for VEGF-induced angiogenesis as a prerequisite for experiencing training related outcomes.

In addition to the angiogenic property, VEGF also plays an important role in acute regulation of sheer stress by increasing blood vessel permeability (Dvorak et al., 1995) and facilitating vascular relaxation (Ku et al., 1993). Mechanistically, it has been shown that VEGF, along withs its receptors, are involved in the activation of eNOS, which is in turn responsible for endogenous production of NO (Hood et al., 1998; Tilton et al., 1999). Clinical trials in humans involving chronic intravenous administration of VEGF have been successful in reducing resting systolic blood pressure (Henry et al., 2001, 2003). Furthermore, pharmacological inactivation of VEGF can significantly reduce endothelial dependent vasodilation (Thijs et al., 2013). Accordingly, some have proposed that the vasomotor effect of VEGF is more prominent at the descending level of the vasculature i.e., arterioles, capillaries and venules (Laham et al., 2003; Walder et al., 1996). This might be vital from an endurance performance perspective as it signifies the final step for O_2 delivery.

In humans, acute bouts of exercise have been shown to increase the expression of VEGF mRNA in activated skeletal muscles. The time-course is such that VEGF mRNA expression peaks between 1–2-h post-exercise and can remain elevated for 6-h, before returning to baseline (Hiscock et al., 2003; Hoier et al., 2013; Hoier & Hellsten, 2014). Contrastingly, changes in muscle VEGF protein concentration have not been conclusive, with some showing a reduction (Gavin et al., 2004) and others reporting no changes post-exercise (Rullman et al., 2007). On the other hand, 10-days of single-leg endurance training was shown to increase resting VEGF mRNA expression and protein levels simultaneously in healthy adults (Gustafsson et al., 2002). Interestingly, extending the same single-leg training to five-weeks had no effect on resting VEGF mRNA and protein levels in recreationally trained adults

(Gustafsson et al., 2007). Likewise, Hoier et al. (2020) reported no changes in the same following two weeks of cycling at 70% $\dot{V}O_{2peak.}$ Although methodological differences exist, mainly in sampling time, training protocol and intervention duration, all the studies mentioned above reported a consistent transient increase in VEGF mRNA expression following acute bouts of exercise. Which indicates that the ability of the myocyte to secret VEGF protein into the interstitial space and its subsequent replenishment by VEGF mRNA following each exercise bout might be more important for training-related angiogenesis compared to elevated basal or resting levels (Hoier & Hellsten, 2014).

With the apparent secretion of VEGF into the interstitial space (Höffer et al., 2003; Hoier et al., 2012), it is logical to assume passage through the circulatory system. Hiscock et al. (2003) reported that 3-h of bilateral knee extension significantly elevated circulating plasma VEGF (venous) in physically active males 1-h post-exercise. Similarly, in well trained female rowers rowing for 1-h at a high intensity, plasma VEGF elevated immediately after the bout and returned to baseline within 30-min (Jürimäe et al., 2018). More importantly, resting VEGF plasma levels seems to be similar in endurance trained individuals compared to sedentary counterparts and acute exercise can elevate circulating plasma VEGF levels irrespective of training status (Kraus et al., 2004). Although it must be noted here that the amount circulating VEGF depends on type of sample analysed, as serum VEGF can be five to six fold greater than plasma VEGF obtained from the same individual (Kraus et al., 2004; Lee et al., 2000; Werther et al., 2002). However, there is a strong correlation between serum and plasma VEGF and, therefore, either can be used to track circulatory VEGF levels (Kraus et al., 2004).

The inconclusive peripheral and systemic changes in resting VEGF after routine training indirectly indicates the tight regulation of this important growth factor. Hypoxia-inducible factor (HIF-1a) is a well-known regulator of VEGF, whereby reductions in partial O_2 pressure (PO_2) results in the binding of a specific promoter gene to HIF-1a, ultimately leading to VEGF transcription (Forsythe et al., 1996). Relating back to a continuous exercise stimulus, which is characterised by repeated cycles of contraction and relaxations and at higher intensities can reduce PO_2 to 3 to 4 mmHg (Richardson et al., 1995), might similarly activate HIF-1a and hence increase VEGF. Acutely, exercise also leads to increase in skeletal muscle PGC1-a expression, which has been linked to VEGF regulation specifically through the estrogen related receptor pathway (Ohno et al., 2012).

2.2.4 Heat shock Proteins

Heat shock proteins (HSP) are the most widely conserved family of proteins present in almost all biological organisms. Under normal conditions, they facilitate folding of newly formed polypeptides, unfolding and refolding of denatured proteins, disaggregation of misfolded proteins and act as chaperons for safe translocation of proteins to the correct cellular destinations (Hartl et al., 2011; Saibil, 2013). Thus, HSPs ensure the complex regulation and maintenance of proteostasis. However, under environmental (temperature) or metabolic (low pH and or disrupted redox state) stress, HSPs are upregulated for the purpose of attenuating protein damage and act as buffers to restore cellular homeostasis and prevent cell death (Whitley et al., 1999).

HSP27

HSP27 is a member of the small HSP family (sHSP) which is mainly present within the cytosol but can migrate towards the nucleus and mitochondria during cellular stress (Bruey et al., 2000). These proteins mainly act as intermediary chaperones that sequestrates unfolded or misfolded proteins for subsequent folding by other HSPs and prevents protein aggregation (Haslbeck et al., 2005). Unlike other HSPs, HSP27 functions in a holdase format and is therefore ATP-independent but are widely involved in growth/development (Singh et al., 2017) and provide protection against thermal (Krueger-Naug et al., 2000) or oxidative stress (Arrigo, 2001). More importantly, HSP27 is present in the myofibrillar compartment of muscle fibers and its phosphorylation is thought to stabilize the actin filaments, while also protecting the cytoskeleton structure from mechanical stress (Dalle-Donne et al., 2001; Landry & Huot, 1995; Lavoie et al., 1993). This indicates an important role of HSP27 in regulation of muscle damage and repair after an exercise stimulus. Likewise, prolonged endurance exercise (half-marathon) has shown to increase circulating HSP27 that returns to baseline only after 24-h (Fehrenbach, Niess, et al., 2000). Similarly, high-intensity exercise lasting only two minutes can significantly elevate serum HSP27 levels immediately post-exercise (Kon et al., 2021), however recovers at a quicker rate (3-h) compared to longer exercise bouts (Fehrenbach, Niess, et al., 2000; Kon et al., 2021). Interestingly, Kon et al. (2021) reported a positive correlation was reported between serum HSP27 and oxidative stress makers (reactive oxygen species). The above relation could be attributed to the indirect scavenging like action of HSP27, which upregulates intracellular levels of glutathione and glucose-6-phosphate dehydrogenase that are known for ROS

detoxification (Arrigo, 2001; Préville et al., 1999). On the other hand, systemic increases in HSP27 have been reported under passive heat stress in animal models (Mohanarao et al., 2014; Krueger-Naug et al., 2000) and also in humans completing exhaustive exercise in the heat (Périard et al., 2012), which is related to the degree to which core temperature is elevated. Here, systemic expression of HSP27 is thought to be produced by the CNS systems for cytoprotection against thermal shock.

HSP70

Out of all the HSP family, HSP70 is the most highly investigated protein that is both highly conserved and ubiquitous in nature (Lindquist & Craig, 1988; Rosenzweig et al., 2019). In normal cellular conditions, HSP70 (HSC70) act primarily as molecular chaperones, assisting in protein synthesis, folding and transportation across cellular membrane (Brocchieri et al., 2008). Under stress, HSP70 is highly inducible and acts as a potent cytoprotective agent, although, both the above functions are ATP dependent making it energy costly (Young, 2010). Initially, the inducible nature of HSP70 was majorly linked to heat stress/hyperthermia (Jolesch et al., 2012) but further investigations revealed that lowering of pH (Rafiee et al., 2006), increased reactive oxygen species (ROS) production and tissue ischemia (Szyller & Bil-Lula, 2021; Xia et al., 2017) can also lead to HSP70 upregulation. Exercise is well known to collectively generate all of the above inducible factors and, therefore, creates a strong link to HSP70. Puntschart et al. (1996) were the first to show that running for 30-min at GET was able to increase HSP70 mRNA concentration in working muscle cells but did not alter protein levels. Later, it was observed that intramuscular HSP70 protein content was elevated only after a week following a moderate-intensity exercise stimulus (Khassaf et al., 2001), depicting a delayed timeframe for exercise-related upregulation. Chronic endurance training (> three-weeks) studies have revealed that HSP70 protein content can be elevated even in well-trained athletes, albeit dependent on training intensity rather than volume (Liu et al., 1999, 2004). Similarly, circulating HSP70 was elevated after a half-marathon race (Fehrenbach, Passek, et al., 2000) but was unchanged following an hour-long exercise at a lower intensity (Shastry et al., 2002). Interestingly, trained athletes displayed a similar intramuscular HSP70 content compared to untrained individuals (Morton et al., 2008); however, circulating HSP70 seemed to be higher in the later (Shastry et al., 2002). Contrastingly, resting blood HSP70 are elevated in previously unacclimated individuals after heat acclimation programme (HA), indicating a higher cytoprotective capacity (McClung et al., 2008; Sandström et al., 2008). Here, the HSP70

response to repeated thermal stress is biphasic, wherein basal HSP is initially reduced (five to six-days of heat exposure) but after further exposure (10-14 days) is elevated (Horowitz, 1998). The systemic increase in HSP70 level might be regarded as a cumulative outcome, allowing for rapid handling of denatured proteins produced during future heat stress (Murray et al., 2022).

2.3 Passive Heating

Passive heating involves the use of an external heating source to artificially expose an individual, usually in a rested or sedentary state, to a tolerable/non-lethal thermal stress for the purpose of promoting positive physiological outcomes. Historically, the thermal challenge induced by passive heating mimics that of traditional HA protocols, which have proposed the rise in core temperature (> 38 ºC) to be the driver of thermoregulatory adaptations (Horowitz, 2016; Périard et al., 2015). However, passive heating protocols that are considered to be milder (Pallubinsky et al., 2017) or do not raise the core temperature above 38 ºC (Carter et al., 2014) have also been shown to elicit beneficial adaptations. Common modalities of PH are briefly described below.

2.3.1 Sauna

Sauna chambers come in a wide variety, with Finish dry sauna being the most common of all. Usually, the air room temperature falls within the range of 80-100 ºC with the floor maintained at 30 ºC and the relative humidity set at 10-20%, making it a hot-dry environment (Helamaa & Aikas, 1988). Typically, individuals will complete 10-30 min exposures with or without rest intervals (coming out of the sauna) in between (Hannuksela & Ellahham, 2001; Kukkonen-Harjula & Kauppinen, 2006). From a thermoregulatory perspective, Finish sauna can elevate skin temperature up to 40 ºC within few minutes (Iwase et al., 2013) and elevate core temperature by 1-2 ºC (Leppaluoto et al., 1986).

Alternatively, Waon sauna therapy involves the use of far-infrared rays maintained at 60 ºC. Miyata and Tei, (2010) were the first to extensively investigate the use of Waon therapy in chronic heart failure and peripheral heart disease. Due to the ability of the infrared rays in seamlessly penetrating the skin, core temperature can rise by 1-1.2 ºC within 15-min, after

which individuals exit the chamber and are wrapped in a thermally insulative blanket to preserve the internal heat for a further 30-min.

2.3.2 Hot water immersion

Hot water immersion (HWI) is the most widely used passive heating modality, primarily due to the lower equipment set-up demands and superior thermal conductivity of water compared to air. Typical water temperature is maintained between 38-42 ºC, with immersion durations ranging from 10-90 min of continuous exposure (An et al., 2019). Likewise, only waist-level immersion (42 ºC) can elevate core temperature by 1 ºC within ~45 min (Rodrigues et al., 2020). Additionally, immersion itself imparts a hydrostatic pressure that results in a central shift of blood volume, increasing cardiac filling volume and, subsequently, resulting in higher SV and $\dot{Q}$ (Begin et al., 1976).

Furthermore, contemporary HA strategies have widely used hot water immersion in a PH format (Zurawlew et al., 2016, 2018b, 2019). The advantages with such a format are not only limited to the economical ease of set-up but also imposes a lower training hindrance compared to traditional HA protocols. Major adaptations reported with the above strategy include lowering of resting core temperature, end exercise core temperature, core temperature threshold for sweating onset and increased whole-body sweat rate while subsequently improving thermal comfort and endurance performance in the heat (McIntyre et al., 2021; Zurawlew et al., 2016, 2018b, 2018a, 2019). More importantly, these adaptations have been recognized with only six consecutive PH exposures (McIntyre et al., 2021), with a recent study reporting significant thermoregulatory improvements reported within three days of commencement, which contributes to 57 % of the total improvements seen after six days of PH via hot water immersion (McIntyre et al., 2022). Here, reduction in resting core temperature seems to be the most important factor explaining 80 % of the thermoregulatory improvements when exercising in the heat (McIntyre et al., 2022), although the physiological mechanisms for the above phenotypic expression is not fully known, alterations in neural wiring within the hypothalamus has been proposed as the likely cause (Tan et al., 2016; Zhao et al., 2017). The remaining 20 % might be explained by the body's ability to lose heat following repeated PH hot water immersions (McIntyre et al., 2022). Likewise, the quicker onset and higher sweat rate reported following such interventions, favor the possibility that a higher proportion of $\dot{Q}$ is reserved for cutaneous circulation. The favorable peripheral adaptations with hot water immersion might

be attributed to the combination of greater efferent activity of neurons located on the skin (Horowitz, 2016), and a lower exercising heart rate that would prolong the time available for ventricular filling allowing greater stroke volumes (Turkevich et al., 1988; Zurawlew et al., 2019). Finally, hot water immersion as a modality is effective in simultaneously increasing skin and core temperature, this dual nature has been proposed to facilitate superior thermal-related adaptions compared to traditional HA or exercise alone (McIntyre et al., 2022; Regan et al., 1996).

2.3.3 Hot water circulating or Steam generation suits

Steam or hot water circulating suits are novel textile innovations that can impart direct skin (local) heating with the added benefit of easily regulating the temperature of the water or steam perfused through them. The common circulating temperatures reported in the literature vary between 44-50 ºC, and can raise both muscle and skin temperature beyond 38.5 ºC (Goto et al., 2011; Kim et al., 2020; Ko et al., 2020). Another advantage of such a system is that isolated or area-specific heating can be conducted and, therefore, protocol duration can vary between 2-8 h, indicating a higher tolerable duration compared to the previously described modalities.

2.3.4 Diathermy

Pulse shortwave and micro-wave are the two most common forms of diathermy that use high-frequency electro-magnetic currents or radiations to generate heat over very specific targeted areas (Fu et al., 2019). Common pulse wave protocols include 800 pulses/s and can rapidly increase localized muscle temperatures by 4.2 ºC (Hafen et al., 2019). On the other hand, micro-wave diathermy can elevate muscle temperature up to 41 ºC (Nosaka et al., 2007). Due to the highly localized heating (covering extremely small body surface areas) treatment duration can vary between 2-8 h and, therefore, this modality has been widely used in limb immobilization studies (Hafen et al., 2019; Hyldahl et al., 2021). This approach remains somewhat limited by the localized treatment, which focuses on one area of interrogation and has no effect on core body temperature (Adair et al., 2001).

2.3.5 Acute effects of passive heating

Changes in body temperature have been one of the main fundamental driving forces for a vast variety of phenotypic and genotypic adaptations across almost all species living on earth. Humans are homeothermic and their survival depends on maintaining internal temperatures (brain temperatures) around a narrow range of ~36-38 ºC (Kenney & Munce, 2003). This makes the human thermoregulatory system extremely sensitive/reactive to stressors (Hot or Cold) that can disturb thermal homeostasis. Skin being the largest sensory organ plays an important role in sending positive or negative feedback (afferent) to the CNS (hypothalamus), which is processed and relayed back as efferent signals to induce either vasodilatory (purpose: loose heat and prevent hyperthermia) or vasoconstrictive (purpose: conserve heat and prevent hypothermia) response (Romanovsky, 2014). As PH creates an hyperthermic environment this section will focus on physiological responses related to the same.

Cutaneous or skin vasodilation can be considered as the initial response of PHT, with the onset occurring by a minimal 0.3 ºC increase in core temperature (Brengelmann & Savage, 1997; Wyss et al., 1974). Likewise, independently heating the skin to ~ 38-40 ºC can also result in maximal dilation of blood vessels supplying the skin (Johnson et al., 1986; Pergola et al., 1993). Local or direct warming of the skin causes vasodilation in a biphasic manner characterised by an initial rapid rise, followed by a sustained maximal plateau in skin blood flow that persists as long as the heating stimulus (Kellogg, 2006). The initial rise is brought about by release of specific neurotransmitters from temperature-sensitive vanilloid receptors located on afferent nerve endings (Charkoudian, 2003; Kellogg, 2006). The secondary plateau phase is thought to be predominantly mediated by NO, with its formation linked to HSP90 mediated eNOS activation (Xi et al., 2005). Although, a variety of other vasoactive substances have been found which are proposed to co-contribute in heat-induced cutaneous vasodilation (Brunt et al., 2013; Wong et al., 2004; Wong & Minson, 2006). The peripheral demands for increased blood flow are met by alterations in central cardiovascular activity. Most notably, hot water immersion or sauna can increase $\dot{Q}$ from ~ 5 to 6 L/min (Cheng et al., 2021; Radtke et al., 2016), facilitating an increase in skin blood flow by ~ 6-8 L/min, that accounts for 60 % of the $\dot{Q}$ (Minson et al., 1998; Rowell, 1974). The increase in $\dot{Q}$ is in turn supported by an elevated heart rate, as such, peak heart rates of ~100 beats/min have been observed during PH (Chiesa et al., 2016; Chou et al., 2018; Henderson et al., 2021). In contrast, stroke volume does not appear to change during passive heating; however, blood pressure and peripheral vascular resistance is significantly reduced (Crandall & Wilson, 2015). More recently, it was seen that an acute bout

of HWI (40.5 ºC for 60-min) reduced mean arterial pressure by ~ 15 %, additionally, 60-min post HWI, mean arterial pressure remained reduced by ~ 8 mmHg, which was comparable to post-exercise hypotension (Francisco et al., 2021).

