The influence of local and whole-body cooling on pulmonary and muscle oxygen uptake kinetics during moderate exercise

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Human Kinetics (MHK)

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	The influence of local and whole-body cooling on pulmonary and muscle oxygen uptake kinetics during moderate exercise	
Name of Candidate Nom du candidat	Beckett-Brown, Nicholas	
Degree Diplôme	Master of	
Department/Program Département/Programme	Human Kinetics	Date of Defence Date de la soutenance March 04, 2019

APPROVED/APPROUVÉ

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ACCESSIBILITY CLAUSE AND PERMISSION TO USE

Abstract

This study investigated oxygen kinetics and blood flow in response to core and muscle cooling at the onset of moderate exercise. Eight males performed two 3-min cycling bouts at moderate intensity (80% of the first ventilatory threshold) with (leg cooling; LC), and without (thermoneutral; TN) cooling of the *vastus laterals* (VL) muscle by 6 °C, and when core temperature was also decreased (whole-body cooling; WBC) by 1 °C. Muscle blood flow, pulmonary (VO_{2p}) and muscle (VO_{2m}) oxygen uptake, and kinetics, as well as cardiovascular hemodynamics kinetics, were assessed. VO_{2p} was higher in WBC (+1.96 $mL \cdot kg^{-1} \cdot min^{-1}$, $p = 0.023$). Despite central (reduced cardiac output, heart rate pH, PO_2, sO_2, and increased VO_{2p}, whole-body A-VO_2Diff, PCO_2) and local changes (decreased muscle blood flow and increased local-leg A-VO_2Diff) induced by core and muscle cooling, oxygen and cardiovascular hemodynamics kinetics remained unaltered. This study demonstrated that local muscle cooling by 6 °C and core cooling by 1 °C do not influence local muscle or pulmonary oxygen kinetics during moderate exercise. Whether muscle priming, antagonist muscle activity, exercise intensity, cooling intensity or modality influenced the lower oxidative capacity of cooled muscles on kinetics remains unclear.

Keywords

Muscle cooling, core cooling, oxygen kinetics, moderate exercise, Near-infrared spectroscopy.

Co-Authorship Statement

Author Contributions:

Nicholas Beckett-Brown assisted with the design of the study, led data collection, conducted the data analysis, and wrote the manuscript.

Dr. Dominique D Gagnon conceptualized the study, supervised the data collection, assisted the data analyses, and reviewed the manuscript.

Dr. Olivier Seresse assisted with the conceptualization of the study, methodological development, and review of the manuscript.

Dr. Juha Peltonen assisted with the conceptualization of the study, methodological development, and review of the manuscript.

Dr. David C Marsh was the supervising medical doctor for the study; he also assisted in the review of the manuscript.

Dr. David A MacLean assisted with the methodological development of the study, data analysis and review of the manuscript.

Alexus McCue assisted in data collection, data analysis, review of the manuscript.

Etienne Gagnon assisted with data analysis and interpretation of the results.

Acknowledgments

Many have helped me immensely to bring this project through to fruition over the last two years.

First, I would like to thank Dr. Dominique Gagnon, who has been my supervisor and mentor over the past couple of years. He has provided endless support and always promoted my growth academically and as an individual as well. His outstanding attention to detail has developed high-quality research abilities within his lab.

Thanks to my committee members Dr. Olivier Seresse and Dr. Juha Peltonen who have been an enormous support throughout the progress of my degree.

Dr. Stephen Ritchie, who helped initiate my interest in research many years ago. You have been an outstanding academic mentor, and although our meetings often got sidetracked with discussions of outdoor adventures, you still managed to provide excellent support and direction.

Thank you to my parents and girlfriend, who have provided endless support throughout all of my academic endeavours.

My colleague and friend, Adrian Nizzero, you have been a mentor in many ways, pushing me beyond what I thought was possible, both in the gym and on the field. We have consumed a substantial volume of Old Rock coffee in a short amount of time, and you always provided a much-needed distraction when workdays were tough.

I want to acknowledge the CROSH for establishing a level of academic professionalism among graduate and undergraduate students. I also want to thank CROSH and the United Steel Workers for providing financial support throughout my studies at Laurentian University.

I would like to thank the Power Corporation of Canada- Queen Elizabeth Graduate Scholarship in Science in technology for financial support throughout my studies.

Thanks to Etienne Gagnon, who developed the data analysis codes, along with troubleshooting during data analysis. We could not have done it without his knowledge and

skills. Undergraduate summer research assistants Michelle Myre and Sara Machum, as well as, visiting researcher Laura Vilen were a considerable help during data collection.

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List of Abbreviations

AO	Arterial occlusion
Amp	Amplitude
BE	Base excess
BL	Baseline
BP	Blood pressure
HbDiff	Difference between oxy – deoxyhemoglobin
HCO_3	Bicarbonate
HHb	Deoxygenated hemoglobin
HR	Heart rate
LT	Lactate threshold
NIRS	Near-Infrared Spectroscopy
O_2	Oxygen
O_2Hb	Oxygenated hemoglobin
PCO_2	Partial pressure of carbon dioxide
PO_2	Partial pressure of oxygen
$\dot{Q}$, CO	Cardiac output
$\dot{Q}_{SFA}$	Leg blood flow of the surface femoral artery
$\dot{Q}_m$	Muscle blood flow
sO_2	Saturation of Oxygen
SV	Stroke Volume
τ	Time constant
T_c	Core temperature
TCO_2	Total carbon dioxide

TD	Time delay
tHb	Total hemoglobin
T_{leg}	Leg surface temperature
T_m	*Vastus lateralis* muscle temperature
$\bar{T}_{sk}$	Mean-weighted skin temperature
VE/ VCO_2	Ventilatory equivalent of carbon dioxide
VE/ VO_2	Ventilatory equivalent of oxygen
VL	*Vastus lateralis* muscle of the quadriceps
VO	Venous occlusion
VO_{2m}	Muscle oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2p}	Pulmonary oxygen uptake
VT	Ventilatory threshold

1 Introduction

The study of pulmonary and muscular oxygen (O_2) kinetics offers insights on oxidative metabolism, and the phosphorylation potential of ATP, the main source of energy for the majority of human movements (Jones & Poole, 2013). Oxygen transport and delivery are influenced by factors such as age, sex, physical fitness level, O_2 concentration, muscle fiber composition, blood flow, disease status, and others (Hughson, 2009; Poole & Jones, 2012). Temperature, is also an important factor to consider because it affects blood flow, neuromuscular function, the rate of chemical reactions and oxygen affinity thus having the potential to influence O_2 kinetics (Abramson, Tuck, Bell, Burnett, & Rejal, 1959; Ishii, Ferretti, & Cerretelli, 1992; Mairbaurl & Humpeler, 1980; Wakabayashi, Oksa, & Tipton, 2015). By studying the O_2 kinetics of the pulmonary and muscular systems during exercise, we can attempt to characterize their dynamic profiles (Poole & Jones, 2012). Changes in core and muscle temperature have the potential to influence pulmonary and muscular processes affecting the time and rate at which they reach steady-state (Brooks et al., 1971; O'Hanlon & Hovarth, 1970; Rowell, 1974; Wilson & Crandall, 2011).

The effects of changes in muscle temperature (T_m) on pulmonary oxygen ($\dot{V}O_{2p}$) kinetics have first been investigated by Koga, Shiojiri, Kondo, & Barstow (1997). They found that after increasing the leg muscle temperature, pulmonary O_2 kinetics was minimally affected. Shiojiri, Shibasaki, Aoki, Kondo, & Koga (1997) conducted a study to examine the effects of reduced body temperature on pulmonary O_2 kinetics. They used a lower-body cold-water immersion technique which resulted in a decrease in muscle and core temperatures. Their results showed a slower $\dot{V}O_{2p}$ on-response with no change in cardiac output kinetics and similar end-exercise $\dot{V}O_{2p}$ values. They suggested this is due

to a higher contribution of anaerobic glycolysis at the start of exercise to account for the decreased O_2 extraction and/or impaired oxidative reactions in the muscle from cooling. Further suggesting that enzyme activity may be reduced because of a temperature-dependent relationship. They concluded that reductions in muscle and core temperature have a prominent influence on slowing the pulmonary O_2 kinetics on-response.

Pulmonary O_2 uptake kinetics has provided valuable insights into whole-body oxygen usage. When examining pulmonary O_2 kinetics, three domains of exercise can be used to distinguish $\dot{V}O_{2p}$ responses (moderate, heavy, and severe), with the most commonly studied being moderate and heavy. These three domains are important because $\dot{V}O_{2p}$ responses are highly dependent on the intensity of exercise being performed (Gaesser & Poole, 1996; Whipp, 1987). Moderate intensity exercise occurs below the lactate (LT) or ventilatory threshold (VT) (Xu & Rhodes, 1999). At this intensity, exercise can be sustained for prolonged periods with no significant increase in blood lactate. Most human movements, whether it be exercise or physical activities, occur in the submaximal range, and the majority of which fall within the moderate-intensity domain of exercise. Therefore, making it a very relevant level of exercise intensity to study because it is applicable to the majority of everyday movements (Poole & Jones, 2012). The $\dot{V}O_{2p}$ response is characterized by several distinct phases. Phase I (also deemed the cardiodynamic component by Whipp in 1987) represents a fast increase in $\dot{V}O_{2p}$ usually observed within the first 15-25 seconds. It is thought to mainly reflect a change in cardiac output ($\dot{Q}$) (Seeto, 2012; Wasserman et al., 1974). The phase II (primary component) response is represented by an exponential increase towards steady-state, whereas, phase III (steady state) refers to the point in time where $\dot{V}O_{2p}$ levels reach steady state, typically

observed by the 3-minute mark of exercise. Beyond this, $\dot{V}O_{2p}$ increases linearly with the work rate (Poole & Jones, 2012; Whipp, 1987; Xu & Rhodes, 1999). The exponential response in phase II has been assumed to reflect changes in $\dot{V}O_{2m}$ at the onset of exercise (Barstow & Molé, 1991; Grassi et al., 1996; Koga et al., 2005). Exercise priming can also contribute to the speed of O_2 kinetics. Priming refers to the pre-activation of muscle groups prior to the intended bout of exercise opening the oxidative pathways and vasodilating the microvasculature, resulting in greater O_2 extraction, as well as, better matching of O_2 between delivery and utilization (Burnley, Davison, & Baker, 2011; Maturana, Peyrard, Temesi, Millet, & Murias, 2018; Rocha, Gildea, O'Shea, Green, & Egana, 1985).

Krogh & Lindhard (1913) were early investigators to study $\dot{V}O_{2p}$ and blood flow responses during exercise. Over a century later, these responses are still being investigated and debated by exercise physiologists. In particular, there has been a great focus on establishing the limiting factors of O_2 utilization at the onset of exercise (Poole & Jones, 2012). At the onset of constant-load exercise, there is an O_2 deficit that occurs. This O_2 deficit is the result of a slow adjustment in VO2 relative to the work rate. In a review, Hughson (2009) proposed a list of factors affecting O_2 kinetics, including physical fitness status, muscle fiber composition, blood flow/cardiac output, the fraction of inspired O_2, prior exercise, intracellular PO_2, metabolic substrates, enzyme activation, and disease status. Most notably, this list did not mention temperature, although it is clearly known to interact with blood flow parameters, metabolic substrates, intracellular PO_2, and has been more recently shown to affect O_2 kinetics (Martineau & Jacobs, 1988;

Rowell, Brengelmann, Blackmon, & Murray, 1970; Severinghaus, 1958; Shiojiri et al., 1997; Tschakovsky & Hughson, 1999).

The underlying cause of slowed O_2 uptake kinetics has been a longstanding debate. A hypothesis proposed by Whipp (1994), is the metabolic inertia hypothesis that attributes an intrinsic slowness of intracellular oxidative metabolism as the limiting factor to adjust to the new metabolic demand. Alternatively, another by Tschakovsky & Hughson (1999) suggested that the finite kinetics of O_2 delivery to the muscle may be the limiting factor. Research has suggested that it may be intramuscular processes rather than O_2 delivery at the onset of exercise, or a combination of both that are responsible for slowed kinetics (DeLorey et al., 2004; Hughson et al., 1996; Jones et al., 2003; Koga et al., 1996; Krogh & Lindhard, 1913; Poole & Jones, 2012; Williamson et al., 1996; Yoshida et al., 1993). However, due to the complexity of the oxidative machinery and O_2-transport pathways along with varying exercise types, it may be more appropriate to indicate that multiple limiting factors are at play (Grassi, 2001). Pulmonary O_2 kinetics is a whole-body approach and has long been thought to reflect muscle oxidative metabolism as well (McCreary et al., 1996), however, without direct measures, it is difficult to make meaningful comparisons between whole-body and local O_2 uptake.