The acute molecular response to passive heating is characterised by the upregulation of cytoprotective or inflammatory proteins, such as HSPs and interleukin- 6 (Ely et al., 2018). Chung et al. (2008) was the first to show that a single exposure of passive heating increased intramuscular HSP72 and downregulated c-jun amino terminal kinase (JNK) phosphorylation in transgenic mice models, deeming it as potential therapeutic tool against insulin resistance. Contrastingly, in humans acute heating using HWI (39-45 ºC for 60 min) did not increase intra monocyte or muscular HSP72 (Hoekstra et al., 2018; Morton et al., 2007). Interestingly, circulating (plasma) HSP70 was elevated after a similar HWI protocol in healthy adults (Faulkner et al., 2017) but was unchanged in sedentary/overweight individuals (Hoekstra et al., 2018), implying baseline body composition might influence the thermal load experienced. Likewise, the expression of IL-6 seems to be dose dependent, with longer HWI (~ 2-h) protocols generating greater responses (laing 2008; Faulkner et al., 2017). Finally, a single bout of lower-body heating (water suit: 48 ºC for 90 min) was able to upregulate mRNA expression of VEGF and multiple HSP families (Kuhlenhoelter et al., 2016), indicating the creation of an angiogenic environment and might explain the increases in plasma NO reported elsewhere (Hoekstra 2018).

2.3.6 Chronic whole-body heating

The acute responses to passive heating drastically mimic those associated with exercise and it, therefore, would be both logical and scientifically rational to assume that passive heating over time could improve cardiorespiratory fitness. Indeed, Ohori et al. (2012) were the first to report that Waon therapy (five times/week for three-weeks) in chronic heart failure patients increased $\dot{V}O_{2peak}$ by 8 %. Likewise, in moderately-active females, eight-weeks of HWI (42 ºC for 30-min, three times/week) increased $\dot{V}O_{2peak}$ by ~3 ml/kg/min, which was similar to a time-matched endurance training programme (Bailey, Cable et al., 2016). Similarly, in sedentary males who underwent six-weeks of passive heating using a heat chamber (40 ºC, 40-50 min, three times/week) a 5 % improvement in $\dot{V}O_{2peak}$ was noted (Hesketh et al., 2019). Once again, the changes in $\dot{V}O_{2peak}$ were comparable to time-matched moderate-intensity endurance training (Hesketh et al., 2019). More recently, ergogenic effects of passive heating on $\dot{V}O_{2peak}$ were

reported in semi-professional soccer players (Bartolomé et al., 2021), indicating that improvements favouring endurance capacity were not limited to sedentary or clinical population. However, it must be noted that the above study used climatic chambers at 100 ºC (40-min, three times/week for three-weeks) and, therefore, higher thermal intensities might be required for already adapted phenotypes.

It is well-established that $\dot{V}O_{2peak}$ is dependent on the integration of O_2 delivery and metabolism (Wagner, 2011a). Considering the above, improved haemodynamic redistribution post chronic whole-body passive heating has been proposed to be the main reason for the positive changes in $\dot{V}O_{2peak}$ reported above. For instance, the 5-8 % improvement in $\dot{V}O_{2peak}$ occurred alongside significant increases (2-6%) in brachial artery FMD (Bailey, Cable et al., 2016; Ohori et al., 2012). Extensive work by others (Brunt, Eymann et al.,2016, Brunt, Howard et al., 2016, Brunt et al., 2018 & 2019) have clearly demonstrated a superior functioning of the vascular system following long-term (eight weeks) HWI (40.5 ºC, 4-5 times/week). FMD in sedentary individuals increased by 5% (Brunt, Howard et al., 2016), which could be attributed to the ability of the endothelial cells to generate NO. The hyperemic response of passive heating creates a vascular wall shear stress stimulus (Chiesa et al., 2016), well known for driving endothelial adaptations (Zhou et al., 2014). Alternatively, repetitive bouts of passive heating might increase expression of HSP90 and indirectly increase the capability of eNOS to generate NO via direct allosteric activation or increasing its affinity for Ca^{2+}/calmodium pathway (Brouet et al., 2001; García-Cardeña et al., 1998). Structurally, eNOS has a Ca^{2+}/calmodium binding site and therefore its enzymatic functioning is proposed to be dependent on the formation of eNOS- Ca^{2+}/calmodium complex (Busse & Mülsch, 1990). Likewise, intra or extracellular manipulation of Ca^{2+} availability can directly alter eNOS activity (Fleming, 2010), however, HSP90 can increase the sensitivity of eNOS to Ca^{2+}/calmodium and can directly facilitate protein-kinase B mediated phosphorylation (Takahashi & Mendelsohn, 2003), ultimately increasing the production of NO. Indeed, following a similar chronic intervention cutaneous microvascular dilation also improved (Brunt, Eymann, et al., 2016), these improvements were lost with pharmacological administration of NO inhibitors. These findings imply that passive heating has the ability to improve both systemic and peripheral delivery of O_2. More importantly, the passive heating model of Hesketh et al. (2019) was successful in increasing capillary density by 21 % and eNOS content by 8% in the vastus lateralis. This is an extremely important finding, specifically from an endurance exercise

standpoint, as the improvements in upstream haemodynamic characteristics, such as conduit artery diameter of vasodilatory capacity, can only be realised if the distal microvasculature surrounding the working musculature is able to effectively facilitate blood distribution and oxygen perfusion (Murias et al., 2010a, 2011) .

The transfer of O_2 to the mitochondria is complex, wherein oxygenated RBC travel down the pressure gradient; capillary wall → interstistium → sarcolemma → inner myocyte housing the mitochondria (Poole, et al., 2022). Here, a greater capillarisation would increase the capillary surface area surrounding the activated musculature, facilitating a superior spread of O_2 across the myocytes and also prolong the time available for mitochondrial O_2 uptake by increasing the RBC transit time (Gayeski et al., 1988; Richardson et al., 1994). The superior perfusive environment created by the above adaptations would aid in preserving muscle oxygenation during exercise, subsequently promoting greater O_2 extraction (Poole, 2019; Wagner, 2015). Furthermore, apart from the vasodilatory effects of NO, this molecule might also play a role in mitochondrial oxidative capacity and contractile function (Poole, et al., 2022). For instance, in an *ex-vivo* mice model (Coggan & Peterson, 2018), it was seen that sodium nitrite administration improved sarcoplasmic reticulum Ca^{2+} handling and delayed fatigue onset. Although, NO-related changes in contractile function of humans have been equivocal (Coggan & Peterson, 2018). Interestingly, 11-days of passive heating (heat chamber; 48-50 ºC, 60 min/day) increased maximal voluntary contraction by 11 % (Racinais et al., 2017); however, no molecular responses were measured and, therefore, based on previous chronic passive heating studies, a potential role of NO cannot be disregarded. Recently, following the same chronic HWI protocol, Brunt et al. (2019), reported a 1.4-fold increase in systemic eNOS i.e., in serum, which would effectively improve NO bio-availability and would render a positive impact on the endurance phenotype (Bailey et al., 2010; Bailey, Blackwell et al., 2016; Giannesini et al., 2011).

2.3.7 Chronic direct-skin heating

Passive heating studies utilising direct-skin (contact heating) have predominantly focused on peripheral adaptations. This is logical, since both skeletal muscles and the microvasculature (capillaries) exhibit a certain level of plasticity, whereby increased demand (exercise → heat → mechanic disturbance → metabolic perturbation) would facilitate growth/proliferation and reduced demand (immobilisation) would result in deterioration. Additionally, localised heating

can rapidly increase the temperature of the area being heated, skin and muscle being closest to the heat source would therefore be the primary site of response. Support for the above was extensively provided, first in preclinical animal models that underwent chronic passive heating (2 - 4 weeks), with improvements reported for muscle mass regulation, mitochondrial biogenesis and angiogenesis (Dodd et al., 2009; Naito et al., 2000; Ohira et al., 2017; Tamura et al., 2015; Yoshihara et al., 2013). These studies collectively indicate positive adaptations, which would be beneficial for endurance capability.

In humans, Goto et al. (2007, 2011) was the first to show to significant increases in muscle cross-sectional area after chronic passive heating (8 -10 weeks) using steam generating sheets. The protocol required very long induction periods (8 h, 4 days/week) and rapidly increased muscle temperature by 2-3 ºC, simultaneously, a three-fold upregulation of the oxidative gene; ubiquinol cytochrome c reductase was reported (Goto et al., 2011). Likewise, using an aggressive diathermy protocol i.e., raising muscle temperature by 4 ºC, albeit with short induction days (6 -10 days, 2-h/day) attenuated vast negative consequences associated with limb immobilisation (Hafen et al., 2018, 2019). The prevention of disuse atrophy was proposed to be related to an increase HSPs (HSP70 and 90) and upregulation of PGC1-a, key molecular regulators of muscle regeneration (Washington et al., 2022) and mitochondrial adaptation (Taylor & Bishop, 2022). More interestingly, Hafen and co-authors also reported increases in inner-mitochondrial content of major electron transport chain proteins; I, IV & V, indicating an improved respiratory capacity at the muscular level. The electron transport chain is imperative for energy metabolism, whereby complex I to IV oxidize metabolic products; NADH and $FADH_2$ creating a proton (H^+) gradient that drives complex V to generate ATP. Accordingly, ETC protein content is well correlated with $\dot{V}O_{2peak}$ (Greggio et al., 2017), which further strengthens the endurance biased effects of chronic passive heating.

Similar to whole-body passive heating, eight-weeks of passive heating using water perfused suits (52 ºC, 90 min for five days/week) in young sedentary adults, increased muscular eNOS content by 18% and 35% at week four and eight, respectively (Kim et al., 2020). Adding further evidence that heat-induced angiogenic improvements are related to endogenous NO expression and availability. Interestingly, the above passive heating protocol increased peak voluntary torque of knee extensors, which is in agreement with previous reports (Goto et al., 2007, 2011). Whether improvements in muscle contractile ability are related to intramuscular NO

availability remain to be investigated. Contrastingly, Labidi et al. (2021) reported no improvements in cross-sectional area or contractile function after six-weeks of single-leg passive heating (heat pads; 8-h/day, five days/week). The results of the above study might be explained by two main reasons; (i) the single-leg heating was limited only to the calf region and, therefore, the area of heating might be an important factor (ii) the participants were recreational (endurance sports) active and therefore an alternative dosing strategy might be required for this sub-group.

To conclude, the adaptations associated with passive heating seem to induce favorable cardiovascular outcomes that mimic those observed with routine endurance training. At present, there is a wealth of evidence favoring passive heating as an exercise replacement (where necessary), adjunct therapeutic strategy or an isolated rehabilitation tool for sedentary and clinical population subgroups. However, it is uncertain whether similar passive thermal interventions can induce the abovementioned adaptations in already trained phenotypes, and whether it can be used concurrently/additively with routine endurance training to maximise training outcomes. The current study aimed to investigate the effects of 20 consecutive post-exercise passive heating sessions across four-weeks. It was hypothesised that supplementing training with a home-based post-exercise passive heating intervention would improve endurance performance to a greater extent compared to exercise-alone controls.

Chapter 3.0 Methods

3.1 Participants

A total of 27 male and 7 female adults volunteered to take part in the study. Participants were randomly assigned to the post-exercise passive heating group (PH) or the control group (CON). Baseline fitness levels and training volume was also taken into consideration (3.3) when assigning individuals into their respective group. A-priori sample size was calculated using G*Power (version 3.1, Universität Düsseldorf, Germany) based on changes reported in $\dot{V}O_{2peak}$ after lower body PH (F = 0.544; Hesketh et al., 2019). Likewise, 15 participants per group was considered to be a sufficient sample size, for achieving a power of 0.80 and $\alpha = 0.05$ using an ANCOVA model with numerator degrees of freedom = 1. To account for dropouts, the study

over-recruited by two participants in each group. Accordingly, there was one drop out in PH and three dropouts in CON, leading to a final sample size of n = 16 in PH and n = 14 in CON. Both groups were required to continue their normal training and record their food intake, with the PH group required to supplement training with limb heating (3.4). Prior to being recruited, participants were initially screened for the inclusion criteria, which were: (i) partaking in regular endurance training (> 150-min per week), (ii) not affected by any cardiovascular or neuromuscular pathologies and (iii) have not taken part in any structured training in the heat in the past year or visited a hot country in the previous three-months. The study was conducted in UK, between the months of January and July (temperature ranging from ~10-21 °C). This timeline ensured all participants to be unacclimatised to the heat. All participants were also informed to avoid using hot baths and saunas throughout the participation period (four-weeks) of the study.

The study was approved by Swansea University, College of Engineering Research Ethics and Governance Committee (KJ_01-10-21). All participants were clearly informed of the study design, experimental procedures and potential risks associated with partaking in the study. After the initial-screening a written informed consent was obtained from each participant.

3.2 Design

After the initial screening (3.1), a familiarisation visit was organised for each participant, one-week prior to them starting the study. Upon arrival, participants were given clear instructions regarding pre-testing guidelines for day one and two of each testing block, which was also electronically shared with each participant. After this, participants were familiarised with the cycle ergometer (3.5), ideal saddle and handlebar positions were recorded for each participant and repeated for all subsequent visits. Likewise, mask fitting was also conducted and the appropriate size was recorded ensuring minimal air leakage during pulmonary gas data assessment (3.5). Furthermore, as the single-leg critical torque test was the most unconventional exercise test of all, it was deemed necessary to familiarise the participants using a reduced (3-min) format of the test (3.6.2). Finally, each participants intended training volume (min) was self-forecasted for the four-week duration of the study and was later used during the group allocation process to ensure matching between groups (3.3).

After the initial familiarisation visit, participants reported to the laboratory on six different occasions, across a four-week period. Data collection was divided into three testing 'blocks' (Figure 1): two consecutive days in the 72-h prior to the first day of intervention (PRE); two consecutive days in the middle of the four-week intervention, following 10 sessions of limb heating (MID); two consecutive days in the 72-h after the final day of the four-week, and 20 limb-heating session intervention (POST). Visit 1 required participants to report to the laboratory in a fasted state for arterial imaging (3.7.1), after which venous blood samples were collected (3.8). After this, participants were given a 60-120 min break to consume food of their choice, and on return, completed a ramp protocol test (3.5.1) to establish $\dot{V}O_{2peak}$. On Visit 2, participants completed a multiple-bout sub-maximal exercise test (3.5.2), followed by a single-leg critical torque test (3.6.2). All testing was conducted at A-STEM research laboratories in the College of Engineering at Swansea University and took place between 7:00-10:00 am in thermoneutral conditions (~20 ℃). Participants were required to report to the laboratory wearing normal sports kit (t-shirt, shorts and running shoes) which was kept consistent for all visits.

The above testing order remained consistent for each testing block and participant throughout the study. To control for the effect of external variables on testing performance, participants were asked to refrain from strenuous exercise, caffeine or use of any other dietary supplements and alcohol in the 24-h prior to and during each testing block. Additionally, participants were required to replicate the same dietary pattern 24-h prior and during each testing block. Email reminders of the testing guidelines mentioned above were sent prior to each visit.

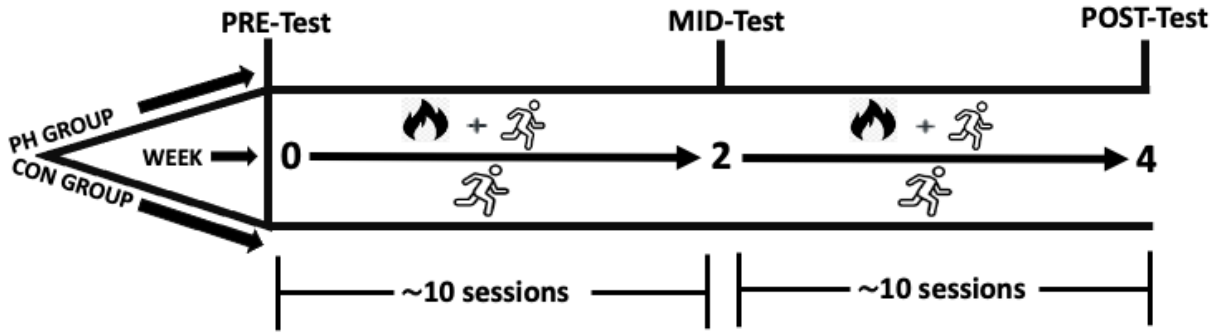

Figure 1. Schematic representation of study timeline. PH GROUP complete 90-120 min of post-exercise passive heating five days/week, CON group are required to carry on with their normal endurance training

3.3 Group allocation

Participants were allocated to one of two groups, but all took part in endurance exercise throughout the four-week intervention. After acquiring baseline scores (PRE), participants were randomly assigned to the post-exercise passive heating (PH) or control (CON) groups. Given that the participants' weekly activity requirements to take part in the current study, it was deemed necessary to account for potential baseline differences in fitness and routine training during the group allocation stage. The randomisation of group allocation was performed using a Microsoft Excel spreadsheet (MinimizeMeansAsRecruit.xls) developed by Hopkins (2010) and ensured that differences in baseline cardiorespiratory fitness (indicated by $\dot{V}O_{2max}$ measured at PRE) and training volume (estimated four-week average time in min) was minimised between groups by taking these two factors as co-variates.