With the emergence of Near-Infrared Spectroscopy (NIRS) in the late 1970s for monitoring cerebral oxygenation (Jöbsis, 1977), NIRS soon began to be used for muscle physiology in the late 1980s (Chance et al., 1988; Hampson & Piantadosi, 1988). Non-invasive, reliable and relatively low cost, NIRS allows for measurements of tissue oxygenation to be possible. NIRS can assess local changes in oxygenated and deoxygenated hemoglobin (O_2Hb/HHb respectively), myoglobin (O_2Mb/HMb

respectively) and total hemoglobin (tHb). The incorporation of venous and arterial occlusions allows for a quantitative measure of microvascular blood flow and oxygen consumption (Skovereng, 2011; Van Beekvelt, 2002). HHb is a proxy for $\dot{V}O_{2m}$ and thus can be used to study muscle O_2 kinetics (Grassi et al., 2003). Ferreira, Koga, & Barstow (2007) found that during ramp exercise, $\dot{Q}_m$ increased at a faster rate than $\dot{V}O_{2m}$ early in exercise and slows towards maximal work rate. Another study investigated the simultaneous adjustment of $\dot{V}O_{2p}$, limb blood flow, and regional muscle deoxygenation during moderate exercise, hypothesizing that blood flow adjustments would be faster than O_2 consumption. They found that $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ responses were similar (Dumanoir, Delorey, Kowalchuk, & Paterson, 2010), however, they noted a slower alteration of $\dot{Q}_m$ relative $\dot{V}O_{2m}$ demand (Dumanoir, Delorey, Kowalchuk, & Paterson, 2010). In addition, $\dot{V}O_2$ responses and kinetics profiles can be highly influenced depending on exercise intensity (Whipp, 1987).

Cold exposure signals thermoregulatory responses, such as changes in core and peripheral blood flow, via redistribution throughout the body. One of the initial responses to cooling of the skin's surface is peripheral and visceral vasoconstriction which occurs independently of cardiac output (Wilson et al., 2007). The vasoconstriction indicates a systemic response to skin-cooling by limiting blood flow to the periphery and extremities to reduce heat loss (Charkoudian, 2003). Prolonged cooling can result in the cooling of deeper tissues, and consequently, a decrease in core temperature (T_c). This decrease in T_c results in blood being relocated from the periphery to the central vascular space, thus increasing cardiac output due to an elevation in stroke volume and consequently decrease in heart rate (Hanna et al., 1975; Raven et al., 1970; Stocks et al., 2004). Studies have

suggested that cooling also causes a decrease in enzyme activity and thus reduced cellular metabolism from the Q_{10} effect (a measure of the temperature sensitivity of enzymatic reactions for a change in 10 °C) (Bennett, 1984; Willford, Hill, & Moores, 1986) and oxygen consumption, therefore, leading researchers to investigate the influence of local muscle cooling (Ferretti, 1992; Merrick et al., 1993; Oksa et al., 2004; Yanagisawa et al., 2007). Mitochondrial function has been proposed to be temperature-dependent and the rate of O_2 consumption to be lower at reduced muscle temperatures (Binzoni, Ngo, Hiltbrand, Springett, & Delpy, 2002). Reduced temperatures also decrease the rate of ATP hydrolysis and/or resynthesize (Ferretti, 1992), resulting in reduced power output in the cold. However, in submaximal conditions, this is compensated for by greater activation of muscle mass (greater recruitment of muscle fibers) to overcome the reduced rate of oxidative reactions and allowing for maintained ATP contribution.

The investigation of reduced local T_m on O_2 kinetics and blood flow has become an area of interest for researchers (Shiojiri et al., 1997; Wakabayashi et al., 2018). Shiojiri et al. (1997) found slower VO_{2p} responses, suggesting that reduced O_2 extraction and oxidative reactions to be responsible. However, it is important to consider the cooling method used in their study. Lower body and whole-limb cold-water immersion techniques result in temperature reductions of all the tissues submerged below the water, this includes the hip, knee, agonist and antagonist muscle. Furthermore, this can also reduce core temperature as blood travelling through the tissues is cooled and circulated throughout the body. There is no specificity in the muscle groups being cooled. Since then, few studies have investigated this response to decreased muscle temperature, and even fewer have investigated the influences of reduced temperature on tissue oxygenation

(Gagnon et al., 2017). Indeed, the relationship of temperature and tissue oxygenation has been studied at rest (Yanagisawa et al., 2007), during activities such as walking and running (Gagnon et al., 2017) and during isotonic hand exercises (Hom et al., 2004). A recent study looked at muscle cooling on $\dot{V}O_{2m}$ and $\dot{V}O_{2p}$ during a 30 minute cycling exercise (Wakabayashi et al., 2018). They found that cooled muscles resulted in greater recruitment of glycolytic metabolism and also a delay of oxygen uptake at the onset of exercise. They attributed this delay to be from a reduced aerobic energy provision due to less blood being supplied to the working muscles.

Mechanisms related to core and muscle cooling impact oxygenation and blood flow (Dumanoir et al., 2010; Shiojiri et al., 1997; Wakabayashi et al., 2018; Wilson et al., 2007). Core cooling results in lower $\dot{Q}$ which has been shown to slow phase I & II kinetics. Meanwhile, muscle cooling results in lower $\dot{Q}_m$, and reduced oxidative processes. Thereby, both core and muscle cooling have been hypothesized to impact the rate of O_2 kinetics. Shiojiri et al. (1997) demonstrated that O_2 uptake kinetics (by measuring $\dot{V}O_{2p}$) at the onset of exercise were slowed but were not able to distinguish whether this was due to impaired circulatory perfusion or delayed muscular diffusion of O_2 to the working muscles. Furthermore, they used a lower-body cold-water immersion technique, which cooled their participant's entire legs (hip, knee, agonist and antagonist muscles) and resulted in a combination of core and muscle cooling. To this day, no studies have examined $\dot{V}O_{2m}$ or its kinetics with local muscle cooling only. Core cooling results in a shift of blood volume from the periphery to the core due to vasoconstriction of cutaneous tissue, thereby reducing the volume of O_2 available for working muscles. It is well established that there is an increase in fat oxidation in cold conditions, which

results in an increase in aerobic metabolism, thus resulting in a greater need for oxygen and a higher $\dot{V}O_2$. However, O_2 availability at rest and during early minutes of exercise (determined by NIRS) is decreased in cold conditions, therefore resulting in an uncoupling between the O_2 and its subsequent availability (Gagnon et al., 2017). By examining $\dot{V}O_{2p}$, $\dot{V}O_{2m}$, $\dot{Q}$ and $\dot{Q}_m$ independently, we can observe how muscle and whole-body cooling affect O_2 delivery and uptake during moderate exercise.

Therefore, the purpose of this study is to assess pulmonary and VL muscle O_2 kinetics simultaneously from the onset to steady-state moderate-intensity exercise under thermoneutral state, VL cooling, and whole-body and VL cooling. Compared to other studies that have employed lower body cold-water immersion, we aimed to solely cool the VL and surrounding quadriceps muscle, without cooling the hip, knee or antagonist muscles. It was hypothesized that decreased oxidative reactions and lower O_2 uptake capacity from muscle cooling would result in slowed O_2 kinetics. It was also hypothesized that, after whole-body cooling, the shift in blood volume from the periphery to the core, would further reduce O_2 transport and availability to the working muscle, thereby further slowing O_2 kinetics.

2 Review of Literature

2.1 Tissue Oxygenation and Oxygen Uptake Kinetics

2.1.1 Pulmonary oxygen uptake kinetics and transport

Muscular exercise requires a complex demand from oxidative machinery and O_2 transport pathways to supply O_2 to the working muscles. The kinetics of respiratory, cardiovascular, and muscular systems all display dynamic profiles in responses to exercise. By examining O_2 uptake from exercise onset, we can attempt to better understand these responses (Poole & Jones, 2012). Pulmonary O_2 uptake responses are measured at the mouth using breath-by-breath gas analyzers and algorithms that estimate the changes in alveolar O_2 uptake (Poole & Jones, 2012). Krogh & Lindhard (1913) were early investigators in the area of regulation of respiration and circulation at

the onset of exercise. By examining alveolar CO_2, $\dot{Q}$, and ventilation during the first few minutes of exercise, they highlighted the abrupt increase in pulmonary ventilation and heart rate, especially in trained individuals. They also noted rapid increases in $\dot{Q}$ and adaptations of the respiratory and circulatory systems to sudden muscular exertions (Krogh & Lindhard, 1913). The increase, in what would later be known as $\dot{V}O_2$, provided an indirect estimation for the proportional increase in $\dot{Q}$ (Whipp, 1987). Another pioneer in the field of O_2 kinetics, Whipp (1987), studied the dynamics of pulmonary gas exchange. He suggested that power outputs below the ventilatory threshold (or lactate threshold) can be maintained for prolonged durations with no, or modest fatiguing due to the O_2 balance allowing for sufficient clearance of lactate (Whipp, 1987).

A delay in the O_2 uptake response has been reported at the onset of exercise (Grassi et al., 1996; Tschakovsky & Hughson, 1999; Whipp, 1987; Williamson et al., 1996). There has been a continued debate between hypotheses regarding the source of slowed O_2 kinetics. A popular hypothesis is the metabolic inertia hypothesis, which attributes an intrinsic slowness of intracellular oxidative metabolism as the limiting factor to the adjustment of the new metabolic demand (Whipp, 1987). Alternatively, Tschakovsky & Hughson (1999) suggested that the main limiting factor may be attributed to the finite kinetics of O_2 delivery to the muscle instead. Emerging evidence now suggests that due to the complexity of the oxidative machinery and O_2-transport pathways along with varying exercise types, it may be more appropriate to indicate that multiple limiting factors are at play (Grassi, 2001; Hoppeler & Weibel, 2000). Taylor & Weibel (1981) put forward the hypothesis of “Symmorphorosis” which, for aerobic work, predicts that all parts of the respiratory cascade can appear as limiting factors for O_2

supply to the muscle. Delays can shift from one level to another and that under certain conditions (age, fitness level, disease status, temperature), some levels may potentially having more resistance than others (Hoppeler & Weibel, 2000).

Wagner (2012) highlighted four main O_2 transport processes that contribute to overall $\dot{V}O_2$ (ventilation, alveolar-capillary diffusion, circulation and muscle diffusion). Therefore, each component can impose limitations to the O_2 uptake process and that there is no single limiting factor. However, many have studied the relationship between $\dot{Q}$ and $\dot{V}O_{2p}$ and its kinetics (DeLorey et al., 2004; Koga et al., 1996; Krogh & Lindhard, 1913; Mortensen et al., 2005; Seeto, 2012; Williamson et al., 1996; Yoshida et al., 1993) and comparisons of $\dot{V}O_{2p}$ uptake and $\dot{Q}$ have been made in attempts to distinguish the abovementioned theories. Using the Fick principle equation ($\dot{Q}=\dot{V}O_2/A\text{-}VO_2Diff$) comparisons of $\dot{V}O_{2p}$ uptake and $\dot{Q}$ kinetics response (typically via cardiac output, stroke volume or heart rate), and insights into the limiting factors of O_2 delivery and/or O_2 utilization have been made. For example, Yoshida et al. (1993) examined the $\dot{Q}$ and pulmonary O_2 kinetics at the onset of exercise and found that $\dot{Q}$ and $\dot{V}O_{2p}$ increase rapidly, with $\dot{Q}$ presenting a slightly faster response than $\dot{V}O_{2p}$, suggesting that O_2 delivery (via blood flow) was not the limiting factor. Other researchers have supported these findings with similar results suggesting that factors other than delivery of O_2 to the working muscles are accountable for slower phase II kinetics (DeLorey, et al., 2004; Hughson et al., 1996; Koga et al., 1996; Krogh & Lindhard, 1913; Williamson et al., 1996).