3.4 Rational and specifications of limb heating intervention

Due to the novel approach in the current study, the dosing strategy had to be designed based on the findings of various thermal interventions. As the current passive heating system imposed direct skin heating, we decided to best mimic the duration and frequency of exposures used in those interventions that primarily focused on local heating modalities (Goto et al., 2007b, 2011a; Hafen et al., 2018b, 2019a; Kim et al., 2020a). For instance, Kim et al. (2020) showed that using hot water circulating suits for 90-min, five days/week for eight-weeks increased peripheral eNOS and HSP content. On the other hand, short-wave diathermy that applied more intense heat stress over a lesser body surface area, required shorter timeframes (120 min/day for 10-days consecutively) to induce positive mitochondrial adaptations (Hafen et al., 2018).

Finally, as PH includes an exercise stimulus, we felt it was necessary to include this in the finalisation of the dosing strategy. All the post-exercise PH studies to date implemented a three day/week strategy (Dalleck et al., 2019; Kirby et al., 2021; Scoon et al., 2007b; Sitkowski et al., 2021); however, modalities applying whole-body heat stress i.e HWI, SAUNA etc. were used and therefore each PH exposure lasted < 30-min. Furthermore, Kirby et al. (2021) showed that extending PH beyond three-weeks had no ergogenic effect on endurance performance. Amalgamating the above findings and taking into consideration that the current systems only heated the lower limbs, it was decided to design an intensive four-week PH strategy, comprising 20 PH sessions, completing 10 sessions prior to MID and the remaining 10 prior to

POST. Participants were required to wear the heating system for 90-120 min immediately (~ 5-min) post-exercise. On rest days they were asked to wear the system at their convenient time.

The limb-heating system comprised an ensemble of three clothing layers.:

(1) An electronic limb-heating garment (HUUB Design, Derby, England) was worn in immediate contact with lower-limb skin's surface, and was set to 43 ℃. The HUUB garments contain electrically-heated elements, wired into both sides of the trouser and is powered by 7.4 V lithium battery that can generate 14.8 W, heating both sides of the garment to 43 ℃ for maximum of 2-h. The anatomical sites covered by the HUUB garment can be seen in Figure 2.

(2) Elasticated soft-fleeced line thermal leggings (HeatGaurd™, China, 0.5 Tog 140 denier 90% polyester & 10% elastane) were worn over the HUUB garment to provide thermal insulation and promote a uniform fit of the garment across the skin's surface.

(3) The outermost thermally insulative layer was a pair of goose down trousers (Naturehike Outdoors, China, 400 tog 20D outer nylon layer, 95% goose down inner fill). Pilot work from our laboratory reveled that the limb-heating system was able to raise and maintain mean thigh skin temperature to ~ 39 °C (Appendix F).

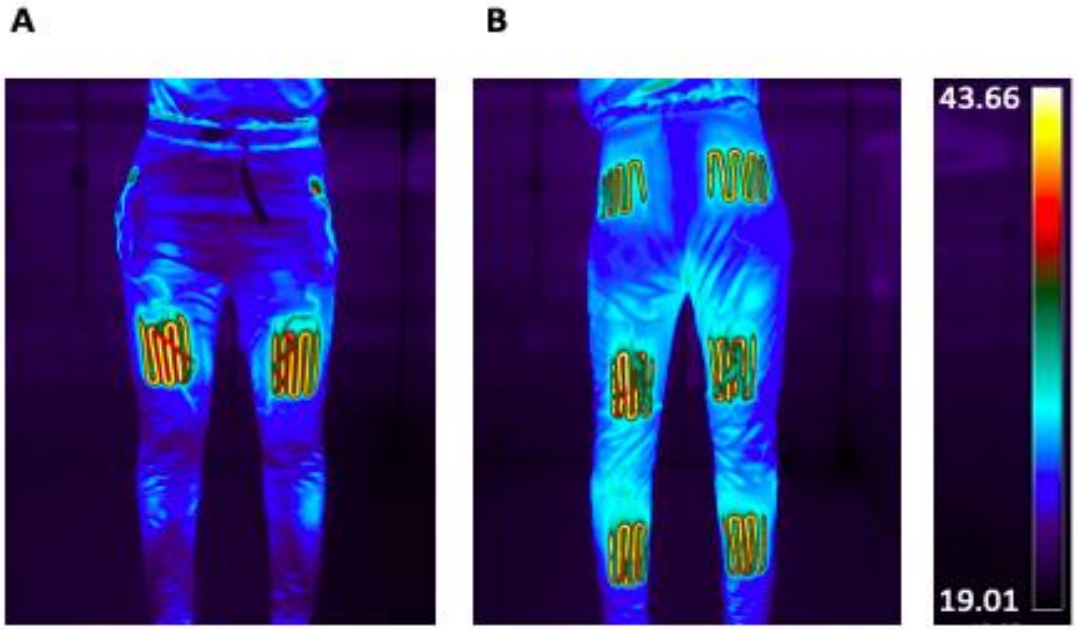

Figure 2. Thermal images of the HUUB trousers. A. anterior view- heating the quadricep muscle region, B. posterior view- heating the glutes, hamstrings and calf muscle regions. Electrical heated elements reaching a maximum temperature of ~ 43 ℃

Participants in both groups completed a minimum of 12 endurance training sessions and were advised to keep their training regime consistent with their previous (typical, pre-intervention) training and continue this throughout the four-week intervention. At pre-screening, it was ensured that the training of participants at the beginning of the intervention did not coincide with a planned overload in intensity or volume. To monitor this, an exercise logging sheet was developed for each participant using Microsoft Excel (version, 2019 16.0.6742.2048). Likewise, training load (TL) for each session was calculated using the method proposed by Foster et al. (2001), which required multiplying training duration (min) and the rate of perceived exertion (RPE) measured using the Borg CR10 scale (1998).

All participants were required to record their dietary intake throughout the study using the MyFitnessPal application. After POST visits, screen-shots of food diary were taken for each day and stored on a password protected laptop. The dietary pattern for each participant will be analysed using the Nutritic software and are currently unreported here.

3.5 Oxygen uptake ($\dot{V}O_2$) response to an incremental exercise test

All pulmonary gas measurements (volume of oxygen uptake (ml) = $\dot{V}O_2$, volume of carbon dioxide exhaled (ml) = $\dot{V}CO_2$, minute ventilation = VE, respiratory exechange ratio = RER) were recorded using breath-by-breath gas analyser system (Vyntus CPX, Carefusion, Hoechberg, Germany 234 GmbH) and measured in litres per minute (L/min). Prior to every testing visit, the gas analyser system was calibrated using known calibration gases (15.94% O_2, 5.00% CO_2). The turbine transducer was volume-calibrated automatically by the system, using flow rates 2 L/s and 0.2 L/s. The calibration procedures mentioned above were completed in accordance to manufacture recommendations. The same gas analyser and cycle ergometer was used for every participant throughout the study.

All exercise tests were carried out on an electronically-braked cycle ergometer (Lode, Excalibur sport 06, Groningen, Netherlands). Optimum saddle and handle bar positions were recorded for each participant during familiarisation and these remained consistent throughout every testing visit.

3.5.1 Ramp test for determination of $\dot{V}O_{2peak}$ and GET

Prior to testing, participants completed a 5-min warm-up, cycling at 50 W with a self-selected cadence. Following a 3-min recovery, the ramp test to exhaustion was conducted, starting at 10 W and progressing by 25 W/min. Participants were also instructed to maintain a steady cadence of 70-80 revolutions/min for the entire duration of the test. Criteria used for test cessation and achieving $\dot{V}O_{2max}$ response was: (i) reaching volitional exhaustion, (ii) unable to maintain a pedal rate >70 revs.min^{-1} and (iii) RER > 1.15. The mean $\dot{V}O_2$ value achieved over the final 30-s of the test was recorded as $\dot{V}O_{2peak.}$

GET was determined using the simplified v-slope method (Schneider et al., 1993) and previous recommendations (Wasserman, 1984). The criteria used for visual detection of GET were: (i) the first point of deviation in linearity between the plots of $\dot{V}CO_2$ (y-axis) & $\dot{V}O_2$ (x-axis), (ii) the first rise in the ventilatory equivalent for O_2 ($\dot{V}E/ \dot{V}O_2$) without any simultaneous increases in the ventilatory equivalent of CO_2 ($\dot{V}E/ \dot{V}CO_2$).

3.5.2 Constant work rate test

On a subsequent visit, participants performed a series of three square-wave transitions from rest to exercise on the same cycle ergometer. Each transition began with a 3-min resting period, sitting stationary on the ergometer, followed by 3-min of unloaded (0 W) pedalling, which was proceeded by an instantaneous transition of pedalling for 5-min against a constant load. The load selected was aimed to elicit 90% of the $\dot{V}O_2$ determined at GET and was adjusted after each testing block depending on changes in GET. Participants were required to maintain a steady cadence of 70-80 revs/min between all exercise transitions. Breath-by-breath pulmonary gas data was collected simultaneously using the same procedure described in 3.5, which was used to determine parameters of the $\dot{V}O_2$ kinetic response, as well as the gross cycling efficiency (GE) of cycling at steady state.

3.5.3 Determination of $\dot{V}O_2$ kinetics

Breath-by-breath $\dot{V}O_2$ data acquired from the trial (3.5.2) was first corrected for errant breaths as a result of normal breath-to-breath fluctuations, coughs and swallows. Likewise, all breaths that lay 3- 4 standard deviations (SD) away from the local mean were removed from the data

set (Lamarra et al., 1987). After cleaning, $\dot{V}O_2$ data for each bout was linearly interpolated to 1 s intervals, time aligned and finally ensemble averaged at each 1 s interval to produce a single rest to exercise $\dot{V}O_2$ response data set. The final data set was then averaged at 5-s intervals and the $\dot{V}O_2$ response after baseline was modelled using a mono-exponential function (Eq. 1) (Lamarra et al., 1987 ; Rossiter et al., 2002; Benson et al., 2017).

$$\dot{V}O_{2(t)} = \dot{V}O_{2,BASE} + A[1\text{-}e^{-(t\text{-}TD)/\tau)}] \quad (1)$$

Where $\dot{V}O_2$ at time point t is equal to the sum of $\dot{V}O_2$ at rest ($\dot{V}O_{2,BASE}$ [ml]) and the exponential function comprising of an amplitude (A [ml]: highest steady-state $\dot{V}O_2$ achieved above $\dot{V}O_{2,BASE}$ following rest to exercise transition), time constant (τ [s]) and a time delay (TD [s]). Here, τ represents the time required to attain 63% of the total amplitude (A) after an initial TD.

The first 20 s of the rest to exercise transition was defined as the cardio-dynamic phase I response (Whipp et al., 1982) and was therefore removed prior to fitting the model. This was done to ensure that the model only characterized the phase II exponential response that is related to muscle O_2 consumption and subsequent achievement of steady-state (Rossiter et al., 1999). Optimal fit of the data set was determined using non-linear least squares regression and parameters of $\dot{V}O_2$ kinetics: A, τ & TD along with their respective SD, was estimated from the line of best-fit. The above data handling and processing procedure was completed using the open-source code (https://github.com/fmmattioni/whippr) developed on R framework and was kept consistent for all participants.

3.5.4 Determination of GE

The $\dot{V}O_2$ and RER values from the final 120-s (steady state) of the final breath-by-breath data set (3.5.3) was averaged and GE was determined using the equations provided in (Garby & Astrup, 1987):

$$GE = \text{Mechanical power output / metabolic input} \quad (2)$$

$$\text{Metabolic input} = (\dot{V}O_2) \cdot ((RER \cdot 4904)+16040)/60) \quad (3)$$

The mechanical power output (W) was taken as the sub-maximal load used (90% 0f GET) during the CWR test. $\dot{V}O_2$ in equation 3 was measured in L/min and represented the new steady-state achieved during the loaded phase of the CWR test. Furthermore, due to the sub-maximal nature of the test, RER values were always below 1.0, therefore no further corrections were necessary (Noordhof et al., 2010).

3.6 Single-leg isometric muscle assessment

3.6.1 Dynamometer

All single-leg assessments were performed with participants seated in the chair of an isokinetic dynamometer (HUMAC NORM^{TM}, Cybex), and the right leg was assessed for every participant throughout the study. Following system warm-up and calibration, the distal tibial region (above the distal tibiofibular joint) was secured using a Velcro strap to the padded end of an extended-lever arm attached to the dynamometer. The chair was positioned such that the lateral epicondyle of the right femur was perpendicular to the axis of rotation of the lever arm. Participants sat with their back rested on the chair ensuring the hip was placed at a 90° angle and the knee held at a 75° angle (complete knee extension defined as the anatomical 0°) for the complete duration of the test. Both shoulders and waist were strapped securely to the chair and participants were asked to hold the fixed side handles when performing isometric muscle contractions. This was done to minimise any external movement and isolate the right quadricep muscle group. The final seating position was recorded during the familiarisation visit and remained constant for all subsequent test visits.

3.6.2 Five-minute all-out critical torque test (5-min AOCT)

Participants were seated on the dynamometer as described above (3.6.1). Following a sub-maximal isometric warm-up, participants performed three maximal voluntary contractions (MVC), forcefully contracting their knee extensors for 3-s with a 60-s rest in between. MVC was defined as the peak torque (N.m) produced between the 3 contractions.

After establishing MVC, participants were given a 3-min rest period. During this period, the participants were made aware of their MVC and were instructed to equal the score during the 5-min AOCT. Participants were also informed to expect extreme feelings of fatigue after the initial contractions but were instructed to produce maximal efforts for the entire duration of the

test. The 5-min AOCT consisted of 60 consecutive contractions, with each contraction cycle consisting of 3-s of MVC followed by 2-s of rest (Burnley, 2009). To prevent a reserve pacing strategy, participants were blinded to the duration or the number of contractions remaining. Due to there being no visual feedback, the test was conducted with verbal ques of "go" and "relax" for the contraction and relaxation phase, respectively. The dynamometer used in the study had a sampling frequency of 100 Hz. The NIRS unit started recording 30-s before the initiation of the 5-min AOCT (3.6.3).

The raw torque data was analysed using a code implemented in Python (version 3.10.8), mean torque was calculated for each 3-s contraction cycle and impulse (N.s) was calculated as the area under the torque-time curve. The mean torque of the last six contractions were averaged to establish critical torque (CT), which represented the last 30-s of the 5-min AOCT (Burnley, 2009). Total impulse (I) was taken as the sum of the impulse calculated for each 3-s contraction cycle. The work done above CT (I') was determined by a modification of the two-parameter model proposed by (Monod & Scherrer, 1965).

$$I = I' + CT.Tlim \qquad (4)$$

Where Tlim represents the duration of the all-out test in seconds (i.e. 300-s).

3.6.3 Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) uses near-infrared light wavelengths ranging from 700-900 nm to monitor oxygenation of various biological tissues (Pellicer & Bravo, 2011). It works on the differential absorption properties of haemoglobin (Hb) and myoglobin (Mb) in the presence (oxy-haemoglobin: O_2Hb) or absence (deoxy-haemoglobin: HHb) of oxygen (Hamaoka et al., 2007). The study used a continuous-wave NIRS unit (PortaMan, Artinis medical systems, Amsterdam, Netherlands) to record relative concentrations of O_2Hb, HHb and estimate tissue saturation index (TSI) of the quadriceps during the 5-min AOCT test. TSI reflects the instantaneous balance between O_2 supply and demand (Boushel et al., 2001), which is calculated as a ratio of O_2Hb to total Hb (O_2Hb + HHb) and expressed as a %. The NIRS unit was placed longitudinally over the right vastus lateralis (VL) muscle, at approximately 50% of the thigh length measured between the greater trochanter and lateral epicondyle of the femur. Hair around the VL was removed prior to placing the NIRS unit. The unit was secured to the

region of interest using an elasticated bandage and then fastened with tape to prevent unit-movement artefacts during contractions. Lastly, the attached unit was covered with a black indelible cloth, taped on the anterior portion of the thigh to prevent external light from interfering with the NIRS signal. The NIRS unit used a dual-wavelength of 760 and 850 nm, with source-detector spacing of 30, 35 and 40 mm. Before initiating the isometric test, the unit was connected to a personal computer via Bluetooth technology and data were acquired in real-time through the OxySoft software (Artinis medical systems, Amsterdam, Netherlands) at a sampling frequency of 10 Hz.

Similar to CT (3.6.2), mean TSI and HHb was calculated for each 3-s contraction. Following this, mean torque for every contraction (60) was expressed as percentage of the MVC. Here MVC was taken as the highest mean torque output achieved during the 5-min AOCT. This was repeated for the mean TSI and HHB signal and maximum mean TSI and HHb was used in place of MVC. Once all the values were expressed as a percentages to their respective maximum, ratios were developed between CT % : TSI % (representing mechanical work for a given O_2 availability) and CT % : HHb % (representing mechanical work for a given O_2 extraction). The graphed trend of the final ratio data followed an exponential decay and, therefore, the data were modelled on GraphPad Prism (GraphPad 9.0 Software, San Diego, CA, United States) using a mono-exponential decay equation, with ratios on the y-axis and time (s) on the x-axis.

$$R_{CT/TSI} = (R0_{CT/TSI} - CR_{CT/TSI}) \cdot e^{(-(X)/\tau)} + CR_{CT/TSI} \quad (5)$$

$$R_{T/HHb} = (R0_{T/HHb} - CR_{T/HHb}) \cdot e^{(-(X)/\tau)} + CR_{T/HHb} \quad (6)$$

Where;

(1) $R_{T/TSI}$ and $R_{T/HHb}$ are the ratios at time point X derived from TSI and HHb signal, respectively

(2) $R_{CT/TSI}$ and $R_{CT/HHb}$ is the ratios when time point X = 0 s

(3) τ is the time constant expressed in the same unit as X, s

(4) $CR_{T/TSI}$ and $CR_{T/HHb}$ is the model predicted critical ratio and represents the interaction of CT with oxygenation and deoxygenation.

3.7 Vascular Screening

Endothelial-dependant functioning of the brachial artery was assessed non-invasively using a high-resolution ultrasound device (ESAOTE, MYLAB9, Genova, Italy). A 10 MHz ultrasound probe was used for simultaneous measurement of brachial artery diameter and blood flow using the B-mode echo and pulse-wave Doppler velocity, respectively. Real-time changes in the arterial diameter was monitored using a specialised edge-detection software program (Cardiovascular suite, version 4.3.0) that recorded data at 1-s intervals. A manual pressure cuff (Hokanson SC5 Vascular Tourniquet) was placed 3-5 cm distal to the antecubital fossa and was used during the occlusion period of the flow mediated dilation test.

3.7.1 Flow Mediated Dilation (FMD)

The FMD assessments was carried out pre-prandial, with participants having fasted for 12-h prior to laboratory visit. Participants were instructed to refrain from caffeine, alcohol, dietary supplements and strenuous exercise 24-h prior to their visit. All tests were carried out in the morning and time of analysis was kept consistent between visits (± 30 min). The FMD protocol was conducted according to recent guidelines (Thijssen et al., 2019)

On arrival at the laboratory, participants rested in supine position in a quiet, dark and temperature-controlled room for 10-min prior to analysis. Following the rest period, participants were asked to lay supine with their right arm placed on a custom designed bench, such that the arm was abducted and rotated externally. This arrangement minimised movement at the arm and prevented loss of image during the test. Once the participant felt comfortable in the position, an ultrasound probe was placed longitudinally on the brachial artery, proximal to the antecubital fossa with a pressure cuff wrapped distal to it on the forearm. After acquiring a clear image of the vascular walls (i.e., lines of pignoli), a region of interest on the image was selected through the software and the test was initiated. Baseline artery diameter and blood flow was measured for 1-min, after which the pressure cuff was manually inflated to 220-230 mmHg for 5-min (occlusion period). Post-deflation, diameter and blood flow was measured for another 4-min to capture the complete hyperaemic response following occlusion.

FMD was calculated as the difference between maximum diameter (D_{MAX} [mm]) achieved post-occlusion and baseline diameter ($D_{BASELINE}$ [mm]). Likewise, FMD % was calculated as follows (Eq. 7)

$$FMD\ \% = [(D_{MAX} - D_{BASELINE})/ D_{BASELINE}] \cdot 100 \qquad (7)$$

Sheer rate (SR [1/s]) was calculated using the Doppler flow velocity waveform using;

$$SR = (4 \cdot V)/D. \qquad (8)$$

Where V and D represent mean velocity (mm/s) and arterial diameter (mm) at a particular time frame respectively. In addition to this, SR area under the curve (SR_{AUC}), which represent maximal vasodilation area, was calculated from the beginning of vasodilation (post-occlusion) until D_{MAX} using the trapezoidal rule (Thijssen et al., 2019; Tinken et al., 2009).

3.8 Blood sampling

Resting venous blood samples were drawn from an antecubital vein into 10 mL serum vacutainers. Samples were allowed to coagulate for 20-min at room temperature after which they were placed on ice for a further 20-min. Tubes were then centrifuged at 2800 rev/min for 10-min at 4 °C before being aliquoted into autoclaved 1.5 mL Eppendforf tubes in a sterile environment within a safety cabinet (Class II Bio Safety Cabinet, Microflow Devices India, India, Chennai). All samples were stored at -80 °C for later analysis.

Serum/plasma concentrations of free vaso-endothelial growth factor (VEGF), soluble VEGF Receptor (sFlt-1), endothelial nitric oxide synthase (eNOS) and Heat shock proteins (HSP) will be determined using commercially-available enzyme-linked immuno-sorbent assay (ELISA). For in-vitro experiments, ex vivo serum will be exposed to in vitro cell culture using human umbilical vein endothelial cells (HU-VECs; ATCC, Manassas, VA). Cells will be cultured (in Kaighn's Modification of Ham's F-12 Medium (F12K), ATCC) and 10% FBS) and characterised for metabolic activity growth curve via Presto Blue, HUVEC specific characterising assay, Fluorometric Spectrophotometry and stain imaging. Cells will then be cultured in F12K media where FBS is gradually replaced with sterile exercise-conditioned serum (in triplicate) and re-characterised as above (expected end volume 10% conditioned

serum). Supernatant will be collected from culture media and assayed for VEGF, (sFlt-1), e-NOS and HSP (potentially others depending on the results of tin vivo experiments) i.e. analysis of the local endothelial cellular environment in response to the intervention administered in the PH group.

3.9 Statistical analysis

All statistical analyses were performed using SPSS 28.0.1.1 (14) (SPSS Inc., Chicago, Illinois, USA). A two-way repeated measures analysis of co-variance (ANCOVA) was used to determine the main and interaction effects of group (PH and CON) and time (PRE, MID and POST) on all physiological and performance variables recorded in the study. The PRE values were treated as co-variates, with the MID and POST values entered as dependent variables. This adjusted baseline values for between-group comparisons and minimised conditional bias (Crager, 1987; Vickers & Altman, 2001). Training load was analysed using a two-way repeated measures (2[group] x 2[time] analysis of variance. Significant main and interaction effects were analysed using Bonferroni-corrected *post-hoc* tests. Statistical significance was set as $p \leq 0.05$. All results are presented as mean ± standard deviation (SD).

Chapter 4.0 Results

Participant characteristics, baseline $\dot{V}O_{2peak}$ and training volume are listed in Table 1. There was no group ($F_{(1,28)} = 0.176$, $p = 0.678$, $\eta_p^2 = 0.006$) or group by time interaction ($F_{(1,28)} = 0.234$, $p = 0.632$, $\eta_p^2 = 0.008$) effects for training load assessed in the study.

The intervention group completed 20 ± 1 post-exercise passive heating sessions, accumulating total heating time of 2245.3 ± 7.73 minutes

Table 1. Baseline participants characteristics and training load.

	Passive Heating			Control		
Age (years)	24.8	±	5.9	29.9	±	7.1
Body mass (kg)	72.5	±	13.6	72.6	±	9.7
Stature (cm)	176.4	±	7.8	177.0	±	8.3
$\dot{V}O_{2peak}$ (L/min)	3.3	±	0.7	3.6	±	0.8
TD (min)	1618.1	±	606.1	1834	±	1059.5
TL1 (min x RPE)	3220.3	±	1258.6	3540	±	2276.1
TL2 (min x RPE)	3092.9	±	1323.9	3279.8	±	1819.4

Note: TL = training load; TL1= TL between PRE to MID, TL2 = TL between MID to POST, $\dot{V}O_{2peak}$ = maximal oxygen uptake; TD= baseline training volume (4-week average).

4.1 Ramp test: $\dot{V}O_{2peak}$ and GET

There was no group ($F_{(1,27)}$ = 3.306, p = 0.080, η_p^2 = 0.109) or group by time interaction effect ($F_{(1,27)}$ = 0.878, p = 0.357, η_p^2 = 0.031) for relative $\dot{V}O_{2peak}$. Similarly, there was no group ($F_{(1,27)}$ = 2.448, p = 0.129, η_p^2 = 0.083) or group by time interaction effect ($F_{(1,27)}$ = 0.697, p = 0.411, η_p^2 = 0.025) for power output achieved at $\dot{V}O_{2peak}$ (Table 2).

There was no group ($F_{(1,27)}$ = 1.936, p = 0.175, η_p^2 = 0.067) or group by time interaction effect ($F_{(1,26)}$ = 0.081, p = 0.778, η_p^2 = 0.003) for $\dot{V}O_2$ at GET. Likewise, there was no group ($F_{(1,27)}$ = 0.694, p = 0.412, η_p^2 = 0.025) or group by time interaction effect ($F_{(1,27)}$ = 0.851, p = 0.364, η_p^2 = 0.031) for GET when expressed as % of $\dot{V}O_{2peak}$. Finally, there was no group ($F_{(1,27)}$ = 2.394, p = 0.133, η_p^2 = 0.081) or group by time interaction effect ($F_{(1,27)}$ = 0.405, p = 0.530, η_p^2 = 0.015) for power achieved at GET (Table 2).

Table 2. Parameters derived from maximal exercise test (mean ± SD), two groups (Passive heating & Control) tested at three time points (PRE, MID & POST)

	Passive Heating			Control		
Parameter	Pre	Mid	Post	Pre	Mid	Post
$\dot{V}O_{2peak}$ (mL/kg/min)	45.4 ± 7.3	46.8 ± 7.5	47.8 ± 7.5	50.0 ± 7.8	49.6 ± 7.4	49.7 ± 7.4
Power at $\dot{V}O_{2peak}$ (W)	289.1 ± 52.9	294.0 ± 50.6	296.1 ± 51.1	312.2 ± 66.5	312.1 ± 66.7	309.8 ± 65.2
GET (mL/kg/min)	28.3 ± 4.3	29.2 ± 4.7	29.2 ± 4.7	31.3 ± 6.0	31.1 ± 6.6	30.2 ± 5.0
Power at GET (W)	141.8 ± 31.3	156.8 ± 37.8	154.6 ± 37.9	161.7 ± 44.1	162.0 ± 43.4	163.6 ± 48.8

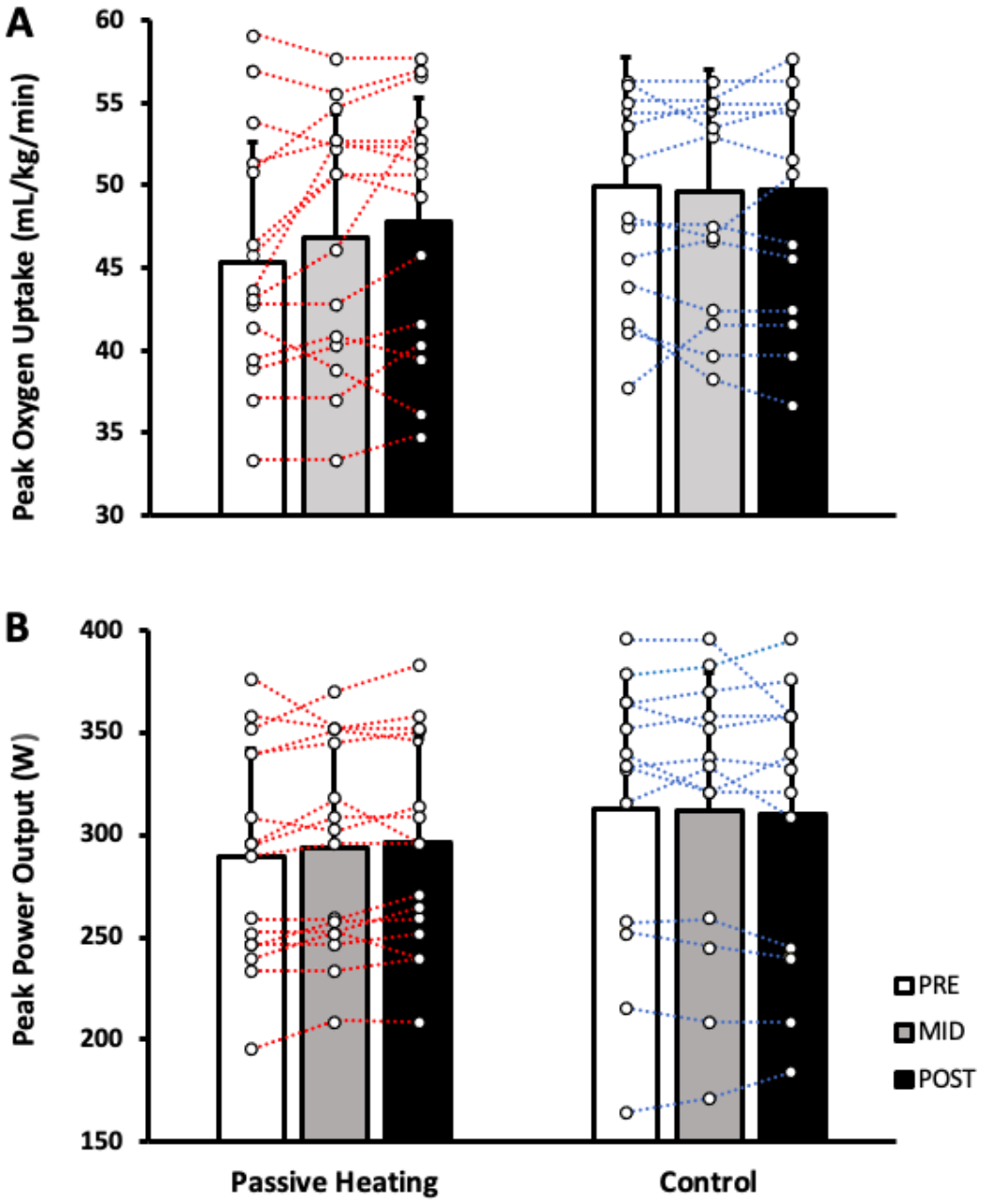

Figure 3. Peak oxygen uptake (A) and peak power output (B) derived from maximal exercise testing. Bar plots and error bars represent mean ± SD. Circle connected with dotted line represent trends for each individual participants across PRE, MID & POST. Red and blue dotted lines are used to differentiate individuals in the passive heating (n = 16) and Control (n = 14) group respectively.

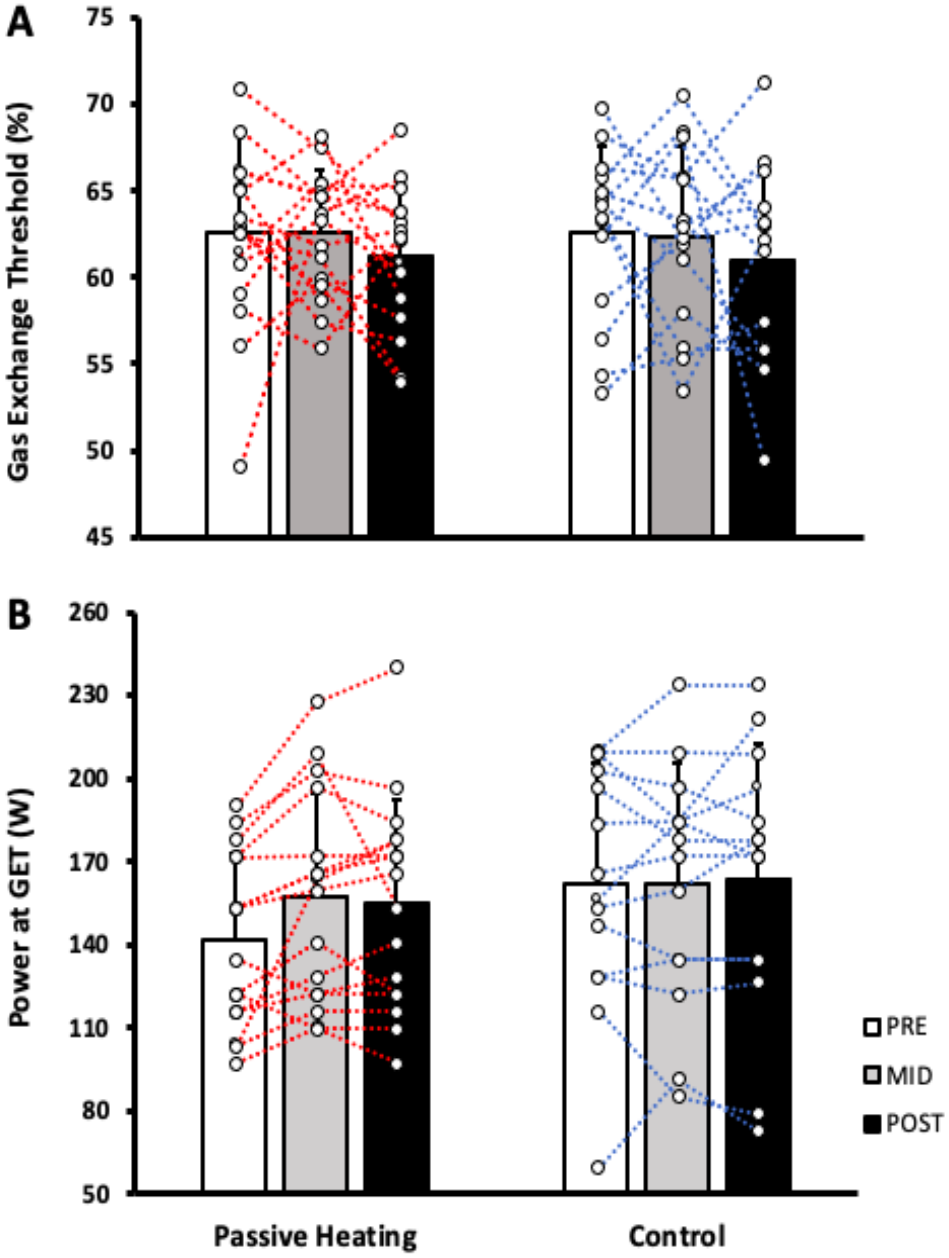

Figure 4. Gas exchange threshold (GET) expressed as % of $\dot{V}O_{2peak}$ (A) and power output achieved at GET (B). Bar plots and error bars represent mean ± SD. Circles connected with dotted line represent trends for each individual participant across PRE, MID & POST. Red and blue dotted lines are used to differentiate individuals in the passive heating (*n* = 16) and Control (*n* = 14) group, respectively.

4.2 Constant work rate test

There was no group ($F_{(1,27)}$ = 2.030, p = 0.166, η_p^2 = 0.070) or group by time interaction effect ($F_{(1,27)}$ = 0.062, p = 0.805, η_p^2 = 0.002) for $\dot{V}O_{2BASE}$ measured prior to the onset of moderate-intensity exercise.