Meanwhile, Bangsbo et al. (2000) and Grassi et al. (1996) suggest that there is sufficient O_2 delivery at the onset of exercise and that the metabolic inertia processes (O_2

utilization) within the cell are limiting. Studies examining O_2 delivery under hypoxia conditions observed an increase in $\dot{Q}$ to compensate for reduced O_2 availability (Koskolou et al., 1997; Rowell et al., 1986). Jones et al. (2003) hypothesized that $\dot{V}O_{2p}$ is modulated by oxidative enzyme inertia and that the repression of nitric oxide synthase would stop the inhibition of mitochondrial $\dot{V}O_2$ and speed the pulmonary O_2 kinetics. The results of that study supported their hypothesis, suggesting that the inhibition of mitochondrial $\dot{V}O_2$ might contribute to the inertia of oxidative metabolism. A review by Poole & Jones (2012) concluded that because there are faster processes like $\dot{Q}$ and $\dot{Q}_m$, there is strong evidence that under normal conditions, O_2 transport does not limit the kinetics of $\dot{V}O_2$. Physiological factors, such as age, may also play a role on pulmonary O_2 kinetics. Therefore, there seems to be an overwhelming breath of evidence to suggest that O_2 delivery is not limiting in pulmonary O_2 kinetics at the onset of exercise and that processes involved with the diffusion of O_2 within the muscle may be responsible.

2.1.2 Muscle oxygen uptake kinetics

Evaluation of muscle oxygen uptake ($\dot{V}O_{2m}$) kinetics provides a targeted measure of metabolic processes in the specific muscle(s) of interest. Millikan first developed near-infrared spectroscopy in 1937, but its methods were not used for studying exercising muscles until the late 1980s (Chance et al., 1992; Chance et al., 1988; Hampson & Piantadosi, 1988; Skovereng, 2011). The NIRS method provides a non-invasive tool to examine local oxygenation and hemodynamics of skeletal muscles (Van Beekvelt et al., 2002). The use of NIRS has expanded over the past two decades and has become a standard method used in oxidative measurement. Its compact, inexpensive and reliable nature allows it to be applied as an objective measure of muscle oxidative metabolism and microvascular blood flow in a variety of settings, including

sports, sports medicine, clinical and therapeutic applications (Hamaoka et al., 1996; Quaresima et al., 2003; Van Beekvelt et al., 2001). NIRS can provide real-time information of both oxygenated and deoxygenated hemoglobin and myoglobin, as well as, tissue blood flow through the Fick equation (Gagnon, 2014; Grassi & Quaresima, 2016; Van Beekvelt et al., 2002; Van Beekvelt et al., 2001).

Near-infrared spectroscopy, as the name implies, uses light in the near-infrared spectrum in the 650-900 nm range (Figure 1). An optical fiber bundle is placed on the skin and emits light with different wavelengths into the tissue. The light that enters the tissue either scatters or is absorbed. That light absorbed by a receiving optical bundle is described to have travelled in a banana-shaped path through the tissue, from the emitting source (Hamaoka et al., 2011; Van Beekvelt et al., 2002). Oxygenated and deoxygenated hemoglobin (O_2Hb/HHb respectively) and myoglobin (O_2Mb/HMb respectively) all absorb different wavelengths of light within the 650-900 nm spectrum, therefore measures of the relative changes in absorption can provide information of tissue deoxygenation (Gagnon, 2014; Skovereng, 2011; Van Beekvelt et al., 2002). The abovementioned method is not a direct measure of $\dot{V}O_{2m}$ or $\dot{Q}_m$ but rather a measure of relative change.

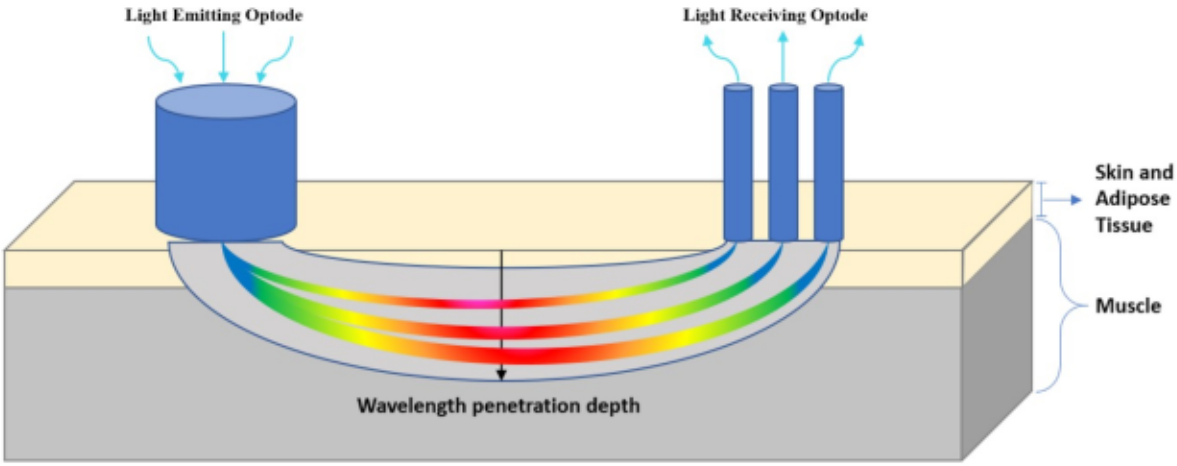

Figure 1. Near-Infrared Spectroscopy optode and penetration depth diagram adapted from Gagnon (2014).

The incorporation of venous and arterial occlusions allows for a quantitative measure of $\dot{V}O_{2m}$ and $\dot{Q}_m$ (Skovereng, 2011). This occlusion method is now used in virtually all studies examining $\dot{V}O_{2m}$ and $\dot{Q}_m$, demonstrating good reproducibility and sensitivity (Grassi et al., 2003; Hamaoka et al., 1996; Jones et al., 2016; Van Beekvelt et al., 2002). For venous occlusions, an inflatable cuff is applied to block venous blood flowing out of the occluded limb, but not arterial blood flowing in. The NIRS device can then measure the venous blood volume and pressure increase in O_2Hb, HHb, and total hemoglobin (tHb). Arterial occlusions block blood flowing in and out of the limb. NIRS can then measure the depletion of local O_2 as oxygenated hemoglobin decreases and deoxygenated hemoglobin increases (Van Beekvelt et al., 2002), thus providing a measure of oxygen consumption.

Blood flow and skeletal muscle perfusion is the primary fuel source (O_2) during exercise. González-Alonso (2012) suggested that muscle O_2 utilization is the critical factor for increased blood flow to the working skeletal muscles. Many have compared $\dot{V}O_{2m}$ to $\dot{V}O_{2p}$ and blood flow during exercise (Keir, Robertson, Benson, Rossiter, & Kowalchuk, 2016; Koga et al., 2005;

Spencer, Murias, Lamb, Kowalchuk, & Paterson, 2011; Wüst et al., 2014). Dumanoir et al. (2010) studied $\dot{V}O_{2p}$, limb blood flow, and deoxygenation kinetics in young adults during moderate-intensity knee-extension exercise and found that there was a greater reliance on O_2 extraction relative to O_2 delivery. Similar results were also observed in other studies where they found $\dot{Q}_m$ increased at a faster rate than $\dot{V}O_{2m}$, suggesting that the processes within the muscle (ATP utilization and decreased calcium release/uptake) were slower than that of O_2 delivery via blood flow and microvascular perfusion (Bangsbo et al., 2000; Ferreira et al., 2007; Grassi et al., 1996, 2003).

2.2 Exercise Intensity and Type

Physiological responses to exercise differ depending on the exercise intensity. Muscle $\dot{V}O_2$ has been reported to increase 50-fold and O_2 delivery up to 10-fold during abrupt increases in exercise (Hamaoka et al., 2011). The exponential response of $\dot{V}O_{2p}$ shows a linear relationship with increasing power output or work rate (Barstow et al., 1993; Xu & Rhodes, 1999), but is highly dependent upon exercise intensity. Exercise intensities are divided into three domains; moderate, heavy and severe (Gaesser & Poole, 1996; Whipp, 1987). However, most human movements, whether it be physical or exercise activities, occur in the submaximal range rather than maximal. During submaximal exercise, O_2 supply and metabolic demand are often closely matched (González-Alonso, 2012). Therefore, it is crucial to investigate $\dot{V}O_2$ and $\dot{Q}$ responses in the submaximal range and especially focusing on rest-to-exercise (or on-) transitions (typically the first 3 minutes of exercise to steady state) as this closely relates to activity patterns performed frequently in our everyday lives (Seeto, 2012).

2.2.1 Moderate intensity

Moderate exercise is classified by a work rate that does not exceed the LT or VT (Xu & Rhodes, 1999). At this intensity, lactate accumulation in the blood does not surpass the rate at which it is cleared, allowing exercise to be maintained for prolonged periods without substantial fatigue. Most human movements occur in the submaximal range and the majority of which fall within the moderate-intensity domain of exercise. Therefore, making it a very relevant level of exercise intensity to study because it is applicable to common everyday movements (Poole & Jones, 2012). Each exercise intensity has distinct, identifiable phases of pulmonary O_2 kinetics. Three Moderate exercise can be divided into three phases. Phase I, also known as the "cardiodynamic component" (Whipp, 1987), and is represented by a fast increase in $\dot{V}O_{2p}$ within the first 15-25 seconds of exercise onset. It is thought to be mainly a change in $\dot{Q}$ (Seeto, 2012; Wasserman et al., 1974). Poole & Jones (2012) described it as the almost immediate increase in pulmonary perfusion occurring within the first breath at exercise onset. Whipp (1987) explained that phase I lasts until the point where there is a change in gas tension from mixed venous blood entering the pulmonary capillaries and an increased muscle gas exchange. Phase I kinetics are not observed in localized muscle $\dot{V}O_{2m}$ (Seeto, 2012). A drop in end-tidal pressure of O_2 determines the end phase I and the beginning of phase II (Jones & Poole, 2005; Seeto, 2012). Phase II (primary component) is the exponential increase towards stead state, caused by the arrival of mixed venous blood returning to the pulmonary capillaries from the working muscle where increased extraction of O_2 and production of CO_2 has occurred (Poole & Jones, 2012; Whipp, 1987; Xu & Rhodes, 1999). Phase III (steady-state) is marked by a steady-state nature of $\dot{V}O_{2p}$ that is achieved, typically around the 3-minute mark of exercise. From this point on, $\dot{V}O_{2p}$ is said to increase as a linear function of work rate, often observed during cycle ergometry at moderate intensity work (Whipp, 1987).

Muscle deoxygenation (HHb) kinetics also have an exponential-like response. It is still uncertain if the underlying processes determining HHb kinetics are truly exponential in nature. However, fitting with a mono-exponential function has been used in previous research (D. S. DeLorey, Kowalchuk, & Paterson, 2005; Dumanoir et al., 2010) and appears to provide a reasonable estimate of the time course for muscle deoxygenation during phase II VO_2 (reflecting the time constant) response. Unlike pulmonary O_2 kinetics, there is a rapid "overshoot" of the HHb response, somewhat opposite to that of the phase I response with VO_{2p}.

2.2.2 Heavy and severe intensity

Heavy and severe exercise occurs at work rated above the LT/VT threshold. This type of vigorous exercise results in the accumulation of lactate at a rate much faster than the body's capacity to clear it. Heavy exercise results in lactate accumulation to the point of the maximum lactate steady-state (MLSS) (Xu & Rhodes, 1999). There is also a disproportional increase in the rate of carbon dioxide output (VCO_2) relative to VO_{2p} along with a systematic increase in the ventilatory equivalent for oxygen (VE/ VO_2), without a rise in the ventilatory equivalent for carbon dioxide (VE/ VCO_2) (Beaver, Wasserman, & Whipp, 2012). In the heavy exercise domain, phase I kinetics remain similar to that of moderate exercise. Phase II still demonstrates an exponential increase in VO_{2p} but instead of plateauing at steady state for phase III, there is a slow but progressive increase in VO_{2p} and an additional component is developed, termed the slow component of VO_{2p} (Barstow & Molé, 1991; Gaesser & Poole, 1996; Whipp, 1994; Xu & Rhodes, 1999). During heavy intensity exercise, lactate accumulates faster than it can be cleared, and therefore our ability to sustain prolonged exercise is significantly reduced. The greater accumulation of lactate results in a greater (and recognizable) slow component during heavy

exercise (Whipp, 1987; Xu & Rhodes, 1999). Severe exercise is considerably less studied because pulmonary O_2 kinetics cannot be well established during this high level of exercise. Severe exercise occurs at the point above the MLSS threshold (Xu & Rhodes, 1999).

2.2.3 Exercise type

The majority of work done on O_2 kinetics during moderate and heavy intensity exercise has used cycle ergometry because it allows for easy control of work-rate, accuracy and stability for data acquisition, compared to treadmill or other exercise modalities (Ettema & Lorås, 2009; Poole & Jones, 2012; Skovereng, 2011). Cycling involves the activation of large muscle mass, and specifically, the *vastus lateralis* and *vastus medialis* because they are highly active during cycling exercise which is the reason they are often selected to study for muscle oxy-deoxygenation purposes (Chance et al., 1992; Poole & Jones, 2012; Skovereng, 2011). Several studies examining the kinetics of $\dot{V}O_{2p}$, limb blood flow, and muscle deoxygenation have used moderate-intensity exercise as the basis for their experiments because of its applicability to everyday movements (DeLorey, et al., 2004; Dumanoir et al., 2010; Spencer et al., 2011). This coupled with the use of cycling as the exercise modality can allow for streamline data collection to assess O_2 kinetics.

The role that moderate and heavy-intensity exercise priming has O_2 kinetics is also an important factor to consider. Priming refers to the activation of muscle groups prior to the intended bout of exercise. This is often observed through bouts of various exercise intensities (moderate and heavy). The effect of priming pre-activates the oxidative pathways and vasodilates the microvasculature, resulting in greater O_2 extraction, as well as, better matching of O_2 between delivery and utilization (Rocha et al., 1985). Studies have shown that priming can

accelerate the pulmonary and muscle O_2 kinetics, and increase the work that can be performed (Burnley et al., 2011; Maturana et al., 2018).

2.3 Exercise Physiology in the Cold

2.3.1 Whole-bod cooling (skin and core)

Whole-body cooling, usually initiated through skin-surface cooling, results in thermoregulatory responses to reduce heat loss. The primary response to skin-surface cooling is to limit blood flow to the periphery and extremities (Charkoudian, 2003). Peripheral and visceral vasoconstriction restrict blood flow as a systemic response that occurs independently of cardiac output (Wilson et al., 2007). The somatic nervous system promotes this vasoconstriction as a protective mechanism (Gagnon, 2014). Prolonged whole-body cooling can cause a decrease in T_c. A reduction in T_c causes cutaneous vasoconstriction, and thus, a shift in blood volume from the periphery to central vascular space. This shift consequently results in an increase in cardiac output due to an elevation in stroke volume and a subsequent reduction in heart rate (Hanna et al., 1975; Raven, Wilkerson, Hovarth & Bolduan, 1975; Stocks et al., 2004).

Several relationships change in a related manner during cold exposure, including respiratory, cardiovascular, and metabolic relationships (O'Hanlon & Hovarth, 1970). Indeed, whole-body cooling has marked effects on blood flow. Reduced blood flow in the periphery also results in an impact on respiratory and metabolic processes. Oksa et al. (2004) found a reduced maximal cardiorespiratory capacity and an increase in submaximal strain during maximal and submaximal exercise in the cold. They also attributed a lower heart rate to less circulating blood, that an increased submaximal VO_2

could be due to thermoregulatory shivering and that coactivation of agonist-antagonist muscle pairs results in reduced mechanical efficiency. Another study suggested that dynamic exercise performance was more disturbed in cold conditions due to decreased nerve conductance and slower ATP utilization (Wakabayashi et al., 2015). Gagnon (2014) suggested that the muscle pump action during rhythmic exercise was powerful enough to re-establish adequate tissue oxygenation even in the presence of peripheral vasoconstriction in the gastrocnemius, concluding that exercise intensity may be a critical factor in the response for microvascular oxygenation to exercise in the cold.

Some researchers have reported the positive effects of exercise in cold conditions. For example, Marsh & Sleivert (1999) found that pre-cooling before exercise improved endurance performance in the short-term, suggesting that it increased central blood volume providing greater blood availability to the muscles, and enhancing O_2 delivery. Another study examined leg muscle metabolism during exercise in the cold. Subjects performed 15-minute cycling bouts at 70-85% VO_{2max}, and found that there was a greater intramuscular lipid utilization that occurred in the cold, suggesting a shift in substrate utilization (Fink et al., 1975). Ishii et al. (1992) found that $\dot{V}O_{2p}$ and its kinetics were not affected by reduced T_m during submaximal steady-state exercise. However, they also reported that $\dot{Q}$ and its kinetics have a remarkable reduction and delay at lowered T_m, but at submaximal work intensities it did not affect pulmonary O_2 kinetics.

2.3.2 Local muscle cooling

Prolonged whole-body cooling or direct local cooling can result in decreases in muscle temperature. Decreased T_m is often desired in clinical and therapeutic settings to reduce pain and to slow cellular metabolism to minimize tissue damage (Merrick et al., 1999). Muscle cooling

results in physiological effects such as reduced ATP hydrolysis, impaired calcium release/uptake in the muscle, decreased nerve conduction velocity/firing rate, and increased demand for O_2 (Bleakley & Hopkins, 2010; Knight, 1995; Oksa et al., 2000; Oksa et al., 2004). Decreased ambient air temperatures, crushed ice/ice-packs, cold-water immersion, or water perfused pads/suits applied to the skin are methods commonly used to achieve muscle cooling. In order to obtain direct and continuous measurements of muscle temperature, an intramuscular probe must be inserted into the muscle at a set depth. A study by Merrick et al. (1993) investigated the effectiveness of intramuscular cooling with ice compared to the ice with compression, suggesting that ice with compression proved to be more effective at cooling the muscle than ice alone. Cold packs have also been proposed to produce significant temperature decreases in cutaneous and subcutaneous tissues (Enwemeka et al., 2002). Another study by Rech (2013), suggested that cold-water immersion can reduce muscle temperature enough to slow cellular metabolism by 2-3 fold (T_m decreased of 10 °C (Bleakley & Hopkins, 2010)) in fewer than 30 minutes.

Reduced T_m effects O_2 uptake kinetics and blood flow as well. Cold results in a leftward shift in the oxyhemoglobin dissociation curve, via a change in the partial pressure of oxygen (PO_2), and the affinity of hemoglobin for O_2 increases. This limits the unloading of O_2 to the muscle, resulting in a decrease in $\dot{V}O_{2m}$ (Gagnon, 2014; Mairbaurl & Humpeler, 1980; Severinghaus, 1958; Willford, Hill, & Moores, 1986). Indeed, whole-body cooling (reductions in T_c) could result in shivering and an associated increase in $\dot{V}O_{2p}$. However, extremity cooling, if done correctly, can have little to no effect on T_c but significant reductions in T_m and changes in $\dot{V}O_{2m}$ and $\dot{Q}_m$ (Beelen & Sargeant, 1991). Yanagisawa et al. (2007) investigated the effects of cooling on skeletal muscle tissue at rest and found that all hemoglobin/myoglobin concentrations decreased during cooling and were slow to recover post cooling. Hom et al. (2004) studied O_2

saturation during isotonic hand exercises and found that the O_2 saturation was higher and that the total hemoglobin percentage decreased after skin (and subsequently muscle) cooling. However, another study by Gagnon et al. (2017) investigated the effects of skin and core cooling on walking and running and found that skin cooling did not influence muscle oxygenation during exercise. Skeletal muscle oxidative metabolism has also been shown to be improved as an adaptation to repeated bouts of muscle cooling (Wakabayashi et al., 2017).

Indeed, cooling also affects mitochondrial function and the rate of ATP hydrolysis/resynthesize. Ishii et al. (1992) studied the effects of muscle temperature on pulmonary O_2 kinetics, muscle blood flow, and accumulation of lactate during rest to exercise transitions. What they found, was that blood flow kinetics were slower and lactate was greater in the cold, concluding that during submaximal exercise, pulmonary O_2 kinetics are not affected by muscle temperature. This is supported by Blomstrand, Kaijser, Martinsson, Bergh, & Ekblom (1986) who found that in cooled muscles (VL), glycolysis is higher, thus accounting for the higher lactate levels and greater rates of muscle glycogen depletion. They also speculated that reduced muscle temperature results in greater recruitment of fast-twitch muscle fibers and as a result, greater participation of glycolytic fibers and higher lactate production. This was further studied by Ferretti (1992), where they examined the effects of muscle temperature on muscular power. Under steady-state conditions, successful aerobic metabolism maintains a balance of ATP splitting and ATP re-synthesis. They noticed that reduced muscle temperatures affect the rate of ATP hydrolysis and/or re-synthesis. However, when testing aerobic power during submaximal exercise, this deficit is met by greater activation of muscle mass to

compensate for the reduced ATP splitting rate. At a maximal state, there is no longer any excess muscle mass to recruit and therefore, a decrease in overall power is observed.

2.3.3 Cardiovascular responses to skin and core cooling

The cardiovascular system plays a crucial role in conserving heat when exposed to cold environments (Wilson et al., 2007). Fluctuations in skin temperature have direct effects on blood flow. In resting thermoneutral conditions, skin blood flow is ~0.25 L/min for a body surface area of 1.8 m^2 (average-sized human) (Charkoudian, 2003). Cold stress can cause a 5-fold decrease in skin blood flow reducing it to only ~0.05 L/min (Kaiyala, 2009). This can drastically reduce the amount of subsequent O_2 delivery prior to the initiation of exercise. Peripheral vasoconstriction, induced by skin cooling, reduces heat loss by limiting blood flow to the periphery, resulting in a decrease in heat dissipation from the skin (Charkoudian, 2003; Wilson et al., 2007). After cold exposure, there is an increase in cardiac output attributed to an elevation of stroke volume and cardiac preload (Hanna et al., 1975; Wilson et al., 2007). Changes in core temperature (T_c) also have a pronounced effect on blood flow. Core cooling causes cutaneous vasoconstriction, relocating blood from the periphery to the central vascular space. A rise in stroke volume, cardiac output, and consequentially a reduced heart rate marks the relocation of blood to the central vascular space (Raven et al., 1970; Stocks et al., 2004). Substantial core cooling also initiates shivering thermogenesis. Heat generation through shivering thermogenesis creates metabolic heat to maintain T_c (Charkoudian, 2003). Therefore, an increase in stroke volume mediates a rise in blood flow needed to support increased tissue oxygenation to fuel shivering muscles (Raven et al., 1970).

Impedance cardiography is a reliable method to measure cardiovascular parameters like cardiac output (CO), stroke volume (SV), and heart rate (HR) in both clinical and applied

research settings. Muller et al. (2010) tested the impedance cardiography method during acute cold exposure and found it to be a reliable measurement method. Portable impedance devices like the PhysioFlow (PF07 Enduro, Manatec Biomedical, France) provide wireless, non-invasive, and real-time monitoring of cardiovascular hemodynamics. The device has been validated against the classic Fick method under resting and maximal exercise settings (Charloux et al., 2000; Richard et al., 2001; Tordi et al., 2004), and provides a promising capability to evaluate rest and exercise under a wide range of environmental conditions.