There was no group ($F_{(1,27)}$ = 2.141, p = 0.155, η_p^2 = 0.073) or group by time interaction effect ($F_{(1,27)}$ = 0.302, p = 0.587, η_p^2 = 0.011) for the A achieved following the onset of moderate-intensity exercise.

There was no group ($F_{(1,27)}$ = 0.573, p = 0.456, η_p^2 = 0.021) but a significant group by time interaction effect ($F_{(1,27)}$ = 4.214, p = 0.050, η_p^2 = 0.135) for τ measured during moderate-intensity exercise. Post-hoc analysis revealed that τ was greater (p = 0.020) at POST compared to MID in the PH group.

Finally, there was no group ($F_{(1,27)}$ = 1.341, p = 0.257, η_p^2 = 0.047) or group by time interaction effect ($F_{(1,27)}$ = 0.569, p = 0.457, η_p^2 = 0.021) for GE measured during moderate-intensity exercise.

Table 3. Parameters derived from sub-maximal exercise test (mean ± SD), two groups (Passive heating & Control) tested at three time points (PRE, MID & POST)

	Passive Heating			Control		
Parameter	*Pre*	*Mid*	*Post*	*Pre*	*Mid*	*Post*
$\dot{V}O_{2BASE}$ (mL/min)	901.8 ± 141.1	873.3 ± 136.4	882.7 ± 158.2	897.7 ± 136.2	895.4 ± 151.5	906.3 ± 161.1
$\dot{V}O_{2AMP}$ (mL/min)	1026.1 ± 284.1	1180.6 ± 338.8	1163.6 ± 348.6	1167.24 ± 404.3	1184.6 ± 380.6	1210.6 ± 459.5
TD (s)	11.3 ± 3.4	11.2 ± 3.2	9.7 ± 3.9	11.7 ± 3.4	12.1 ± 2.3	12.0 ± 3.2
τ (s)	25.0 ± 7.2	24.6 ± 8.3	27.5 ± 6.2*	23.3 ± 5.1	23.7 ± 6.2	23.6 ± 6.5
GE (%)	19.4 ± 1.5	20.0 ± 1.5	19.8 ± 2.0	20.3 ± 1.7	20.3 ± 1.3	19.9 ± 1.5

*Note: * = significantly different between Mid and Post in the passive heating group.*

4.3 Five-min all-out critical torque test

There was no group ($F_{(1,27)}$ = 0.362, p = 0.552, η_p^2 = 0.013) or group by time interaction effect ($F_{(1,27)}$ = 1.680, p = 0.206, η_p^2 = 0.059) for CT.
There was no group ($F_{(1,27)}$ = 0.614, p = 0.440, η_p^2 = 0.022) or group by time interaction effect ($F_{(1,27)}$ = 0.055, p = 0.817, η_p^2 = 0.002) for I'.

4.4 NIRS-derived kinetics of Torque/saturation (T/TSI) and deoxygenation ratio (T/HHb)

There was no group ($F_{(1,18)}$ = 0.350, p = 0.561, η_p^2 = 0.019) or group by time interaction effect ($F_{(1,18)}$ = 0.274, p = 0.607, η_p^2 = 0.015) for $\tau_{1/2}$T/TSI derived from T/TSI curve.

There was no group ($F_{(1,18)}$ = 0.493, p = 0.491, η_p^2 = 0.027) or group by time interaction effect ($F_{(1,18)}$ = 0.033, p = 0.858, η_p^2 = 0.002) for the critical ratio ($CR_{T/TSI}$) derived from T/TSI curve.

There was no group ($F_{(1,18)}$ = 3.656, p = 0.072, η_p^2 = 0.169) or group by time interaction effect ($F_{(1,18)}$ = 0.020, p = 0.890, η_p^2 = 0.001) for $\tau_{1/2}$T/HHb for derived from T/HHb curve.

There was no group ($F_{(1,18)}$ = 2.864, p = 0.108, η_p^2 = 0.137) but a significant group by time interaction effect ($F_{(1,18)}$ = 4.686, p = 0.044, η_p^2 = 0.206) for $CR_{T/HHb}$ derived from T/HHb curve. Post-hoc analysis revealed that $CR_{T/HHb}$ was significantly higher (p = 0.044) at POST compared to MID within the CON group. Additionally, the CON group had a significantly higher (p = 0.031) $CR_{T/HHb}$ values at POST compared to PH.

Table 4. Parameters derived from the five-min critical torque test in combination with NIRS (mean ± SD). Critical ratio calculated using TSI and HHb signal for passive heating (*n* = 12) and control (*n* = 9) group across PRE, MID & POST

	Passive Heating			Control		
Parameter	Pre	Mid	Post	Pre	Mid	Post
CT (N.m)	67.9 ± 32.0	73.5 ± 29.0	78.2 ± 32.0	74.2 ± 23.1	78.8 ± 26.1	78.2 ± 21.7
I’	4651.0 ± 2031.0	4167.0 ± 2218.1	4102.0 ± 1958.8	4914.8 ± 1944.6	4017.8 ± 1553.1	3825.7 ± 1750.7
$CR_{T/TSI}$	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
$\tau_{1/2}T/TSI$ (s)	54.5 ± 20.8	53.6 ± 21.4	61.8 ± 28.0	65.2 ± 27.1	60.2 ± 34.0	54.5 ± 26.0
$CR_{T/HHb}$	2.7 ± 1.7	3.7 ± 2.5	2.8 ± 1.1	2.9 ± 1.6	4.4 ± 4.2	7.1. ± 6.4*#
$\tau_{1/2}T/HHb$ (s)	67.0 ± 21.3	66.3 ± 28.2	70.3 ± 28.2	81.4 ± 36.3	61.8 ± 34.4	61.5 ± 20.5

*Note: * = significantly different between PRE and MID in Control group; # = significantly different from passive heating at that time point.*

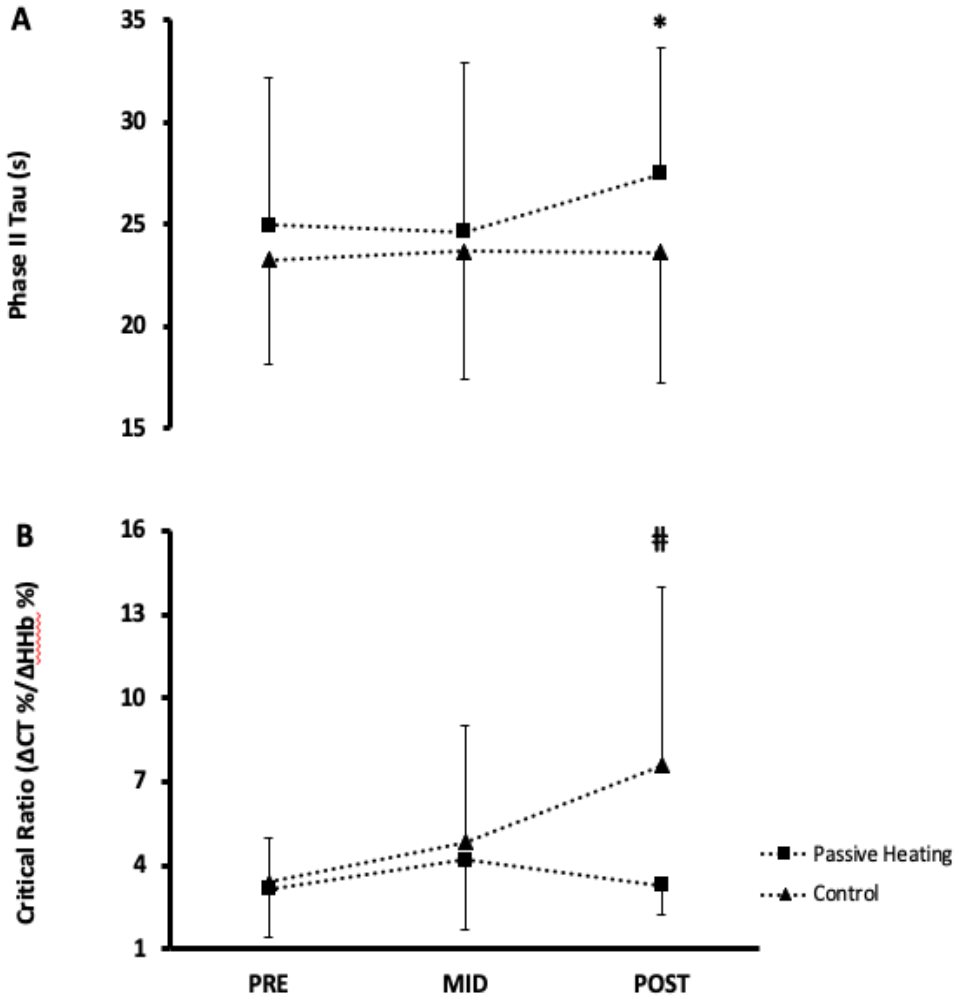

Figure 5. Group mean phase II tau (A) and critical ratio calculated from torque and deoxy signal; $CR_{T/HHb}$ (B). Data represented as mean ± SD. * = significantly different from PRE & MID, # = significantly different from passive heating.

4.5 Flow mediated dilation

There was no group ($F_{(1,10)}$ = 1.980, p = 0.190, η_p^2 = 0.165) or group by time interaction effect ($F_{(1,10)}$ = 0.419, p = 0.532, η_p^2 = 0.040) for flow mediated dilation.

There was a significant group ($F_{(1,10)}$ = 18.415, p = 0.002, η_p^2 = 0.648) and group by time interaction effect ($F_{(1,10)}$ = 4.734, p = 0.055, η_p^2 = 0.321) for baseline diameter. Post-hoc analysis revealed that baseline diameter increased (p = 0.05) from MID to POST in PH. Additionally, baseline diameter was higher (p = 0.002) in PH compared to CON at POST.

There was a significant group ($F_{(1,10)}$ = 22.056, p < 0.001, η_p^2 = 0.688) and group by time interaction effect ($F_{(1,10)}$ = 6.643, p = 0.028, η_p^2 = 0.399) for maximal arterial diameter achieved following occlusion. Post-hoc analysis revealed that maximal arterial diameter increased (p = 0.045) from MID to POST in PH. Additionally, maximal arterial diameter was higher (p < 0.001) in PH compared to CON at POST.

There was no group ($F_{(1,10)}$ = 0.129, p = 0.727, η_p^2 = 0.013) or group by time interaction effect ($F_{(1,10)}$ = 0.135, p = 0.721, η_p^2 = 0.013) for maximal vasodilation area achieved post-occlusion.

Table 5. Parameters derived from brachial artery flow mediated dilation test (mean ± SD).

	Passive Heating			Control		
Parameter	*Pre*	*Mid*	*Post*	*Pre*	*Mid*	*Post*
Flow Mediated Dilation (%)	6.6 ± 2.9	6.8 ± 2.8	6.7 ± 1.9	6.2 ± 2.9	5.9 ± 2.8	5.0 ± 0.9
Baseline diameter (mm)	3.9 ± 0.6	3.9 ± 0.6	4.1 ± 0.6 *#	3.9 ± 0.3	3.8 ± 0.4	3.8 ± 0.2
Max. arterial diameter (mm)	4.1 ± 0.5	4.2 ± 0.6	4.3 ± 0.6 *#	4.1 ± 0.3	4.1 ± 0.3	4.0 ± 0.2
Max. vasodilation area (s^{-1})	19,449 ± 5,343	18,980 ± 8,189	23,476 ± 14,371	17,531 ± 1,521	17,652 ± 8,258	18,122 ± 8,227

*Note: * = significantly different from PRE and MID, # = significantly different from control at that time point.*

Chapter 5.0 Discussion

This study is the first to investigate the effect of chronic (four-weeks) post-exercise passive heating, using a bespoke electrical heating system, on markers of endurance performance in thermoneutral conditions. The primary finding of the study was that inclusion of a home-based passive heating stimulus in recreationally trained individuals did not have any ergogenic effect, reflected by no changes in maximal ($\dot{V}O_{2peak}$, PO_{peak} & I') and submaximal (GET, PO_{GET}, GE, & CT) components of endurance performance, compared to exercise only controls. In contrast to our hypotheses, the intervention resulted in an overall slowing of $\dot{V}O_2$ kinetics (τ) during moderate-intensity exercise and seemed to suppress improvements in torque/O_2 extraction ratio ($CR_{T/HHb}$) during maximal isometric contractions, as observed in the CON group. Despite some descriptive improvements in $\dot{V}O_{2peak}$ (5.4% *vs.* -0.4%), PO_{peak} (2.4% *vs.* -0.75%), PO_{GET} (9% *vs.* 1.2%) and CT (14.9% *vs*. 5.4%) in PH compared to CON respectively, no statistical difference between groups was found. Additionally, the significant slowing of τ (25.0 ± 7.2 s *vs.* 27.5 ± 6.2 s) in PH, along with the simultaneous improvement of $CR_{T/HHb}$ in CON relative to PH (7.1. ± 6.4 *vs.* 2.9 ± 1.6), from PRE to POST, has led to the rejection of the original hypothesis.

It is well documented that various training modalities, differing in volume and intensity have the potential to improve $\dot{V}O_{2peak}$ (Tabata et al., 1996; Wen et al., 2019). The improvements are brought about by adaptations of central and peripheral origin, dictating O_2 delivery and metabolism, respectively (Sutton, 1992). Passive heating, due its thermogenic effect, has gained attention as an exercise mimetic for initiating cardiovascular adaptations similar to that of traditional endurance training (Cullen et al., 2020). Acutely, passive heat stress raises skin temperature (T_{sk}) and evokes a biphasic cutaneous vasodilatory response (Charkoudian, 2003). This is commonly characterised by an initial rapid peak response (3-5 min), regulated by afferent sensory nerves, followed by a secondary prolonged plateau (> 10-min) mediated by NO (Kellogg, 2006), facilitating maximal skin blood flow for the purpose of heat dissipation. To support the above peripheral hemodynamic redistribution, $\dot{Q}$ can increase up to 8-13 L/min (Koroxenidis et al., 1961; Rowell et al., 1970), thus allowing skin blood flow to reach a maximum of 7-8 L/min (Rowell, 1974). Pilot data from the current study were consistent with these findings, demonstrating that our limb heating system elicited substantial increases in skin temperature across all limb areas (Δ 6 °C) and an increased resting heart rate response. This response also appeared to attenuate the rate of decay in post-exercise core temperature. Based on these data and the *a-priori* theoretical foundation, we hypothesised that chronic application would induce changes to the endurance phenotype, particularly when coupled with a strict endurance training regimen.

5.1 Ergogenic effect of passive heating.

In the current study, all markers of the endurance phenotype did not change in the PH group. This was surprising because chronic application (>3-4 weeks) of passive heating has been reported to induce skeletal muscle capillarisation (Hesketh et al., 2019) and myofilament hypertrophy (Goto et al., 2011), haematological expansion (Stanley et al., 2015) and mitochondrial biogenesis (Hafen et al., 2019). Collectively, these adaptations are indictive of peripheral adaptations favouring metabolic support for prolonged exercise (i.e., aerobically biased). Consistent with the notion that the $\dot{V}O_{2peak}$ is a compound measure and the manifestation of an in-series system (Wagner et al., 2011a), we reasoned that the combined acute and chronic responses to passive heating would improve $\dot{V}O_{2peak}$ and perhaps other endurance determinants. It is possible that the mode of heating was responsible for the lack of improvement in the current study. For example, three studies (Dalleck et al., 2019; Kirby et al., 2021; Scoon et al., 2007) have reported an ergogenic effect of PH on endurance performance in thermoneutral conditions, which is in contrast to the results of the current study. However, while the improvements in $\dot{V}O_{2peak}$ occurred across a similar time-course, the studies used either SAUNA or HWI. It is thought that the expansion of blood volume (BV) explains the translational effect of chronic PH on thermoneutral $\dot{V}O_{2peak}$ responses (Kirby et al., 2021), which is logical based upon the rapid time-course of PV expansion of between 4 and 10-days (Pokora et al., 2021; Stanley et al., 2015) and its relationship with $\dot{Q}$ (Bonne et al., 2014). The current study did not measure changes in PV or other potential markers of $\dot{V}O_{2peak}$ adaptation (i.e. HB mass; Lundby et al., 2017), and it is therefore possible that the current intervention did not elicit the physiological responses necessary to improve it. Alternatively, it is possible that our consistently trained endurance group baseline fitness could not be enhanced passively using the home-based model used herein, despite its clear effect upon skin and core temperature.

5.2 Explanation for null effects of hallmark endurance markers in response to PH

Whilst we can speculate that the differences in the methodologies (Heating garments *vs.* SAUNA vs. HWI) used for PH between the current and previous studies might be responsible for the varied outcomes, the thermal effects imposed by each type of intervention often prevents direct comparison. For instance, individuals using SAUNA, are whole-body enveloped, whilst inhaling a combination of hot (89 °C) and dry (5-10% relative humidity) air that might increase intra-thoracic (T_{IT}) temperatures. Investigations using animal models (Lin et al., 2009; Ruan et al., 2005) have shown that raising T_{IT} to 39.2 °C can increase the activation of vagal nerves innervating the lungs and lead to a broncho-constrictive response, and similar responses have also been reported in asthmatic patients (Hayes et al., 2012). In these conditions, changes in core temperature are also reported (Δ

1°C). On the other hand, HWI protocols that require immersion to the clavicle or neck level, exert a hydrostatic pressure on the submerged individual, resulting in increased central blood pressure and fluid regulating hormone secretion (Arborelius et al., 1972), which is a well-known precursor of diuresis (Epstein, 1976). The electrical heating system used in the current study is restricted to the ~50% of whole-body peripheral tissue (i.e. skin) and causes some mild cardiovascular effects, but is largely affecting the local skin temperature, quite aggressively so, alongside other thermo-effector responses, such as sweating (Nadel et al., 1971). Therefore, while these interventions may appear similar, the different physiological effects induced by each intervention could cause different outcomes across time.