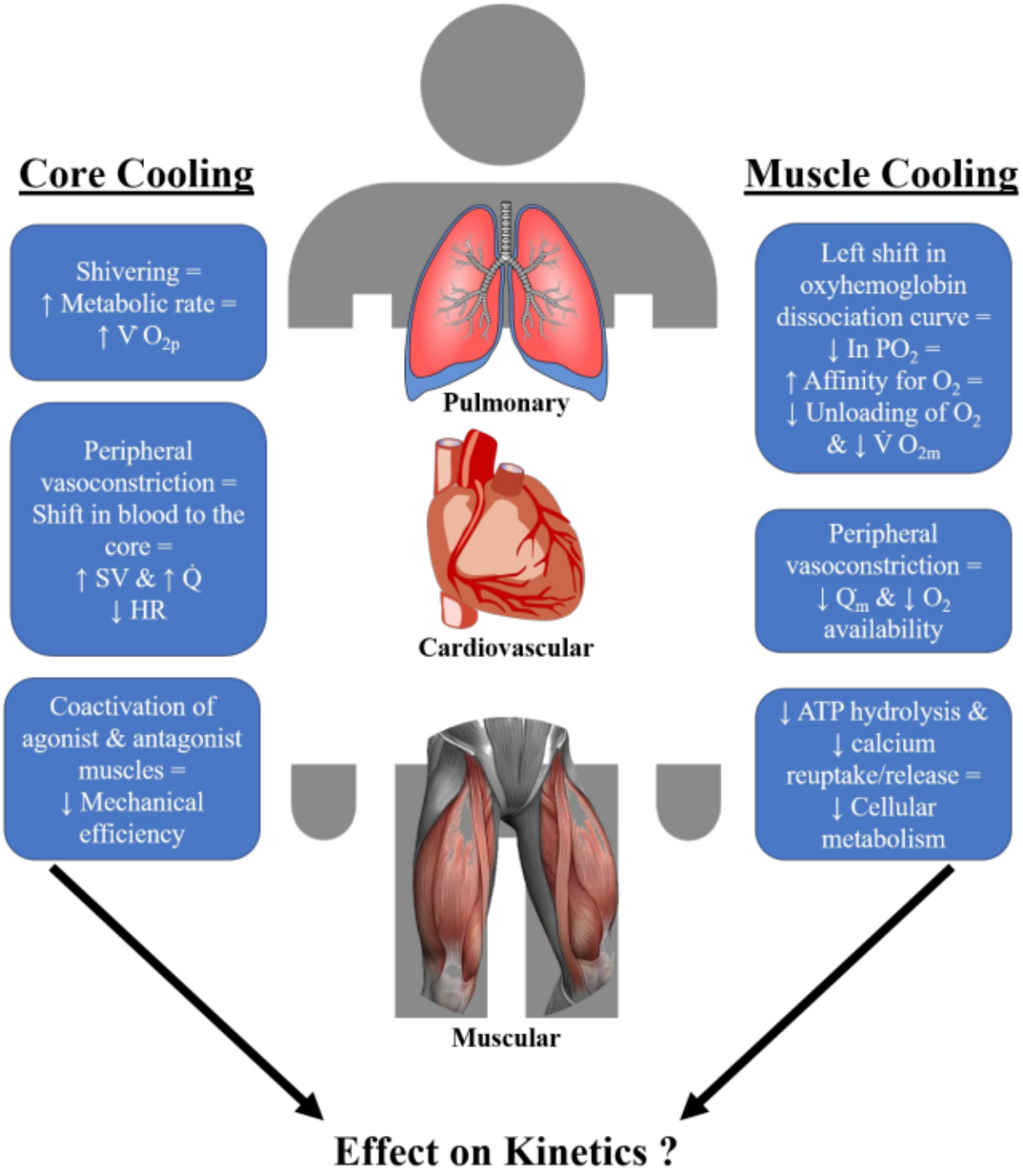

Figure 2. Mechanistic schematic diagram of the effects that core and muscle cooling have on the pulmonary, cardiovascular and muscular systems.

Therefore, it is evident that skin, core, and muscle cooling all influence oxygenation and blood flow during moderate-intensity exercise. The study conducted by Shiojiri et al. (1997) demonstrated that pulmonary O_2 kinetics at the onset of exercise are slowed in some form, and although they were not able to distinguish whether this was due to impaired circulatory perfusion or delayed muscular diffusion of O_2 to the working muscles, they highlighted the need for this discrepancy to be studied further. Since there is a greater need for O_2 in the cold, and its reduced availability due to the shift in the oxyhemoglobin dissociation curve, there is an evident need to address this missing link. Whether this disconnect is due to O_2 transport or diffusion has yet to be explained. With the emergence of newer technologies and techniques to measure continuous T_m, $\dot{V}O_{2m}$, and cardiovascular hemodynamics, we aim to provide further information on O_2 uptake kinetics during the onset of exercise in the cold. Therefore, the purpose of this study is to assess pulmonary and VL muscle O_2 kinetics simultaneously from the onset to steady-state moderate-intensity exercise under thermoneutral state, VL cooling, and whole-body and VL cooling.

3 Aims of the Study

This study aimed at investigating the effects that whole-body and local-muscle cooling have on pulmonary and VL muscle O_2 uptake kinetics, blood flow, and cardiovascular hemodynamics during three-minute rest-to-exercise cycling transitions. Below are the specific aims of this study:

1. To compare the effects of whole-body and VL muscle cooling on $\dot{V}O_{2p}$ and muscle O_2 kinetics during rest to steady-state moderate cycling exercise.
2. To compare the effects of whole-body and VL muscle cooling on cardiovascular hemodynamics and $\dot{Q}_m$ during rest to steady-state moderate cycling exercise.

The hypotheses were:

1. That whole-body and VL muscle cooling would result in slower phase II O_2 kinetics (marked by a longer time delay and/or time constant) at the onset of exercise compared to thermoneutral conditions. Whole-body cooling will result in slower pulmonary (longer time constant) and muscle O_2 kinetics (longer time delay and time constant), while VL muscle cooling will only result in slower muscle O_2 kinetics (marked by longer time delay).
2. It was hypothesized that under VL muscle cooling, there would be a decrease in $\dot{Q}_m$, and therefore a decrease in O_2 availability for uptake. Under whole-body cooling, $\dot{Q}$ will be lower and there would be a further decrease $\dot{Q}_m$.

4 Methods

4.1 Participants

Eight participants completed the full requirements of the study. They completed both the familiarization session and the experimental session. Selection criteria were: a good to very good fitness level (VO_{2max} between 47.2-55.5 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$), a low to moderate body fat % (average or better, based on age category), an adipose tissue thickness of less than 1 cm over the VL, and in the age range of 18-40 years old. Participants were fully aware of the risks related to the experiment. They provided written informed consent, were screened with a Physical Activity Readiness Questionnaire, and screened for respiratory and cardiovascular conditions that could be exacerbated by cold air exposure or exercise. The participation requirements were set because there are marked differences in O_2 kinetics between young and old populations and also in trained and untrained individuals (DeLorey et al., 2005; Kalliokoski et al., 2005). Furthermore, the influence of increased adipose tissue thickness on the NIRS signal causes a significant scattering effect (Jones et al., 2016; Leahy et al., 2012; Van Beekvelt et al., 2001).

The mean (±SD) age of the participants was 25 ± 5 yrs, height 175 ± 7 cm, VO_{2max} 53 ± 6 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$. Table 5 provides a full summary of the participant's anthropometric measurements and physical characteristics. Prior to the commencement of this study, it was approved by the Laurentian University Research Ethics Board and conformed to the declaration of Helsinki.

4.2 Experimental Design

4.2.1 Familiarization session

During the familiarization session, we collected anthropometric measurements, physical characteristics, and assessed the participant's $\dot{V}O_{2max}$. Skinfold calipers were used to determine the percentage of body fat at four sites (triceps, biceps, subscapular, supra iliac). The skinfold measurements were used to calculate body density and subsequently estimate body fat percentage (Durnin & Womersley, 1974). The estimated body fat percentage was used to calculate fat mass and fat-free mass. Height and weight were used to estimate body surface area (DuBois, 1916). A Doppler ultrasound (Portable Ultrasonic Diagnostic Devices, L7m-a Linear probe, Chison Medical Imaging Co., Ltd, Shanghai, China) was used to measure the tissue thickness over the VL muscle and resting limb blood flow.

Participants performed a $\dot{V}O_{2max}$ test to assess their maximal oxygen consumption on a cycle trainer (Flux Smart, Tacx, The Netherlands). They followed a protocol that started at 25W and increases 20W/min at 80rpm and continued to exhaustion (modified from Murias et al., 2013). The first VT was determined following traditional visual methods where the point of $\dot{V}O_2$ where $\dot{V}CO_2$ began to increase out of proportion to $\dot{V}O_2$ along with a systematic increase in the ventilatory equivalent for oxygen (VE/ $\dot{V}O_2$), without a rise in the ventilatory equivalent of carbon dioxide (VE/ $\dot{V}CO_2$) (Caiozzo et al., 1982). The VT may vary slightly under different temperature conditions, however, using the VT established under thermoneutral conditions will provide the best representation of the participant’s actual VT (Neder & Stein, 2006; Smolander, Kolari, Korhonen, & Ilmarinen, 1986). The purpose of this was to determine the specific moderate work rate that corresponds to 80% of the participant's VT (Barstow, Buchthal, Zanconato, &

Cooper, 1994). During the $\dot{V}O_{2max}$ test, participants had cardiorespiratory, metabolic, and hemodynamic variables assessed.

4.2.2 Experimental session

Each participant performed three experimental conditions which included; (1) TN, (2) LC, (3) WBC (Figure 2) all on the same day. This removed any day-to-day variability and equipment calibration for collected variables and specifically for the NIRS, where placement is critical. The first condition was always TN and then subsequently balanced LC and WBC. Participants arrived at the lab between 7:00-10:00 AM, wearing shorts and a t-shirt. They were asked to refrain from consuming alcohol, caffeine, tobacco, and performing vigorous exercise 24 hours before the experimental session. They were instrumented with the rectal core temperature probe and then asked to lay in an examination bed while a researcher landmarked the VL and position for the NIRS device. An intramuscular thermocouple was implanted into the midpoint of the VL under local anesthetic at a 30° angle to a depth of 1 cm within the muscle. The NIRS device was secured to the leg over the VL. It was positioned 10 cm up from the patella and 5 cm lateral, ensuring it was on the muscle belly of the VL. Following positioning of the NIRS, resting arterial (AO) and venous occlusions (VO) were done to get a measure of baseline $\dot{V}O_{2m}$ and $\dot{Q}_m$. A sphygmomanometer measured resting blood pressure (BP).

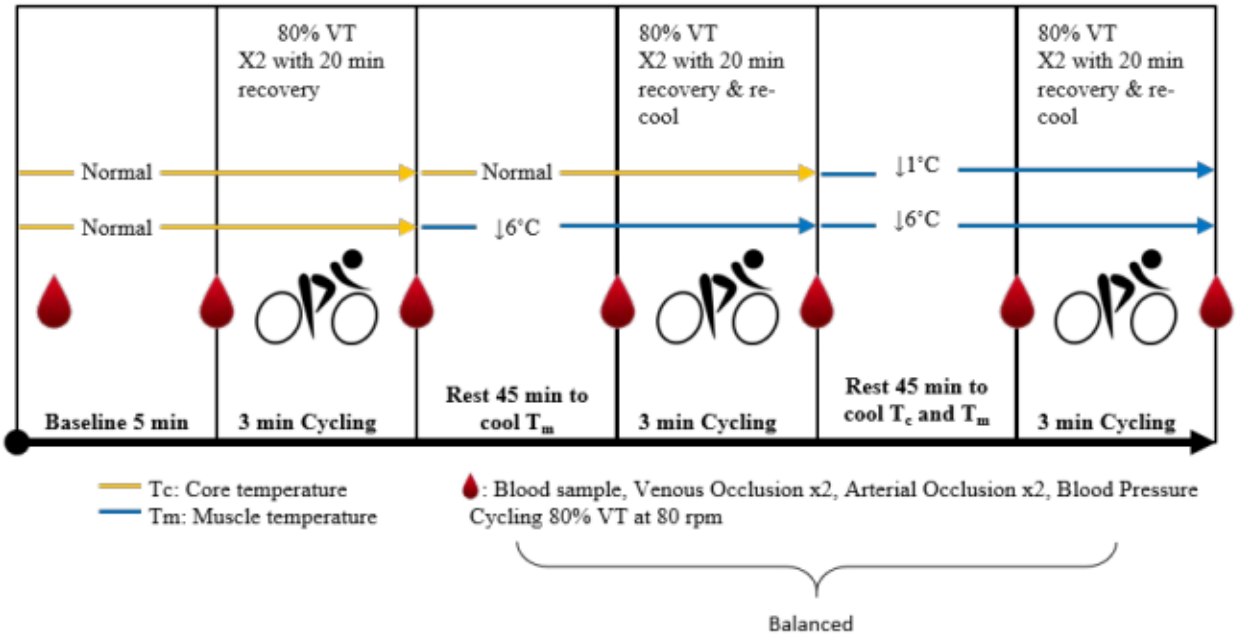

Figure 3. Study design schematic for the experimental testing session.

Participants then had a venous catheter installed in the antecubital vein to collect 2-3 mL of blood for blood analysis, and a baseline measure of blood gases and metabolites was collected. Following the blood sample, participants were then instrumented in the environmental chamber room under thermoneutral conditions and equipped with the bioelectrical impedance device for cardiovascular hemodynamics, ibuttons to record skin temperature and fitted with a mask for the metabolic cart. See Figure 3 for a schematic representation of the study protocol. The trial order consisted of two exercise bouts in the thermoneutral condition, followed by two exercise bouts with VL cooling and whole-body cooling conditions in a balanced order. Following each exercise bout was a minimum of 20 minutes of rest. The 20 minutes of rest allowed for adequate recovery which would not influence the succeeding transition (Spencer et al., 2011). On average the time cooling/rewarming between conditions was 96 minutes. The

thermoneutral (22 °C) condition began with a 5-minute baseline followed by a pre-exercise measure of $\dot{Q}_m$ and $\dot{V}O_{2m}$ by performing two VO at 80 mmHg and two AO at 300 mmHg. A measure of pre-exercise BP and a blood sample were collected. Following the pre-exercise measures, participants began their three-minute cycling on the bike at a work rate that corresponded to 80% of their VT at a cadence of 80 rpm. Immediately following completion of the three-minute cycling bout, post-exercise measures of VO & AO's, a blood sample and BP were taken. Participants were then asked to rest for 20 minutes before repeating the same procedure.

Following the two thermoneutral exercise bouts, depending on the balanced order of the conditions, participants either underwent VL and quadriceps cooling or whole-body and quadriceps cooling. For the VL cooling protocol, participants had an ice water perfused cuff affixed over their VL and surrounding quadriceps muscles, along with another cuff wrapped around the shin and tibialis anterior muscle to help cool any pooling blood below the VL. The participant's intramuscular temperature was monitored, and the cooling continued until the point at which their T_m had decreased by 6 °C. The same procedure outlined for the thermoneutral condition was then repeated. Pre-exercise VO, AO, blood sample, BP were all taken before the start of exercise. Immediately following the three-minute exercise bout, we collected the same post-exercise measures. The participant then rested for a minimum of 20 minutes or until their T_m returned back -6 °C. After which, we repeated the procedure. For the whole-body and VL muscle cooling protocol, participants sat in the environmental chamber set at 0 °C, while wearing a cooling vest. Their VL muscle and surrounding quadriceps muscles were also cooled following the abovementioned procedure. They remained in the environmental chamber until their T_c decrease by 1 °C and T_m decrease by 6 °C (but not more). Again, once we reached the target

temperatures, participants underwent the same pre-exercise measurements, three-minute exercise cycling bout, and post-exercise measurements. They then rested for a minimum of 20 minutes in the thermal chamber or until they reached the target temperatures. The exercise protocol was then repeated one last time.

4.3 Instrumentation and Measurements

4.3.1 Core, skin and intramuscular temperatures

Core temperature was continuously recorded using a rectal core temperature probe (Type-T thermocouple probe, RET-1 by Physitemp Instruments, Clifton, NJ, USA) inserted 10 cm into the rectum. The T_c data was recorded using thermal recording software (DASYLab, by Measurement Computing™). Skin temperature was measured at six sites (forehand, chest, hand, lateral forearm, lower back, lateral thigh) using portable skin temperature loggers (DS1922L, iButtonLink Technology, WI, USA) and recorded using data software (Thermodata Viewer, Thermodata Pty. Ltd., Australia). The Palmes & Park, (1947) formula for weighted average was used with the six locations to estimate mean weighted skin temperature ($\overline{T}_{sk}$).

(1) $\overline{T}_{sk} = 0.14(T_{forehead}) + 0.19(T_{chest}) + 0.05(T_{hand}) + 0.11(T_{lat\ forearm}) + 0.19(T_{lower\ back}) + 0.32(T_{lat\ thigh})$

An intramuscular temperature probe (flexible implantable microprobe, IT-18 Thermocouple by Physitemp Instruments, NJ, USA) was inserted at the mid-point of the leg into the VL in line with the muscle fibers under local anesthetic. An 18G hollow catheter was used to puncture the skin and travel down to the muscle at a 30° angel to a depth of 1 cm within the muscle. The depth was guided from a measure of adipose tissue thickness derived from Doppler Ultrasound

(Beelen & Sargeant, 1991). The temperature probe was threaded into the catheter where it sat for the duration of the experimental session. The wire was taped to the thigh to prevent movement throughout the trial. The T_m data was also recorded using thermal recording software (DASYLab, by Measurement Computing™).

4.3.2 Pulmonary oxygen consumption

Pulmonary oxygen consumption ($\dot{V}O_{2p}$), carbon dioxide release ($\dot{V}CO_2$), respiratory rate (RR), tidal volume (V_t), minute ventilation ($\dot{V}E$), and ventilatory threshold ($\dot{V}E/\dot{V}O_2$) were assessed using an open circuit ergospirometer measured at the mouth using breath-by-breath analysis from a metabolic cart (Ultima CPX, MGC Diagnostics, St-Paul, MN, USA). The gas analyzers were calibrated using two air tanks, one containing 26% oxygen and a nitrogen balance, the other containing 5% carbon dioxide, 12% oxygen, and a nitrogen balance. For calibration of volumes, the metabolic cart's bidirectional flow sensor was calibrated with a 3-L syringe.

4.3.3 Muscle oxygen consumption

Local oxygenation profiles were assessed on the VL using a continuous wave Near-Infrared Spectroscopy (NIRS) system (Portamon, Artinis Medical Systems, Zetten, The Netherlands). The use of NIRS has been validated for use in human and exercise (Mancini et al., 1994), confirming it to be a useful non-invasive method to monitor tissue oxygenation. The NIRS device was placed on the skin at the medial axis of the VL at a position 10 cm above and 5 cm lateral from the patella. The NIRS device was affixed to the skin using microporous hypoallergenic tape (Transpor™, 3M, MN, USA) and covered in a cloth tensor (TENSOR™, 3M, MN, USA) to block out any external sources of light. It was positioned over the belly of the VL and double-checked to ensure it was affixed to the leg. The distance between the light-emitting and receiving optodes was 30 mm, 35 mm, and 40 mm. The optical wavelengths were

760 nm and 850 nm as light sources. The sampling rate for the intensity of incident and transmitted light was recorded at 1 Hz. This provided concentration changes of baseline for O_2Hb, HHb, tHb, and the difference between oxy – deoxyhemoglobin (HbDiff) content in the VL to be measured. It is important to note that myoglobin chromophores also have a similar optical wavelength as hemoglobin and are subsequently assessed within the NIRS signal as well, however, its contribution is minimal (~ 5%) and considered to be negligible. HHb is a proxy for $\dot{V}O_{2m}$ and thus can be used to study muscle O_2 kinetics (Grassi et al., 2003).

4.3.4 Limb blood flow and leg adipose tissue thickness

Resting limb blood flow ($\dot{Q}_{SFA}$) of the femoral artery and adipose tissue thickness (ATT) above the VL was assessed pre-exercise using a linear transducer probe (Portable Ultrasonic Diagnostic Devices, L7m-a Linear probe, Chison Medical Imaging Co., Ltd, Shanghai, China) on a Doppler Ultrasound (Digital Color Doppler Ultrasound System, ECO 5, Chison Medical Imaging Co.,Ltd, Wuxi 214142, Jiangsu, China) on the right leg. To measure ATT, the probe measurement was taken on the body of the VL as indicated for NIRS placement (therefore ensuring the measurement was synonymous with the placement of the NIRS). The VL ATT measurement was taken with the Doppler Ultrasound in basic two-dimensional scanning mode, using a still image of the target area and an imbedded measurement tool within the Doppler Ultrasound software to measure from the surface of the skin to the surface of the muscle. To assess leg blood flow of the femoral artery, subjects laid supine on an examination bed with their hip abducted and externally rotated. Using the liner transducer probe at a frequency of 7.5 Hz, and the Doppler frequency at 6.5-7.5 Hz, with the machine set in colour-coded CFM mode, the transverse femoral arterial diameter was identified. The probe was then rotated 90° to run parallel with the artery to obtain a longitudinal view of the artery. The frame of reference was then adjusted to fit inside the view of

the femoral artery. With the Doppler Ultrasound set in PW mode (used to examine speed, direction, and spectral contrast in regards to blood flow), it recorded the sound image of the blood flow. The gain was adjusted until any noise was eliminated. Once 8-12 heart cycled were recorded, saved, and averaged to obtain the final $\dot{Q}_{SFA}$ measure.

4.3.5 Occlusions

Muscle blood flow ($\dot{Q}_m$) was measured via two 10-second venous occlusions (De Blasi et al., 1994; Hamaoka et al., 2011). An occlusion cuff (E20, Hokanson Inc, Bellevue, WA, USA) was placed proximally to the NIRS optode and intramuscular probe. Venous occlusions consisted of using an automatic pressure cuff to collapse the venous outflow while maintaining the arterial inflow. This was achieved with 80 mmHg of pressure to the limb. The increase in tHb from this occlusion resulted in a slope used to determine blood flow (De Blasi et al., 1994; Van Beekvelt et al., 2001). The following equation was used to calculate $\dot{Q}_m$:

(2) $\dot{Q}_m = (((\Delta tHb x 60)/(([Hb] x 1000)/4)) x 1000)/10$ in $mL \cdot min^{-1} \cdot 100ml^{-1}$

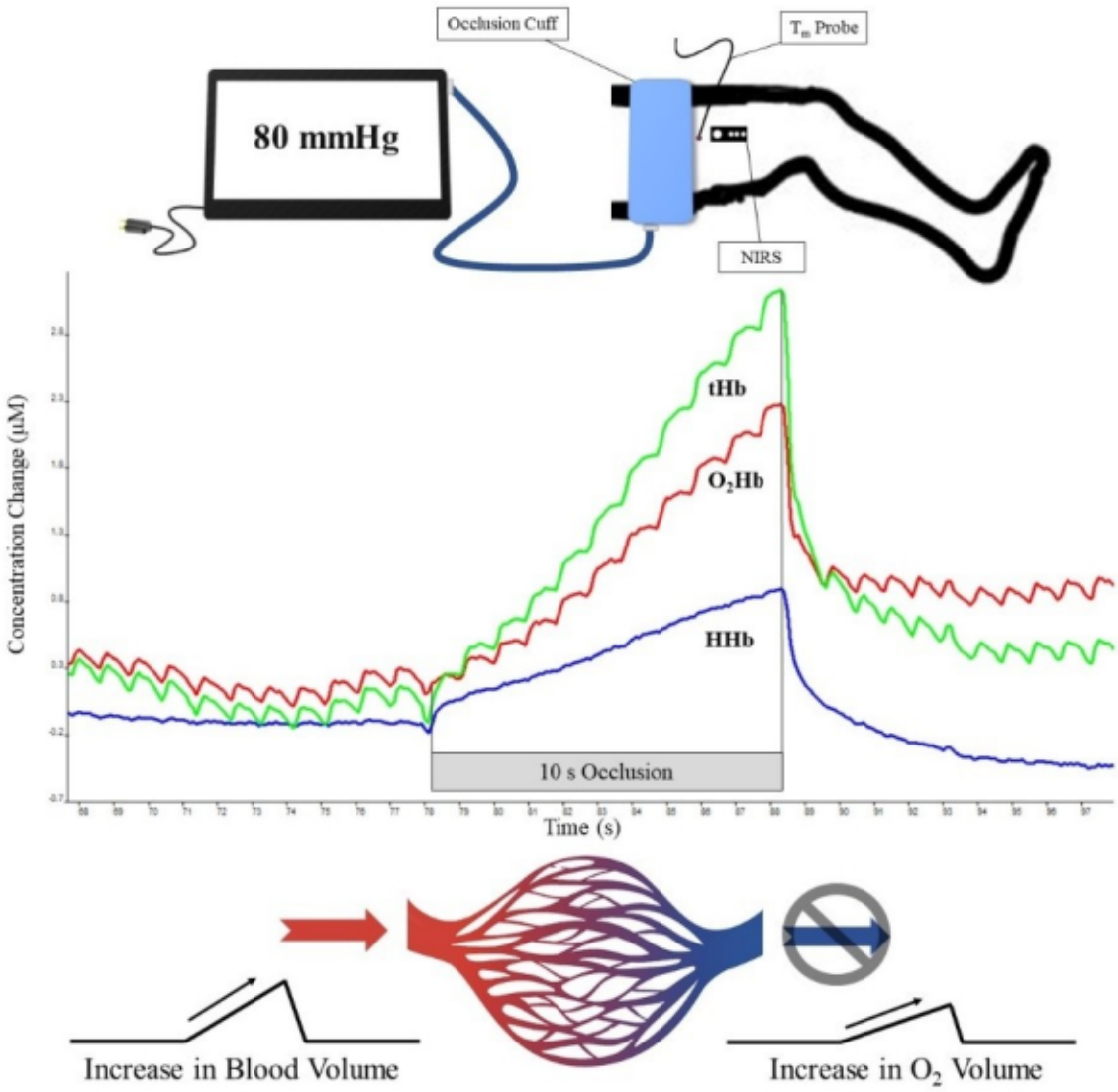

Figure 4. Venous occlusion diagram.