One factor that might explain the non-significant changes in $\dot{V}O_{2peak}$ follow PH in the current study is baseline fitness. This argument stems mainly from active HA studies (exercise in the heat), where it is suggested that fitter individuals have lower adaptative potential, as greater training loads experienced in this group inherently provide a stimulus for partial heat adaptation (Taylor & Cotter, 2006). However, recent studies (Alkemade et al., 2021; Corbett et al., 2018) have provided evidence that baseline $\dot{V}O_{2peak}$ did not dampen hallmark physiological adaptations to HA, at least in recreational or well-trained individuals. Though it can be argued that active HA protocols differ fundamentally from passive heating, the fact that elite (Scoon et al., 2007) and highly trained (Kirby et al., 2021) individuals have previously experienced an ergogenic benefit with PH, contradicts notions regarding the confounding influence of baseline fitness. Interestingly, the participants in the current study had similar baseline $\dot{V}O_{2peak}$ levels compared to two recent studies (Dalleck et al., 2019; Sitkowski et al., 2021); however, only Dalleck et al. (2019) reported a significant ergogenic effect of PH. In addition, we also observed no trend in our data to demonstrate that higher baseline fitness was associated with greater responses (see Figure 3).

Finally, the dosing strategy employed in the current study could also explain differences in outcome between studies. In this regard, we consider the thermal stimulus (i.e., temperature exposed to), PH duration (time per session), total number of induction days and the number of 'recovery' (days between) of each PH session. Here, external thermal impulse can be defined as the product of the heating-source temperature (°C) and volume of PH (duration x no. of PH sessions; mins). This differs from traditional thermal impulse, which is the time an individual spends at a core temperature > 38.5 °C (Fox, 1963). However, external thermal impulse might be a more practical indicator of 'dose' (rather than the response), as it includes the external thermal stimulus, which is dependent on the method used (well reported in all studies). Indeed, the internal Tc response might also be impractical to monitor for each PH session, across each participant, particularly during home interventions. Importantly, both Dalleck et al. (2019) and Kirby et al. (2021) had participants complete nine PH

sessions, distributed across three-weeks, whereas the current study required participants to complete 20 PH sessions in four-weeks. Therefore, the external thermal impulse for the current study was 9.6 x 10^4 °C . min compared to 2.8 x 10^4 °C . min (Kirby et al., 2021) and 1.0 x 10^4 °C . min (Dalleck et al., 2019), which might be indictive of an over-loading thermal stimulus, with insufficient recovery. We feel that this argument is the most convincing to explain the current null intervention effects, since in relation to other parameters of endurance discussed in later sections of this thesis, there appeared to be a retardation of natural training enhancement across the four-weeks of PH in comparison to the control group.

Further investigation into previous studies also supports the contention that training load and recovery was perhaps mismanaged in the current study. For example, using a more even training load distribution of three day/week format (Sitkowski et al., 2021), endurance training (60-min cycling at 50-60% $\dot{V}O_{2peak}$) with or without PH (SAUNA; 89 °C for 30-min) has resulted in a significant but similar thermoneutral performance gain for $\dot{V}O_{2peak}$ (5.5 *vs.* 3.4 %) and P_{MAX} (5.7 *vs.* 9.2 %), respectively. Due to the absence of an interaction effect, the authors concluded that the addition of PH was not ergogenic in recreational athletes. It is important to note here that the athletes in the above study completed ~300 min/week of habitual exercise on top of the 180 min/week of supervised training (Sitkowski et al., 2021), despite lower frequency of sessions. Therefore, further addition of PH could have possibly resulted in overloaded training stimulus (exercise + passive heating), yet despite this, positive gains in endurance performance was noted in both training regimes. In conjunction with the results reported previously (Sitkowski et al., 2021), it is possible that our data demonstrate the importance of sufficient recovery between PH sessions, alongside management of individual training load during PH interventions. Contrastingly, high-responding participants (Dalleck et al., 2019) completed only 96 min/week of supervised training, providing further support that the total over-loading stimulus (exercise + PH) in the current and previous study might have suppressed greater performance gains in the PH group.

According to Selye's seminal general adaptation syndrome (1950), a novel stress applied on a living system leads to an initial decline in function (alarm phase), which is followed by an adaptative response (resistance phase), allowing the system to develop immunity or defence towards the same stress. This theory has laid the foundations for training periodization in various sports (Gamble, 2006; Hawley et al., 1997), and is mainly characterised by a period of over-loading followed by super-compensation, provided adequate recovery is allowed. Likewise, it has been found that $\dot{V}O_{2peak}$ immediately drops from baseline at the start of the over-loading stimulus but, following a taper (reduced training load) or complete removal of the stimulus, a new peak is achieved (Aubry et al., 2014; Rønnestad et al., 2017). Similar interactions between $\dot{V}O_{2peak}$ and recovery following HA has

been noted in a recent meta-analysis (Waldron et al., 2021), wherein, $\dot{V}O_{2peak}$ improved as the gap between HA cessation and post-intervention testing widened, albeit within a seven day period. More importantly, the same group (Waldron et al., 2019), noted that the ergogenic benefit of HA (10 days) on thermoneutral $\dot{V}O_{2peak}$ was only realised 96-h post cessation of the intervention. Extrapolating these findings to the current study, a 24-h recovery period prior to $\dot{V}O_{2peak}$ testing might have been inadequate to reduce the accumulated fatigue and bring forth the full potential of the intervention. Additionally, Sitkowski et al. (2021) conducted $\dot{V}O_{2peak}$ testing across a week following completion of PH, which might have allowed a greater recovery from the over-loading stimulus. Future investigations are therefore required to elucidate the kinetics of $\dot{V}O_{2peak}$ response following the cessation of PH intervention.

5.3 Group Differences (PH vs. CON) in peripheral components of endurance performance

Our study is the first to show an unfavourable slowing of $\dot{V}O_2$ kinetics after four-weeks of PH. The τ represents the rate at which phase II pulmonary $\dot{V}O_2$ reaches steady-state following exercise onset (Whipp, 1971). Attainment of steady-state signifies the point at which external energy demands are met completely by aerobic metabolism and, therefore, τ is used as a reliable non-invasive measure to estimate exercising skeletal muscle $\dot{V}O_2$ kinetics (Rossiter et al., 1999). For healthy adults performing upright exercise in normoxic conditions, it is generally agreed that τ is dependent on the oxidative capability of mitochondria to generate energy (ATP) (Poole & Jones, 2012) or to a lesser extent, microvasculature blood flow distribution, denoted by improved matching of pulmonary and muscle oxygen uptake (Chopra et al., 2022; Murias et al., 2010a). Here, the initiation of mitochondrial respiration is closely linked to the appearance of ADP, which is a by-product of ATP hydrolysis and is a potent stimulator of OXPHOS. However, at the onset of exercise, PCr breakdown and anaerobic glycolysis are the dominant providers of ATP, which in turn are ADP consuming processes and therefore act as temporal buffers attenuating ADP accumulation, ultimately delaying the activation of OXPHOS (Grassi, 2005; Kindig, Hewlett, et al., 2005). Subsequently, NO can interfere with the electron transport chain by competing with O_2 at the binding site of cytochrome-c oxidase (Jones et al., 2003; Kindig et al., 2002), providing an alternative explanation for the inertia in initiating OXPHOS. Koga et al. (1997) were the first to show that limb-heating prior to moderate-intensity exercise had no effect on τ. The current results might indicate that repetitive but not acute thermal exposures has the potential to influence muscle $\dot{V}O_2$ kinetics. Whichever might be the mechanism i.e. an over-reliance on anaerobic metabolism or cross-interference of chemical compounds, the significant slowing down of τ in the PH-group might imply a mal-adaptative response in mitochondrial functioning following the somewhat aggressive PH intervention.

Another novel approach in the current study was the simultaneous measurement of peripheral O_2 kinetics using NIRS during the 5-min AOCT test. Similar to GE, which reflects the ratio of metabolic cost to mechanical work done (Coyle et al., 1992), $CR_{T/TSI}$ and $CR_{T/HHb}$ depict the ratio of CT to quadricep O_2 availability or extraction, respectively. CT is analogous to critical power and or speed and represents the highest exercise intensity at which aerobic metabolism can be sustained without the progressive accumulation of intramuscular metabolites i.e. Pi, ADP, K^+ and H^+ (Jones et al., 2008; Poole et al., 2016). Interestingly, a progressive improvement in $CR_{T/HHb}$ was noted in the CON group, with no changes $CR_{T/TSI}$ in either groups. The HHb signal derived from NIRS is blood flow insensitive and is used to measure O_2 extraction at the micro-circulatory level during muscular contractions (De Blasi et al., 1994). A higher $CR_{T/HHb}$ ratio, therefore, indicates that the CON group became more efficient (i.e. lesser O_2 extraction) in achieving similar CT values compared to PH group. The improvements noticed in the CON group might be explained in two ways. Firstly, none of the participants in the current study had prior knowledge or experience of the 5-min AOCT and, therefore, the possibility of a learning effect (PRE, MID & POST) cannot be completely excluded. However, to minimise this, a familiarisation visit was conducted before PRE and furthermore both groups started with similar experience levels at baseline. Thus, we would anticipate the same improvements as a function of time alone in both groups. Secondly, the CON group most likely benefited from the training alone. Whilst all participants were asked to continue with the same exercise regime as reported at baseline, there is an unavoidable natural improvement in some variables during the training process. When coupled with the requirement to log each training session, might have improved training adherence. Therefore, given the increase in the CON group and lack thereof in the PH group in this variable, we interpret this as a suppression of the natural adaptation in metabolic efficiency during maximal, exhaustive muscle contractions following this form of passive heating. Physiologically, training (not PH) might have resulted in preferential intracellular adaptations, subsequently enhancing mitochondrial function, rather than O_2 delivery related improvements. It is noteworthy that two submaximal parameters of endurance performance (τ & $CR_{T/HHb}$), mainly dependent on mitochondrial function, displayed a dampening-like response to the PH intervention. As discussed above, this could relate to the load of the programme or lack of sufficient recovery during the programme or prior to testing.

5.4 Theoretical basis for peripheral adaptations and suppression

Liu and Brooks, (2012) were the early pioneers to show that repeated heat stress (40 °C, 1 h/day for 5-days) promoted mitochondrial biogenesis in *in-vitro* mouse C2C12 myotubes. The authors also reported an upregulation of key regulatory proteins mainly; HSP's, AMP-activated protein kinase (AMPK), sirtuin 1(SIRT1) and PGC1-α. It is well accepted that AMPK and SIRT1 are key sensors

of metabolic energy homeostasis (Richter & Ruderman, 2009) and their expressions are heightened in conditions of increased AMP:ATP ratio, commonly seen with exercise (Cantó et al., 2009) or heat stress (Han et al., 2013). Increased expression of AMPK and SIRTI, leads to activation of catabolic pathways via direct phosphorylation of PGC1-α (Jäer et al., 2007). PGC1-α is proposed to be the master regulator of mitochondrial biogenesis and co-activates several transcription factors that increase the expression of genes responsible for encoding mitochondrial proteins (Scarpulla, 2011; Villena, 2015). The *in-vitro* observations by Liu and colleagues were recently replicated *in-vivo* in humans (Hafen et al., 2018), wherein six consecutive days of shortwave diathermy improved mitochondrial OXPHOS capacity. Additionally, both studies observed an increased synthesis of HSP70 and/or HSP90, which are specifically known for their chaperone-like cytoprotective activities, such as protein folding and transportation of translated mitochondrial proteins required for mitochondrial adaptations (Morton et al., 2009; Voos & Röttgers, 2002).

Consistent with the understanding that a training stimulus ceases to be beneficial when it is over-loaded, overexpression of the heat-induced signalling-cascade mentioned above can also lead to mitochondrial dysfunction or apoptosis (McCormick et al., 2021; Tamura & Hatta, 2017). The exercise component of PH can be considered as the initial thermal load placed on an individual. For instance, during exercise 70% of the metabolised energy is liberated as heat (Hargreaves & Spriet, 2020), and leads to a steady rise in muscle and core temperature as exercise continues. Using intra-muscular needle thermistors, Morton et al. (2006) showed that running for 45-min at moderate-intensity can increase muscle temperature up to 40 °C. Likewise, performing exercises with a lower eccentric load, such as cycling, can also raise Tc to 38.5 °C in temperate conditions (Verdel et al., 2021). On the other hand, skin temperature kinetics are more complex, characterised by an initial decline at exercise onset followed by a sustained rise as exercise continues (Balci et al., 2016; Nakayama et al., 1981). Altogether, the disturbances in metabolic and thermal homeostasis leads to formation of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (King et al., 2016; Slimen et al., 2014), with skeletal muscles being the primary ROS producing site during exercise. These molecules are widely known to impart oxidative stress; however, they also play a key role in upregulating signalling pathways that facilitate muscle adaptation. During the recovery period, expression of key antioxidant defence pathways, such as PGC1-α, MPAK, HSP's, VEGF and eNOS are elevated, facilitating cell repair and biogenies (Radak et al., 2013). This further indicates the significance of post-exercise recovery period and making it an important determinant of training outcomes.

Passive heating, as applied herein, is unique in the sense that the heating stimulus is applied immediately post-exercise, with the idea of thermally clamping or preserving the metabolic heat

produced during exercise. Interestingly, cutaneous blood flow is reduced almost immediately with the cessation of exercise (Wilkins, Minson & Halliwill, 2004), reducing the body's capacity to lose heat. The decrements in thermoregulatory capacity has been linked to the urgent need for restoring blood pressure (Kenny & Mcginn, 2017). It is well known that exercise (aerobic) cessation, specifically those that activate large muscle groups, is followed by a vasodilatory hyperaemic response that can elevate muscle blood flow up to 90-min post-exercise (Williams et al., 2005). This directional flow of blood, along with venous pooling, contributes to post-exercise hypotension, which is marked by a reduction in mean arterial pressure (MAP) (Halliwill, 2001). Likewise, the drop in skin blood flow to regain homeostatic pressure combined with muscle blood pooling would limit convective heat exchange and instead reverse the direction of heat transfer i.e., muscle to core (Kenny & Mcginn, 2017). In line with the above, core and muscle temperature can remain elevated by 0.4 °C and 1.5 °C above baseline respectively, for extended durations (> 60-min) post-exercise (Kenny et al., 2003; Kenny & Mcginn, 2017). Furthermore, the magnitude of thermal disturbance is dependent on prior exercise intensity (Kenny et al., 2006) and external environmental conditions (Nakamura et al., 2021). The PH intervention in the current study might have elongated the post-exercise hypotension response, ultimately delaying thermal homeostasis. Highly insightful investigations by Chiesa et al. (2016) showed that raising leg Tsk to 39 °C levels significantly increased blood flow through the major arteries supplying the skin and muscles. Pilot testing revealed that the layered heating system raised and held Tsk to 39 °C for the entire duration (2-h) of the PH bout. Thus, it is highly likely that the participants in the current study might have also experienced similar haemodynamic alterations. Here, the increase in skin blood in response to direct skin heating is achieved by a combination of elevated sympathetic vasodilatory drive and eNOS expression, the latter being more important due its role in directly producing the vasodilatory NO molecule (Kellogg et al., 1998, 2009). Additionally, the objective of the layering system was to create a hot microclimate surrounding the leg and therefore prevent any heat loss. Altogether this system would have created a physiological challenge, whereby the blood reaching the skin would have gained heat as result of a narrowing in core-to-skin-to-environment temperature gradient. It can also speculated that longer PH duration of the current study could have led to greater peripheral ROS formation, specifically those linked with NO that can react with O_2 anions to form peroxynitrite, which are highly cellular damaging agents (Brown, 1999). This coupled with the consecutive nature of the intervention might have resulted in a negative shift in redox balance, implying a greater net production of ROS than the net stimulation of the antioxidant defence system. Ultimately, this could have altered the cellular signalling cascade mentioned earlier and led to unfavourable and unchecked period of oxidative stress. Though these signalling pathways were not directly measured, it provides a reasonable explanation for the results of the current study, as both the peripheral submaximal endurance variables were assessed using quadricep dominant exercise tests, which were the primary sites of direct skin

heating. Likewise, the combination of exercise and PH along with the selected regime of the intervention could have created a thermal challenge that might have been less beneficial compared to previous studies.

5.5 Adaptation of in-series, systemic components

We demonstrated that the post-exercise passive heating regime was successful in facilitating brachial arterial remodelling, as depicted by a higher baseline (resting) arterial diameter in the PH group after 4-weeks of the intervention. This is significant for two main reasons. First, the current heating system was limited to the lower-body, and despite this, upper-body/upstream vascular adaptations were induced, implying that local heating can have positive systemic consequences. Furthermore, this outcome also shows the superiority of post-exercise passive heating regimes compared to passive heating alone, wherein, longer (60-90 min; 8-10 weeks) and more aggressive HWI protocol improved aspects of arterial functioning in the absence anatomical improvements (Brunt et al., 2019; Brunt, Howard, et al., 2016). This implies that protocols integrated with exercise that are less thermally challenging might be better tolerated and still provide peripheral cardiovascular benefits.