Mean muscle oxygen consumption (VO_{2m}) was measured through two 10-second arterial occlusions (Cheatle et al., 1991; Colier, Meeuwsen, Degens, & Oeseburg, 1995; Roberto A. De Blasi, Cope, Elwell, Safoue, & Ferrari, 1993). With the already placed occlusion cuff, arterial occlusions were used to completely stop the inflow and outflow of blood in the muscle. This was achieved with 300 mmHg of pressure to the limb. The decrease in the variable O_2Hb following the occlusion provided us with a slope to determine how much oxygen the muscle is consuming at a given time (i.e., VO_{2m}) (De

Blasi, Almenrader, Aurisicchio, & Ferrari, 1997). The following equation was used to calculate $\dot{V}O_{2m}$:

(3) $\dot{V}O_2m = Abs(((\Delta O_2Hbx60)/(10x1.04))x4)x22.4/1000$ in $mLO_2 \cdot min^{-1} \cdot 100g^{-1}$

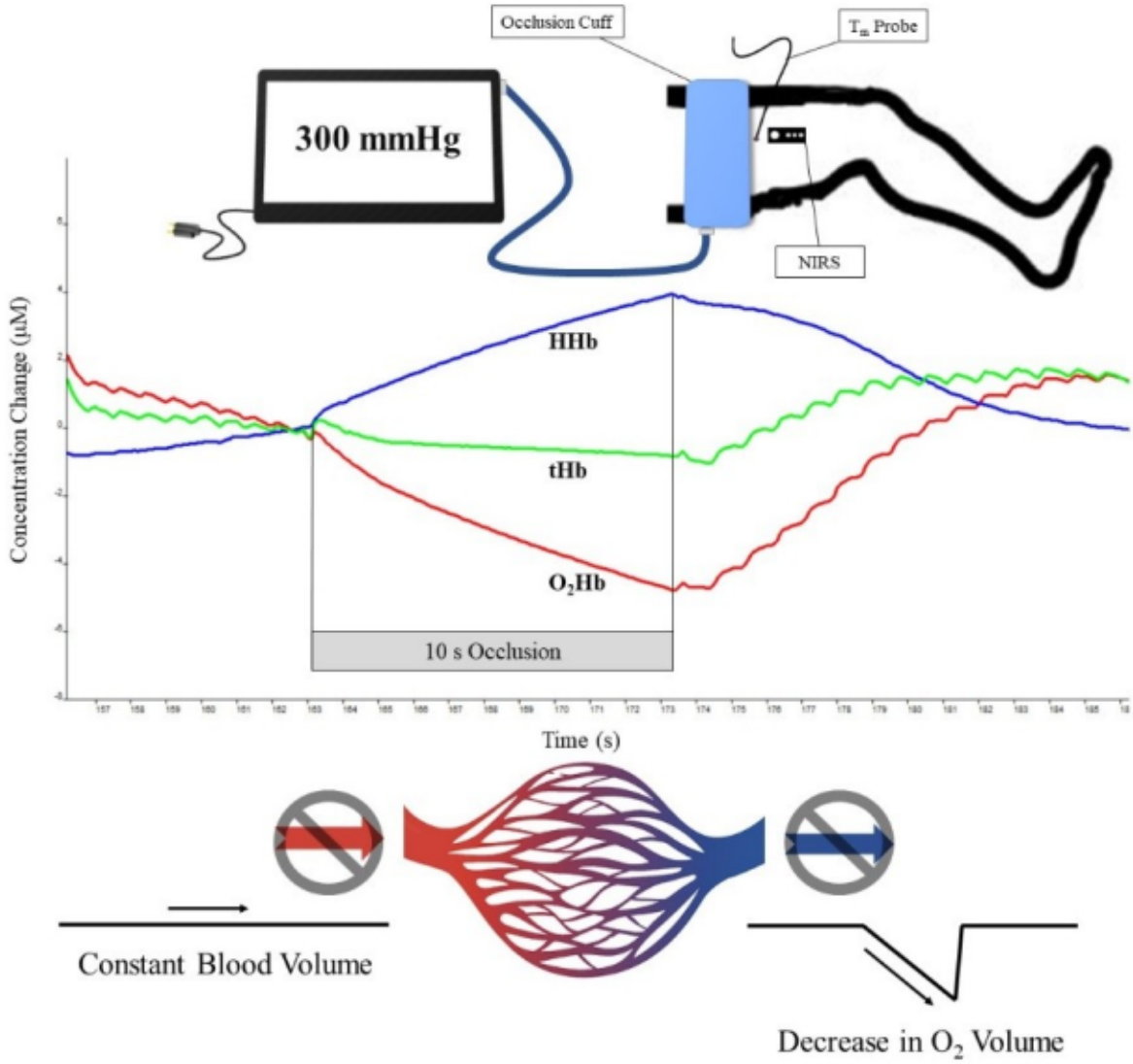

Figure 5. Arterial occlusion diagram.

4.3.6 Thoracic electrical bioimpedance

A portable thoracic electrical bioimpedance device was used (PhysioFlow PF07 Enduro, Manatec Biomedical, France) to evaluate hemodynamics parameters non-invasively and in real-

time. Previous studies have described the methodology of the PhysioFlow device in detail (Charloux et al., 2000; Gordon, Abbiss, Maiorana, Marston, & Peiffer, 2018; Tonelli, Alnuaimat, Li, Carrie, & Mubarak, 2011). In short, the device works by generating a high frequency (66 kHz), low magnitude (4.5 mA peak to peak) alternating electrical current towards the thorax between two sets of electrodes. Electrodes (Ag/AgCl, Skintact FS-50) were placed on the participant, each with a "transmitting" and "sensing" electrode. Two electrodes were placed above the supraclavicular fossa at the base of the neck on the left side. An additional two electrodes were placed along the xiphoid process, and two placed at the V1/V6 position of the chest for ECG monitoring. The alternating current is time-correlated with the simultaneous ECG recording. Before electrode placement, the placement locations were shaved, cleaned with 70% isopropyl alcohol swabs and then prepared with abrasive gel (NuPrep®, Weaver and Company, Colorado, USA) to provide better skin conductance. After entering pertinent anthropometric information including height, body mass, and age. The device was auto-calibrated following the manufacturer's guidelines. The procedure used 30 heart cycles of the resting participant in an upright seated position. The auto-calibration stores the largest impedance variation during systole ($Z_{peak} - Z_{min}$) and the largest rate of variation of the impedance signal, also called the contractility index (dZ/dt_{peak}). Stroke volume index (SVi_{cal}) calculation was dependent on the thoracic flow inversion time (TFIT) (measured by the time in ms from the first mathematical derivative of the impedance signal) and the two other parameters mentioned above during the autocalibration phase of 30 consecutive heart cycles. The stroke volume index equation is as follows, $SVi_{cal} = k \cdot [(dZ/dt_{max}) / (Z_{max} - Z_{min})] \cdot W(TFIT_{cal})$, where k is an empirically adjusted constant and W is a propriety correction algorithm. Following the auto-calibration process, the participant's systolic/ diastolic blood pressure values were entered using a digital blood pressure monitor (Life Source,

UA-787EJ, A&D Medical, Milpitas, CA, USA). During data collection the device calculates stroke volume (SV; in mL), stroke volume index (SVi), heart rate (HR; bpm), cardiac output (CO; in $L \cdot min^{-1}$), cardiac index (CI; $L \cdot min^{-1} \cdot m^2$), systolic atrial blood pressure (SABP), diastolic atrial blood pressure (DABP), mean atrial blood pressure (MABP; mmHg), contractility index (CTI), ventricular ejection time (VET; ms), early diastolic filling ratio (EDFR; %), left cardiac work index (LCWi; $kg \cdot m^{-1}/m^2$), systemic vascular resistance index (SVRi; $Dyn \cdot s^{-1} \cdot cm^5 \cdot m^2$), end-diastolic volume (EDV; mL), left ventricular ejection fraction (EF, %). Specifically, the device calculates SV according to the equation: $SV = SVi_{cal} \cdot ((dZ/dt_{max}) / (dZ/dt_{max})_{cal} \cdot TFIT_{cal} \cdot TFIT)^{1/3} \cdot BSA$. The device calculates body surface area (BSA; m^2) using the Haycock equation: $BSA = 0.024265 \cdot height^{0.3964} \cdot weight^{0.5378}$ (Haycock, Schwartz, & Wisotsky, 1978). Using the R-R interval of the first derivative from the ECG signal ($dECG/t$) collected, heart rate is determined. It uses the derivative of the ECG signal because it provides a more stable signal. Following this, it calculates cardiac output according to the equation: $\dot{Q} = HR \cdot SVi \cdot BSA$ (Gordon et al., 2018). See Gordon et al. (2018), for the further equations and calculations on the remaining variables calculated by PhysioFlow. In previous studies by Charloux et al. (2000), Richard et al. (2001) and Tonelli et al. (2011), this device was validated against the direct Fick method at rest and during maximal exercise.

4.3.7 Blood pressure

A digital blood pressure monitor (Life Source, UA-787EJ, A&D Medical, Milpitas, CA, USA) was used to assess blood pressure at rest, prior to each exercise bout and during the last minute of exercise. The device provided a measure of systolic blood pressure (SBP) in mmHg, diastolic blood pressure (DBP) in mmHg, and a measure of heart rate in beats/minute. Mean arterial pressure (MAP) was then calculated using the following equation:

(4) MAP = ⅓ SBP + ⅔ DPB.

With calculated MAP, total peripheral resistance can then be calculated using $\dot{Q}$, following the equation:

(5) Total Peripheral Resistance = MAP / $\dot{Q}$.

Following the Fick principle of $\dot{V}O_2 = \dot{Q} \times a\text{-}vO_2Diff$, we can rearrange the equation to calculate $a\text{-}vO_2$ difference using known values for $\dot{V}O_2$ and $\dot{Q}$ for both whole body and local levels. Where:

(6) $a\text{-}vO_2Diff = \dot{V}O_{2p} / \dot{Q}$.

4.3.8 Blood gases and metabolites analysis

Lactate, pH, PCO_2 (partial pressure of carbon dioxide), PO_2 (partial pressure of oxygen), TCO_2 (total carbon dioxide), HCO_3 (bicarbonate), BE (base excess), sO_2 (saturation of oxygen) were analyzed via a portable blood gas analyzer (i-STAT, Abbott Point of Care Inc., Princeton, NJ, USA) with specifically-designed blood gas collection cartridges (CG4+). Following laboratory biohazard and safety guidelines, we installed a 20G venous catheter in the antecubital vein of the participant. A mandarin/stylet was then put in place to lock the catheter. Blood was collected in 3ml syringes from the catheter and immediately transferred on the cartridges for analyses.

4.4 Data Processing

Temperature data for T_c and T_m were collected in second by second and analyzed using an average of 5 minutes before exercise to establish a BL, then an average of 10s preceding each minute of exercise at 0 s, 60 s, 120 s, 180 s. Temperature data for T_{leg} and $\bar{T}_{sk}$ were collected

minute by minute and analyzed using an average of 5 minutes before exercise for BL, then the minute by minute data points throughout exercise.

All kinetics variables ($\dot{V}O_{2p}$, $\dot{V}O_{2m}$, $\dot{Q}$, SV, and HR) used continuous data in second-by-second format with a 60 s BL and 180 s of exercise. The continuously collected data from the two exercise transitions corresponding to each temperature condition were time-aligned and averaged for each participant. The responses to exercise were modelled using a mono-exponential curve fitting function set to fit from 20 sec into exercise to the end of the three minutes, following previously described methods (Bell et al., 2003; Benson, Bowen, Ferguson, Murgatroyd, & Rossiter, 2017):

(7) $$Y_{(t)} = Y_{BL} + \text{Amp}\ \{1 - e^{[-(t - TD)/\tau]}\}$$

where Y represents the variable at any time (t), BL is the baseline value of Y before the start of exercise, Amp is the amplitude of the increase in Y above the baseline value, TD is the time delay observed during the start of exercise to the end of phase-1 for $\dot{V}O_{2p}$ and the initial increase in following a rapid overshoot in $\dot{V}O_{2m}$ at the start of exercise, and τ is the time constant of the response corresponding to the time it takes to reach 63% of the steady-state response.