It could be reasonably speculated that remodelling of upstream vasculature is indicative of peripheral angiogenesis i.e. increased capillarisation at the muscular sites directly exposed to the thermal stimulus (Bloor, 2005; Prior et al., 2004). This is significant, as it means that the current post-exercise passive heating regime would have improved vascular dynamics, both, bulk O_2 delivery and peripheral perfusion around skeletal muscles. Thus, these improvements should positively influence endurance performance, based on features of the Fick principle, yet this was not the case in the current study. However, the null effects of the current intervention can potentially be explained by the concept of an in-series, integrative system that underpins maximal oxidative capacity (Wagner, 2011a). Here, every component of the oxygen transport cascade, from the uptake of atmospheric O_2 to its metabolism at the skeletal muscle (mitochondria) must function in a coordinated manner to facilitate optimal delivery and utilisation of O_2 at both sub-maximal (i.e. GET & CT) and maximal rates (i.e. $\dot{V}O_{2peak}$). As with endurance training, where different components of the integrative-system adapt at different rates (Murias et al., 2010a, 2011), it is possible that the improvement in the O_2 delivery components (at the arterial level) occurred earlier than at the mitochondrial level. The delayed cellular adaptation could, theoretically, be explained by an inability of the PH group to recover from the additional thermal challenge placed by the intervention while endurance training. It is therefore possible that the current data highlight the importance of better understating the dose-response relationship of heating interventions and accurately tracking the time-course of adaptation and recovery to elicit ergogenic outcomes.

Limitations

The major limitation of the current study was that training parameters (type, intensity and volume) and PH implementation were not directly controlled by the investigators and, therefore, relied on self-reporting or logging by participants. Participants recruited for the study were recreationally active and took part in various sports such as; football, cross-country, cricket, netball and triathlon. The heterogenous nature in training type could have affected the findings; however, training load was similar between groups. Likewise, quality/severity of the PH intervention, would depend on the time between cessation of exercise and initiation of the heating stimulus (Heathcote et al., 2019). Based on the self-reported data, all participants (PH-group) wore the layered heating system within 5-min of exercise cessation and so it can only be assumed that they adhered to the study instructions.

Another important limitation of the study was that cardiovascular responses were not directly measured during the PH intervention. For instance, Chou et al. (2018) clearly showed the chronotropic effect of whole-body passive heating, which elevated Tsk to 39 °C and significantly increased heart rate (20 beats/min) compared to resting conditions. The above changes in cardiac contractility occurred alongside a reduction in stroke volume and mean arterial pressure (Chou et al., 2018), which are hallmark responses, facilitating $\dot{Q}$ mediated haemodynamic redistribution (Crandall & Wilson, 2015). The mechanistic insights of passive thermal stress have been investigated using hot water perfusing suits (Chiesa et al., 2016; Chou et al., 2018) and therefore future investigations are required to elucidate the cardiovascular responses when using the lower-body heating systems developed in the current study. Pilot testing revealed that the PH protocol prevented the recovery of core (rectal) temperature (0.4 °C above baseline) following moderate-intensity running (Appendix E), which might have some cardio-vascular implications during the recovery period post-exercise.

The final limitation of the study was that markers of recovery following each PH bout was not assessed. The post-exercise period in PH interventions are spent in a thermally stressed state, the vasodilatory demand from raising the skin temperature, coupled with trapping of internal heat generated from exercise via narrowing core-to-skin temperature gradient, can lead to parasympathetic attenuation and sympathetic reactivation (Gagnon et al., 2015; Low et al., 2011; Lynn et al., 2009). Interestingly, Peçanha et al. (2017) showed that passive heating prior to exercise delayed heart rate recovery (HRR) in the immediate post-exercise period. This might have important implications for the current study, as majority of the participants anecdotally expressed that they trained in the evening, which meant that most PH bouts were completed closer to a period of sleep. It is possible that the demands of the PH intervention might have affected sleep quality (Osborne et al., 2022),

perhaps contributing to an overreaching effect. Future studies are required to clearly understand the time-course of achieving physiological homeostasis following PH interventions.

Conclusion

This is the first study to conduct a non-laboratory-based PH intervention in recreationally trained athletes. We have demonstrated that four-weeks of PH, comprising 20 consecutive applications of 90-120 min, had no ergogenic effect on determinants of endurance performance. In contrast to our hypothesis, we report that the above skin heating regime, in combination with routine endurance training, can elicit some negative effects on components of oxidative metabolism, as demonstrated by an apparent suppression of the $\dot{V}O_2$ primary component adaptation, alongside a degradation of force production-O_2 extraction coupling during maximal leg exercise in the PH group. Despite these negative changes, the PH group improved their resting arterial diameter and vasodilation area relative to CON, demonstrating a potential enhancement of macrovascular structure and function across the four-weeks. We explain these findings as the result of differential adaptations across sites of an in-series, integrative system, whereby positive changes observed in conduit vessels appeared to precede the delayed downstream adaptations, which would most likely occur at a mitochondrial level. Further research is required to elucidate this, but it is possible that the overall thermal load, combined with frequency of training in this group, hampered the anticipated changes in the endurance phenotype. In accordance with this notion, a greater period of recovery post-intervention might have revealed a potentially delayed endurance response, which is known to occur in response to heat training. Further research is required to identify the optimum dose and recovery required to elicit an ergogenic effect using the PH intervention proposed in the current study.

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Appendices

Appendix A: Ethical approval form

LEAD APPLICANT NAME: Kevin John
DISCIPLINE/DEPARTMENT: SPEX
PROJECT TITLE: Investigating the effect of repeated lower-limb passive heating on endurance performance and markers of vascular adaptations using in-vivo ex-vivo and in-vitro analysis.
APPLICATION REFFERENCE NUMBER: Kevin_John_01-10-21

Swansea University
Prifysgol Abertawe

Date of review board: November
Committee members in attendance: Chairs, RM

Date: Monday 22nd November

Dear Kevin,

Thank you for your recent ethics application.
This decision letter is to inform you that the ethics application for the above titled project has been reviewed and approved. The ethical approval number for this application is KJ_01-10-21 approved from 19-11-21– end of approval 01-10-23.

This letter is for Swansea University, College of Engineering Research Ethics and Governance approval only. Local Health and Safety, in addition to appropriate risk assessment guidelines are required separate to this approval, unless otherwise stated herein, and must be adhered to.

Associated researchers must not deviate from the approved protocol or extend beyond the approval end date. Any desired deviations or approval date extensions are subject to the ethical approval amendment process. Upon completion of the approved project researchers responsible for this application must submit a final (short) statement to the ethical committee stating the completion of the project, unless a time extension is being requested through the amendment process.

Any significant un-anticipated adverse effects/events (i.e. not those predicted and stated in section 8 of the ethics application form) must be reported to the Ethics committee upon researcher realisation (email: coe-researchethics@swansea.ac.uk with the subject title including the study approval number followed by "Adverse Effects/Events").

If you have any further questions relating to your application, please contact: coe-researchethics@swansea.ac.uk

Please keep note of your approval number for future reference and correspondence relating to this application.

Best of luck with your research.

Warm regards,

Aynsley Fagan

(on behalf of the College of Engineering Research Ethics and Governance Chair)

College of Engineering Ethics and Governance Committee Administrator
College of Engineering | Y Coleg Peirianneg
Swansea University | Prifysgol Abertawe
Fabian Way | Ffordd Fabian Crymlyn Burrows
Swansea | Abertawe
Wales | Cymru
SA1 8EN

Email: coe-researchethics@swansea.ac.uk

Appendix B: Participant information sheet

PARTICIPANT INFORMATION SHEET
(Version 1.1, Date: 01 /10/2021)

Project Title:

Investigating the effect of repeated lower-limb passive heating on endurance performance and markers of vascular adaptations using in-vivo, ex-vivo and in-vitro analyses

Contact Details:

Kevin John (Lead researcher)
Email – [redacted]
Phone no. - [redacted]

Dr Mark Waldron (Supervisor)
Email – [redacted]

1. Invitation Paragraph

You are invited to take part in a research project. Before you decide whether you would like to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully. Please ask if there is anything that is unclear, or if you would like more information. This study follows the ethical guidelines of Swansea University and has received ethical approval from the Research Ethics Committee.

2. What is the purpose of the study?

Passive heating (PH) is a way of heating the body without exercise and has been shown to improve exercise performance and other important aspects of your cardiovascular system, without altering your normal endurance training programme. This has raised interest within the sporting world regarding the efficacy of PH as a supplementary tool to aid training outcomes. This study aims to assess physiological and performance-related changes associated with PH by using a home-based limb-heating intervention.

3. Why have I been chosen?

You have been chosen because you are between 18 and 40 years, healthy and take part in weekly endurance exercise. Participation is entirely voluntary, it is your decision whether or not to take part. If you decide to take part, you will be given this information sheet to keep and asked to sign separate consent and health screening forms. If you decide that you do not wish to participate then please appropriately discard this information sheet. Regardless of your decision, we thank you for your time. You can withdraw your participation at any time without reason or penalty, up until the study is submitted for publication

4. What will happen to me if I take part?

After you have been health screened, you will be required to visit the laboratory on 6 different occasions and complete a passive heating intervention for 4-weeks. All laboratory visits will be conducted at Swansea University Bay campus in the engineering east sport and exercise science (SPEX) laboratories The heating intervention will require you to wear electrical heating garments post-exercise (2 hrs at 43˚ C for 5 days/week) for total of ~20 sessions; however, this will be completed in your own free-living space. A total of 5 different laboratory tests (please refer to the table below which provides a brief outline) will be conducted across two days at week 0 (pre-intervention), week 2 (mid-intervention) and week 4 (post-intervention), which include: resting ultrasound measurements, venous blood samples, maximal and sub-maximal exercise test on a bike in a laboratory, and a test of your leg muscle strength-endurance (critical torque test). These tests will be repeated at three stages: pre, mid and post-intervention, where each stage will require two visits. The testing order for the first visit are: FMD venous blood samples and VO_{2max} exercise test and on the second visit are: sub-maximal VO_2 kinetics test and a critical torque test. This testing order will remain consistent through-out the study. All visits will take place at Swansea University Sport & Exercise science laboratories. The total time commitments for laboratory visits will be 15 hours.

Test	Procedure
FMD (ultrasound) Location B133A	You will be required to lie down on a raised bed for 10 mins. After which a cuff will be placed on the forearm. A probe will be placed around the right biceps to get images of your artery. The complete test will last for 20 mins wherein you will be only required lay (supine) relaxed on the raised bed without movement. *This test helps us analyse your vascular health (arterial blood vessels). Total test time (TTT):40 mins*
Venous blood samples (Location B133A)	The blood collection will involve taking only ~20 ml of blood from the medial cubital vein via a butterfly needle. Care will be taken for the test to be conducted such that minimal or no pain will be experienced. All samples will be collected from researchers trained in phlebotomy. *These samples will be used in our serum studies that will help determine changes in markers such as (VEGF, eNOS & HSP). These markers have shown to promote overall health and endurance performance. TTT: 10 mins*
VO_{2max} exercise test (Location B108B)	This test will be carried on a cycle ergometer and will only require you to cycle till volitional exhaustion. Usually this test will be completed in 5-10 minutes. *This test tells us about the maximum amount of oxygen that your body can metabolise during exercise. TTT: 60 mins*
Sub-maximal VO_2 kinetics test (Location	This test will be carried on the same cycle ergometer and will only require three submaximal efforts interspersed with active rest periods of easy pedaling. *This test helps us in analyzing the way in which your body consumes oxygen.TTT: 40 mins*

B108B)	
Critical torque test (Location B133A)	This test will be conducted on an equipment designed as a knee extensor machine seen in the gym. You will be required to produce maximal effort knee extensions for 5 min. The test will be conducted only with the right leg. *This test allows us to better understand the force generating capability of your muscle (quadriceps) during a fatiguing exercise bout. TTT: 20 mins*

5. What are the possible disadvantages of taking part?

There are no major disadvantages of taking part in this study but the exercise testing will be quite strenuous and you will need to be prepared for each testing visit.

There has been extensive research conducted addressing the safety of VO_{2max} exercise testing. Possible complications associated with the test are: stroke, difficulty in breathing, myocardial infraction (heart attack), chest pain and death. However, a previous study conducted by Gibbons et al., (1989) showed that out of 71,914 tests only 6 complications were reported. All the 6 patients that showed complications had a previous history of a heart condition.
The current study will only recruit healthy individuals with no underlying cardiovascular complications therefore making maximal exercise testing very safe (99 %) to administer.

The critical torque test could result in delayed onset of muscle soreness, muscle injuries i.e strain or tears and feeling nauseous or light headedness during maximal exercise. However, the occurrence of such incidents is extremely low.

The garments used for heating is programmed to heat the skin to 43° C. This temperature is expected cause mild to high thermal discomfort.

The lead researcher (Kevin John) has been first aid trained and holds a globally recognised CPR/AED certification which is valid till 2022. He will be present at all times during any testing carried out during the entirety of the study.

6. What are the possible benefits of taking part?

The benefits of taking part in this study are the knowledge that you are contributing to the advancement of science. Throughout the study you will also gain information about your own physiological and performance scores, which can be taken forward as bench marks and used in training. Finally, if the PH is successful it will positively impact, you're cardiovascular health and help you recover and perform better in training or competition.

7. Will my taking part in the study be kept confidential?

All information collected about you will be kept strictly confidential. Data will be stored safely and securely and held for the duration of the research project, after which it will be destroyed, in line with the Data Protection Act 2018. At no time will information be disclosed or the data used for other purposes than those described here. Should any abnormal physiological results be identified,

you will be informed of this and recommended to visit your general practitioner should you feel concerned or need further advice. Any measures taken during this study are not diagnostic and the study team are not qualified to provide medical interpretation. Data will be retained electronically for 3 years on a password protected computer. Any information that leaves Swansea University will have your personal details removed so that you cannot be recognized. Names will be replaced with participant numbers to ensure all data collected remains anonymous. Once the study has been completed it will be written up for a Master's thesis and for publication. The overall study findings will be made available to you if desired. It will not be possible for anyone to see your individual results or identify that you participated.

Data Protection and Confidentiality

Your data will be processed in accordance with the Data Protection Act 2018 and the General Data Protection Regulation 2016 (GDPR). All information collected about you will be kept strictly confidential. Your data will only be viewed by the researcher/research team.

All electronic data will be stored on a password-protected computer file only accessible to the research team. All paper records will be stored in a locked filing cabinet situated on Bay campus engineering-east (room A101) and only accessible by the research team. Your consent information will be kept in a separate cabinet situated in the location mentioned above in order to minimise risk in the event of a data breach.

Please note that the data that will be collected for our study will be made anonymous, each participants' name will be replaced with a numerical code after the first visit, this will be done on a password-protected spreadsheet and stored on a password-protected device. Therefore, if at the end of this research you decide to have your data withdrawn, please let us know before you leave.

The lead researcher (or supervisor) will take responsibility for data destruction and all collected identifiable data will be destroyed on 1st January 2024.

Data Protection Privacy Notice

The data controller for this project will be Swansea University. The University Data Protection Officer provides oversight of university activities involving the processing of personal data, and can be contacted at the Vice Chancellors Office.

Your personal data will be processed for the purposes outlined in this information sheet.
Standard ethical procedures will involve you providing your consent to participate in this study by completing the consent form that has been provided to you.

The legal basis that we will rely on to process your personal data will be processing is necessary for the performance of a task carried out in the public interest. This public interest justification is approved by the College of Engineering Research Ethics Committee, Swansea University.

The legal basis that we will rely on to process special categories of data will be processing is necessary for archiving purposes in the public interest, scientific or historical research purposes or statistical purposes.

How long will your information be held?

All personal data collected during the study will be kept for a period of 3 years after the completion of the study. This would allow the researchers to give feedback if participants request further feedback on the study outcomes and personal test scores. After the 3 years the password-protected

spreadsheet containing the participant names and their respective codes will be deleted, however, all the remaining data will be retained, albeit anonymously for 10 years on a password protected computer. All anthropometric data of the participants will be stored safely and securely for the duration of the research project, after which point it will be destroyed as per the Data Protection Act 2018.

What are your rights?
You have a right to access your personal information, to object to the processing of your personal information, to rectify, to erase, to restrict and to port your personal information. Please visit the University Data Protection webpages for further information in relation to your rights.

Any requests or objections should be made in writing to the University Data Protection Officer:-

University Compliance Officer (FOI/DP)
Vice-Chancellor's Office
Swansea University
Singleton Park
Swansea
SA2 8PP
Email: dataprotection@swansea.ac.uk

How to make a complaint
If you are unhappy with the way in which your personal data has been processed you may in the first instance contact the University Data Protection Officer using the contact details above.

If you remain dissatisfied then you have the right to apply directly to the Information Commissioner for a decision. The Information Commissioner can be contacted at: -

Information Commissioner's Office,
Wycliffe House,
Water Lane,
Wilmslow,
Cheshire,
SK9 5AF
www.ico.org.uk

8. What if I have any questions?
Further information can be obtained from the researcher contact stated above. Also state that "the project has been approved by the College of Engineering Research Ethics Committee at Swansea University. If you have any questions regarding this, any complaint, or concerns about the ethics and governance of this research please contact the Chair of the College of Engineering Research Ethics Committee, Swansea University: coe-researchethics@swansea.ac.uk. The institutional contact for reporting cases of research conduct is Registrar & Chief Operating Officer Mr Andrew Rhodes. Email: researchmisconduct@swansea.ac.uk. Further details are available at the Swansea University webpages for Research Integrity. http://www.swansea.ac.uk/research/researchintegrity/."

School of Sport and Exercise Sciences –Health Screening Questionnaire

Name:

Risk Factors		Risk Factor	No Risk Factor
Q 1. Age = _____ *years*	Males	≥ **45**	**< 45**
	Females	≥ **55**	**< 55**
Q 2. Have any parents, brothers or sisters had a heart attack, bypass surgery, angioplasty, heart transplant, pacemaker or defibrillator implanted, treated for an irregular heartbeat or suffered from **sudden death prior to 55 years (male relatives) or 65 years (female relatives). If 'Yes' detail:**		**Yes***	**No**
Q 3. Do any of your parents, brothers or sisters suffer from a serious medical condition or at less than 50 years of age been treated for recurrent fainting or suffered from unexplained seizure problems, drowning while swimming or a car accident? **If 'Yes' detail:**		**Yes**	**No**
Q 4. Are you currently a smoker – have you quit within the past 6 months – are you exposed to environmental tobacco smoke?		**Yes**	**No**
Q 5. In the past 3 months have you performed at least 30 minutes of moderate intensity physical activity or equivalent on at least 3 days of the week (total of 90min mod. act.)?		**No****	**Yes**
Q 6a. Body mass index = _____ $kg.m^{-2}$ (weight divided by height squared)		≥ **30**	**< 30**
Q 6b. Waist girth = _____ cm	Males	**> 102**	**≤ 102**
	Females	**> 88**	**≤ 88**
Q 7a. Do you take blood pressure medication		**Yes**	**No**
Q 7b. Resting blood pressure: SBP = _____ *mmHg, DBP* = _____ *mmHg*		**≥140/90*** **	**< 140/90**

TOTAL NUMBER OF RISK FACTORS

***** If Yes to **early sudden death** in family history advise pre-participation screening for SCD
** If currently sedentary consider whether max test is essential
*** If BP ≥140/90mmHg treat as High Risk and advise pre-participation screening for SCD

Exercise History

Q 8. How would you describe your current physical fitness status?	**Very unfit (sedentary) / Unfit / Somewhat Fit / Moderately Fit / Very Fit (e.g. competitive sportsperson)**	
Q 9. How frequently do you exercise? (times per week)		
Q 10. How often do you undertake exercise of a maximal nature?	**Never / Sometimes / Often**	
Q 11. Do you get tired more quickly than your friends do during exercise? **If 'Yes' detail:**	**Yes**	**No**

Signs or Symptoms

	Yes S/S	No S/S
Q 12. Do you ever have pain or discomfort in your chest or surrounding areas (neck, jaw, arms or other areas)?	**Yes**	**No**
Q 13. Are you ever short of breath at rest or with mild exertion?	**Yes**	**No**

Q 14. Have you ever experienced dizziness or loss of consciousness during or shortly after exercise?	**Yes**	**No**
Q 15. Have you ever been short of breath at rest in the recumbent position or had an attack of breathlessness in the middle of the night which was relieved by sitting up?	**Yes**	**No**
Q 16. Do your ankles ever become swollen (other than as a result of an injury)?	**Yes**	**No**
Q 17. Do you ever have palpitations (=the unpleasant awareness of the heart beating in your chest) or an unusual period of rapid heart rate?	**Yes**	**No**
Q 18. Do you ever suffer from cramp-like pains in your legs, brought on by exertion and relieved after 1-2 minutes of rest?	**Yes**	**No**
Q 19. Has a doctor ever said you have a heart murmur or arrhythmia?	**Yes**	**No**
Q 20. Have you ever been told you had rheumatic fever?	**Yes**	**No**
Q 21. Do you feel unusually fatigued or find it difficult to breathe with usual activities?	**Yes**	**No**
SIGNS/SYMPTOMS OF DISEASE	**YES / NO**	

History of Disease

	History of Disease	**No History of Disease**
Q 22. Heart disease	**Yes**	**No**
Q 23. Heart disorder	**Yes**	**No**
Q 24. Peripheral vascular disease	**Yes**	**No**
Q 25. Cerebrovascular disease (e.g. stroke)	**Yes**	**No**
Q 26. Chronic obstructive pulmonary disease (emphysema/chronic bronchitis)	**Yes**	**No**
Q 27. Asthma	**Yes**	**No**
Q 28. Interstitial lung disease	**Yes**	**No**
Q 29. Cystic fibrosis	**Yes**	**No**
Q 30. Diabetes mellitus	**Yes**	**No**
Q 31. Thyroid disorder	**Yes**	**No**
Q 32. Renal disease	**Yes**	**No**
Q 33. Liver disease	**Yes**	**No**
Q 34. High blood cholesterol	**Yes**	**No**
Q 35. Epilepsy or recurrent seizures	**Yes**	**No**
Q 36. Recurrent fainting	**Yes**	**No**
HISTORY OF DISEASE	**YES / NO**	

If 'Yes' please provide details:

Other Conditions and Additional Information

	Condition	**Condition**
Q 37. Do you have any bone or joint problems such as arthritis or a past injury that might get worse with exercise, or any current muscle, joint of back injury? **(Exercise testing may need delaying or modifying) If 'Yes' detail:**	**Yes**	**No**
Q 38. Do you have any other problem that might make it difficult for you to do strenuous exercise? **If 'Yes' detail:**	**Yes**	**No**
Q 39. have you ever been told to give up sports / exercise because of health problems? **If 'Yes' detail:**	**Yes**	**No**

Q 40. Have you had to suspend any normal activity due to ill health or injury in the last month?**If 'Yes' detail:**	**Yes**	**No**
Q 41. Have you had severe viral infection (e.g. myocarditis or mononucleosis) within the last month? **If 'Yes' detail:**	**Yes**	**No**
Q 42. Have you ever suffered from heat stroke, heat exhaustion, sunstroke, cold illness or injury (non-freezing cold injury or frostbite), poor circulation (e.g. Raynaud's) or peripheral neuropathy? **If 'Yes' detail:**	**Yes**	**No**
Q 43. Have you ever suffered an adverse event in the heat (e.g. dizziness, fainting)? **If 'Yes' detail:**	**Yes**	**No**
Q 44. Do you have a disease / condition or take any medications that may inhibit the sweating process? **If 'Yes' detail:**	**Yes**	**No**
Q 45. Have you consulted your doctor within the last 6 months? (*except for contraception*) **If 'Yes' detail:**	**Yes**	**No**
Q 46. Please give details of any overnight hospital admissions you have had. **Detail:**	**Yes**	**No**
Q 47. Are you on any prescription medications? **If 'Yes' list:**	**Yes**	**No**
Q 48. Have you routinely taken any medications in the past 2 years? **If 'Yes' list:**	**Yes**	**No**
Q 49. Have you any other past medical history we have not already asked about? **If 'Yes' detail:**	**Yes**	**No**
Q 50. Are you or have you recently been pregnant?	**Yes**	**No**
Q 51. Have you donated blood in the last week?	**Yes**	**No**
Q 52. Do you have any allergies? (please include those to dressings e.g. elastoplasts) **If 'Yes' detail:**	**Yes**	**No**
Q 53. Are you a regular user of any dietary supplements (e.g. caffeine, creatine)? **If 'Yes' detail:**	**Yes**	**No**
Q 54. Do you drink alcohol? If 'Yes' do you drink occasionally or every day? **Detail:**	**Yes**	**No**
Q 55. Have you travelled abroad in the last 2months? **If 'Yes' detail:**	**Yes**	**No**
Q 56. To the best of your knowledge are there any other reason(s) that may prevent you from successfully completing the tasks that have been explained to you by the lead academic / principal investigator and as described in the Participant Information Sheet? **If 'Yes' detail:**	**Yes**	**No**

Appendix D: Participant consent form

PARTICIPANT CONSENT FORM
(Version 1.1, Date: 01/10/2021)

Project Title:

Investigating the effect of repeated lower-limb passive heating on endurance performance and markers of vascular adaptations using in-vivo, ex-vivo and in-vitro analysis

Contact Details:

Kevin John
Email – [redacted]
Phone no. - [redacted]
Swansea University,
Bay Campus
SA1 8EN

Please initial box

1. I confirm that I have read and understood the information sheet dated//20 (version number ……) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of data obtained may be looked at by responsible individuals from the Swansea University or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access these records. ☐
4. I understand that data I provide may be used in reports, academic publications and future research in anonymous fashion and by the research team only. ☐
5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix E: Pre laboratory testing guidelines

Pre-Testing Laboratory Visit Information

Dear Participant,

In order to control our measurements and ensure they are as accurate as possible, we have a set of preparation requirements for you to follow prior to each visit. Please read the list below and arrive at the laboratory as suggested:

•Please do your best to maintain a similar dietary intake on the days of testing and 24 hours prior to testing throughout all 3 testing windows.

- E.g.: if you have chicken and rice in between tests on day 1, try and have the same between these tests through all 3 visits.

TESTING DAY 1 (FMD, blood sample, and VO_2max)

•No eating any type of food from 7 pm the previous evening

- You will be given some time to eat after FMD (subject to your own schedule) prior to VO2max
- YOU ARE ALLOWED TO DRINK WATER IN THE MORNING (300-500 ml)

•No consumption of coffee/caffeine/energy drinks e.g. red bull, coca cola

•No alcohol 24 hours prior to testing and throughout the duration of the testing visits (3 days total)

•No exercise 24 hours prior to test visit i.e COMPLETE REST

TESTING DAY 2 (VO_2 submaximal kinetics and critical torque)

•No consumption of coffee/caffeine/energy drinks e.g. red bull, coca cola

•No alcohol 24 hours prior to testing and throughout the duration of the testing visits (3 days total)

•No exercise 24 hours prior to test visit i.e COMPLETE REST

Upon arrival at Bay Campus (Swansea University Bay Campus, Fabian Way, Crymlyn Burrows, Skewen, Swansea, SA1 8EN), please contact Kevin [redacted] or Erik ([redacted]) and they will meet you outside the Engineering East building/car park. If you have any queries, contact the research team at [redacted] or [redacted]

Appendix F- Pilot testing one bout of post-exercise passive heating (PH)

Description – The pilot consists of a sample size of *n* =1, who was a recreationally trained male. The test was conducted in a thermoneutral chamber (20 °C, 50% RH). Skin thermistors were used to measure the skin surface temperature of the lower leg (left) for the duration of the test and data were collected at 5-s intervals using Grant squirrel data logger (SQ2010; Grant Instrumrnts, Cambridge, United Kingdom). The participant rested for 15-min, after which they began a treadmill run at a self-selected pace (~ 11.5 km/h) for 30-min. This was immediately followed by 90-min of PH using the layered system explained in section (3.4) of the current thesis. During the PH period, the participant were seated on a chair, which was interrupted by walking intervals of 1-min on a treadmill every 20-min. This mimicked the conditions that would be expected at home or in office settings. The location of the skin thermistors can be seen in Figure 1, with core temperature measured via rectal probe. The changes in skin and core temperature during the experiment are presented in in Figure 2.

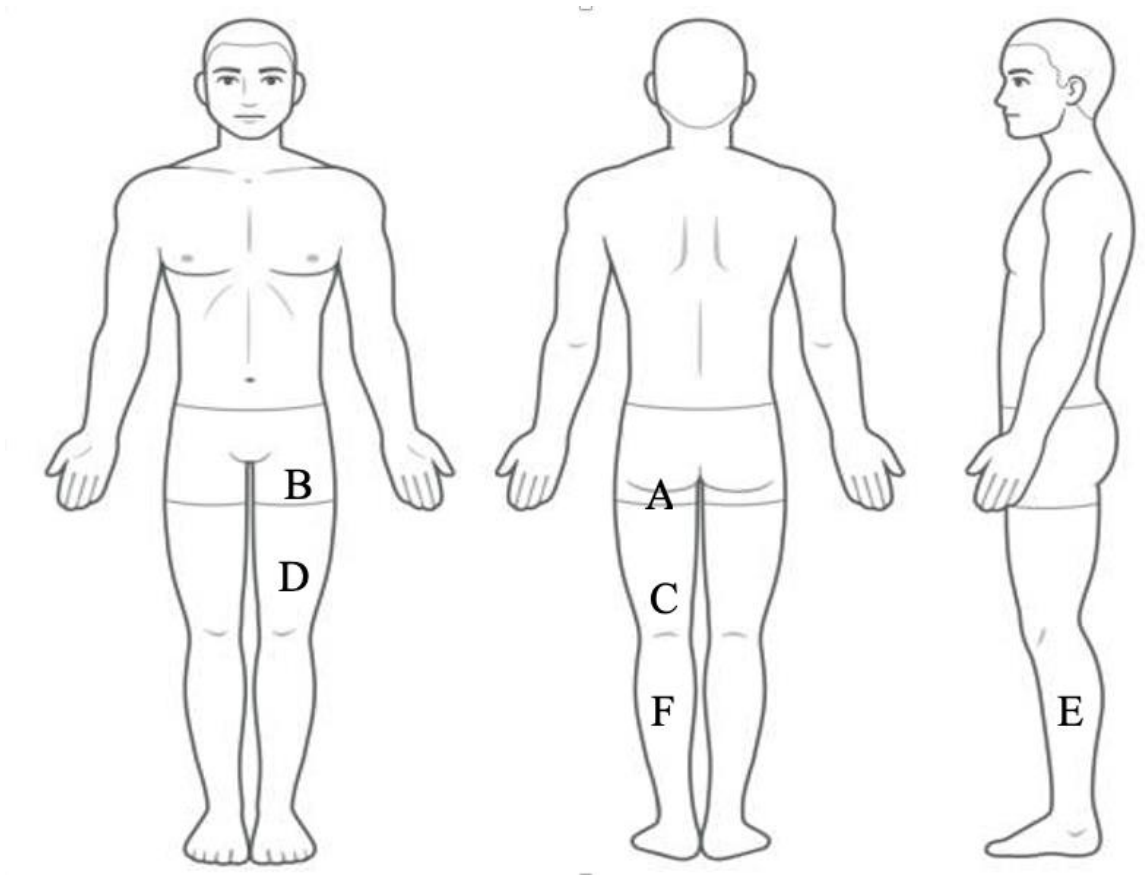

Figure 1. Anterior plane: Two skin thermistors; B- Front-upper and D- Front-lower, Posterior plane: Three skin thermistors; A- Back-upper, C- Back-lower and F- Calf-back and Lateral plane: One skin thermistor; E- Calf-left. Thermistors D, A, C and F were in close proximity to the heating element.

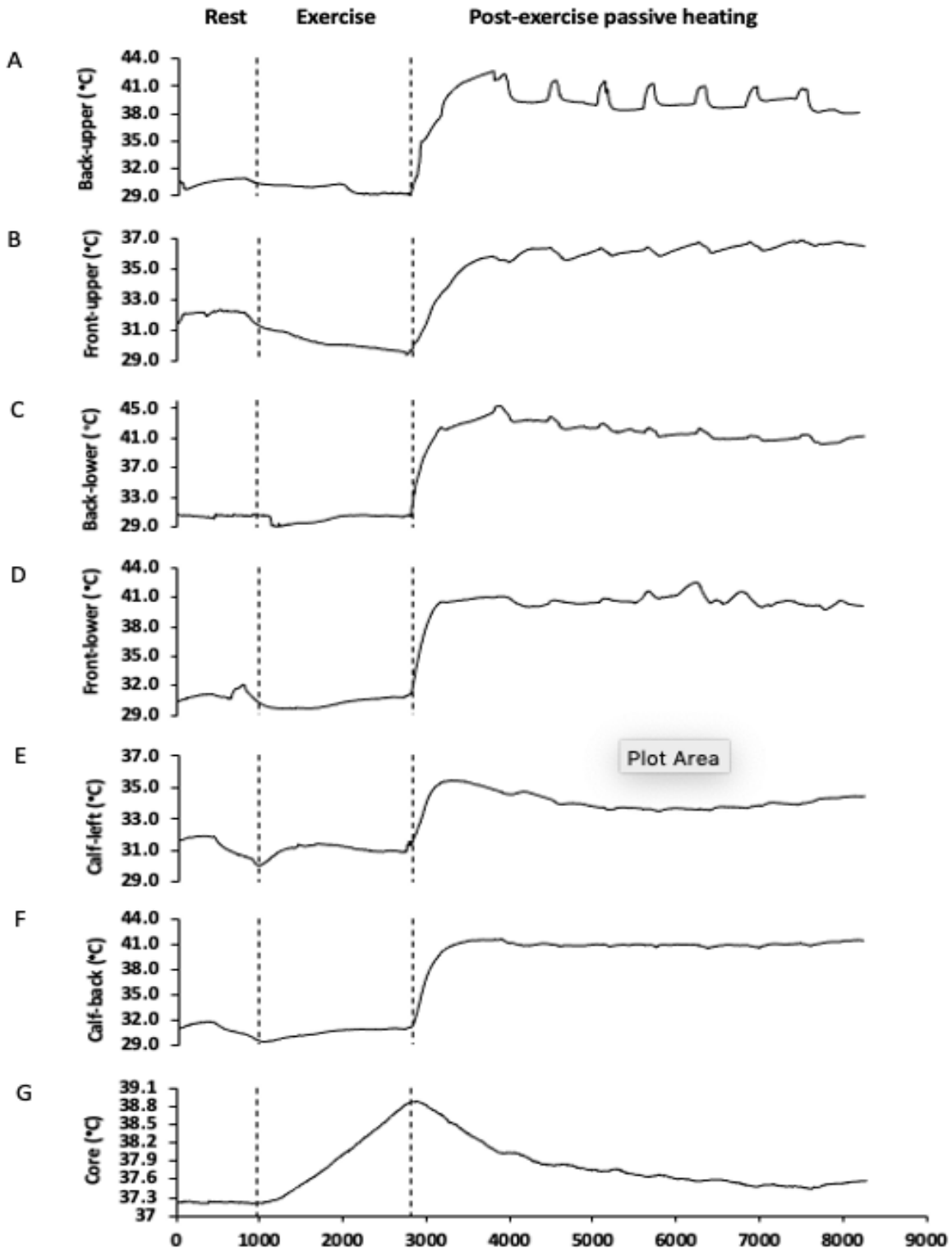

Figure 2. Skin (A-F) and rectal core (G) temperatures during rest, exercise and post-exercise passive heating phase.