Data for average $\dot{V}O_{2p}$ used an average of 60 s in a resting TN state to establish a BL. Pre-exercise values were determined using an average of 30 s preceding the start of exercise, and post-exercise values used an average of 30 s preceding the end of exercise. $\dot{V}O_{2m}$ and $\dot{Q}_m$ values were established using arterial and venous occlusions as previously described. Hemodynamics data (CO, SV, and HR) used an average of 60 s in a resting TN state to establish a BL. Pre-exercise values were determined using an average of 30 s

preceding the start of exercise and post-exercise values were determined using an average of 30 s preceding the end of exercise.

4.5 Statistical Analysis

Figures and tables include the mean ± SD from the mean where applicable. T_c, T_m, T_{leg}, $\overline{T}_{sk}$, $\dot{V}O_{2p}$, $\dot{V}O_{2m}$, $\dot{Q}_m$, $\dot{Q}$ averages, SV averages, HR averages, WB A-VO_2Diff, WB TPR, pH, Lactate, PO_2, PCO_2, Beefc, HCO_3, TCO_2 and sO_2 were all normally distributed as determined with the Shipiro-Wilks test by having a $p > 0.05$. Repeated measures ANOVA were used, followed by Bonferroni post hoc tests to compare the means and recognize if there were differences between conditions.

The baseline and amplitude, along with the kinetics values for TD and τ of $\dot{V}O_{2p}$, $\dot{V}O_{2m}$, $\dot{Q}$, SV, and HR were all normally distributed. This was determined with the Shipiro-Wilks test by having a $p > 0.05$, and therefore one-way repeated measures ANOVA followed by Bonferroni post hoc tests were used to compare the means and recognize if there were differences between conditions.

Leg A-VO_2Diff was not normally distributed as determined with the Shipiro-Wilks test by having a $p < 0.05$. Therefore, Freidman's tests followed by Wilcoxon Signed Ranks tests were then used to test for differences between conditions.

Due to poor signal quality, one participant's data were removed from analysis for $\dot{V}O_{2m}$, $\dot{Q}$ kinetics, SV kinetics, HR kinetics and subsequently the calculated WB A-VO_2Diff, resulting in a sample size of seven for these variables. We were also unable to effectively install and extract blood from the venous catheter on one participant, which

resulted in a sample size of seven for the blood gas and metabolite variables as well. Statistical analysis was conducted using SPSS (version 24.0; IBM Corp., Armonk, N.Y., USA) and graphing in SigmaPlot (version 14.0, Systat Software, Inc.).

5 Results

5.1 Temperature

Table 1. Mean core, muscle, leg, and skin temperature.

Mean (±SD)	*Thermoneutral*	*Muscle Cooling*	*Whole-Body Cooling*
T_c (°C)			*
Baseline	37.16 (±0.15)	37.16 (±0.24)	36.37 (±0.40)
0s	37.13 (±0.22)	37.16 (±0.24)	36.35 (±0.42)
60s	37.13 (±0.21)	37.17 (±0.24)	36.32 (±0.44)
120s	37.14 (±0.21)	37.18 (±0.24)	36.31 (±0.45)
180s	37.15 (±0.21)	37.18 (±0.25)	36.30 (±0.45)
T_m (°C)	*		
Baseline	35.42 (±0.84)	30.27 (±1.32)	29.77 (±2.02)
0s	35.43 (±0.88)	30.40 (±1.45)	30.01 (±1.92)
60s #	35.61 (±0.93)	31.03 (±1.35)	30.42 (±1.80)
120s #	35.84 (±1.02)	31.68 (±1.42)	30.98 (±1.80)
180s #	36.05 (±1.06)	32.41 (±1.56)	31.63 (±1.80)
T_{leg} (°C)	*		
Baseline	33.32 (±1.00)	27.38 (±2.62)	27.41 (±1.51)
0s	33.32 (±1.00)	27.85 (±2.36)	27.69 (±1.75)
60s	33.26 (±1.11)	28.29 (±2.26)	28.10 (±1.82)
120s	33.23 (±1.12)	28.48 (±2.11)	28.23 (±2.01)
180s	33.13 (±1.15)	28.63 (±1.89)	28.35 (±2.13)
T_{sk} (°C)			*

Baseline	32.53 (±0.88)	33.17 (±1.56)	25.66 (±2.16)
0s	32.55 (±0.88)	33.08 (±1.43)	25.89 (±2.17)
60s	32.58 (±0.89)	33.13 (±1.33)	26.09 (±2.15)
120s	32.41 (±0.85)	33.03 (±1.26)	26.12 (±2.22)
180s	32.32 (±0.88)	32.89 (±1.17)	26.15 (±2.32)

(* significant difference from the other conditions, # significant difference from the other time points)

5.1.1 Core temperature – T_c

A significant difference between conditions (p = 0.000) was indicated. WBC T_c (36.33 ± 0.43 °C) was significantly lower than TN (37.14 ± 0.24 °C, p = 0.001) and LC (37.17 ± 0.20 °C, p = 0.001). TN and LC were not significantly different (p = 1.000). Between time points of BL (36.90 ± 0.47 °C), 0 s (36.88 ± 0.48 °C), 60 s (36.88 ± 0.50 °C), 120 s (36.88 ± 0.51 °C) and 180 s (36.88 ± 0.52 °C) no differences were indicated (p = 0.332), thus no further analysis was conducted. Core temperature remained very constant throughout the 3 minutes of exercise in all conditions.

5.1.2 Muscle temperature – T_m

A significant difference between conditions (p = 0.000) was indicated. TN T_m (35.67 ± 0.98 °C) was significantly higher than LC (31.16 ± 1.63 °C, p = 0.000) and WBC (30.56 ± 1.99 °C, p = 0.000). LC and WBC were not significantly different (p = 0.986), indicating that the T_m cooling method was proficient in reducing the VL muscle temperature significantly. Between time points, there was a significant difference (p = 0.012). All time points (BL; 31.82 ± 2.95 °C, 0 s; 31.95 ± 2.88 °C, 60 s; 32.35 ± 2.71 °C, 120 s; 32.84 ± 2.59 °C, and 180 s; 33.36 ± 2.45 °C) were significantly different, with the exception of BL and 0s for TN and LC. During exercise, muscle temperatures all increased significantly.

5.1.3 Leg temperature – T_{leg}

A significant difference between conditions (p = 0.000) was indicated. TN (33.25 ± 1.08 °C) was significantly higher than LC (28.13 ± 2.31 °C, p = 0.006) and WBC (27.95 ± 1.89 °C, p = 0.000). LC and WBC were not different (p = 1.000). Between time points (BL; 29.37 ± 3.34 °C, 0 s; 29.62 ± 3.17 °C, 60 s; 29.88 ± 2.99 °C, 120 s; 29.98 ± 2.92 °C, and 180 s; 30.04 ± 2.82 °C) a significant difference was also indicated (p = 0.018). Further analysis revealed the only significant difference was between BL and 60 s (p = 0.037). No other statistically significant differences were indicated. VL leg skin surface temperature did not warm significantly during exercise.

5.1.4 Mean weighted skin temperature – $\overline{T}_{sk}$

A significant difference between conditions (p = 0.000) was indicated. WBC $\overline{T}_{sk}$ (25.98 ± 2.21 °C) was significantly lower than TN (32.48 ± 0.88 °C, p = 0.000) and LC (33.06 ± 1.36 °C, p = 0.000). TN and LC were not significantly different (p = 1.000). Between time points BL (30.45 ± 3.77 °C), 0 s (30.51 ± 3.64 °C), 60 s (30.60 ± 3.55 °C), 120 s (30.52 ± 3.49 °C) and 180 s (30.45 ± 3.44 °C) no differences were indicated (p = 1.000), thus no further analysis was conducted.

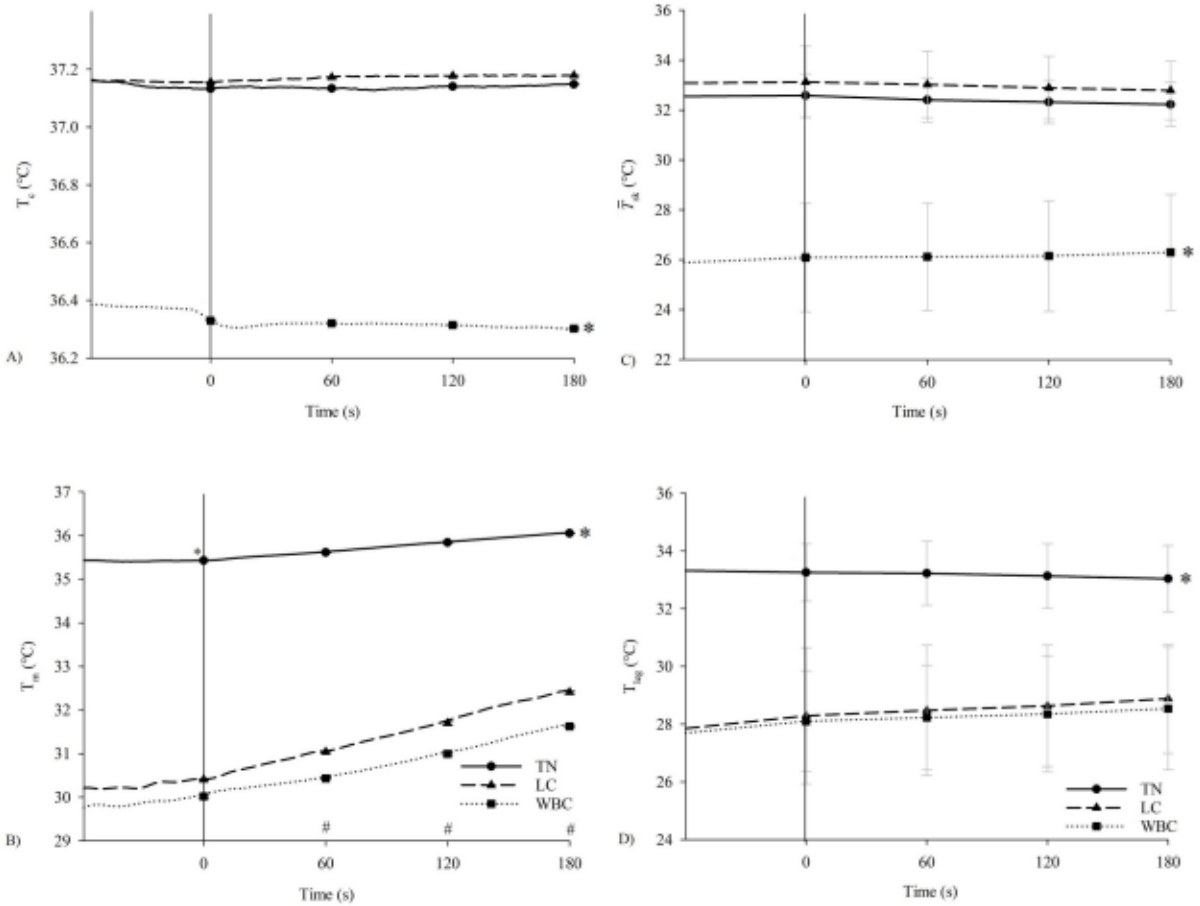

Figure 6. Continuous mean core temperature (T_c) for each condition (A); continuous mean muscle temperature (T_m) for each condition (B); minute by minute mean leg skin surface temperature (T_{leg}) of the VL (C); minute by minute mean skin temperature ($\bar{T}_{sk}$) (D). * Significant difference between WBC *vs.* TN and LC for (A), TN *vs.* LC and WBC for (B), WBC *vs.* TN and LC for (C), and TN *vs.* LC and WBC for (D). # Significant difference between each time points in (B).

5.2 Kinetics

Table 2. Mean O_2 and cardiovascular hemodynamic kinetics.

Mean (±SD)		*Thermoneutral*	*Muscle Cooling*	*Whole-Body Cooling*
VO_{2p}				
	Baseline ($mL \cdot kg^{-1} \cdot min^{-1}$)	5.21 (±0.55)	5.48 (±1.10)	8.58 (±3.59)
	Amplitude ($mL \cdot kg^{-1} \cdot min^{-1}$)	25.29 (±5.38)	25.18 (±6.73)	24.30 (±5.85)
	Time Delay (s)	20.10 (±1.98)	16.85 (±5.45)	17.02 (±4.45)
	Time Constant (s)	51.96 (±4.74)	49.36 (±8.18)	50.56 (±11.50)
VO_{2m}				
	*Baseline (*µm)	12.65 (±3.43)	12.33 (±3.04)	16.04 (±5.08)
	*Amplitude (*µm)	7.20 (±5.07)	7.64 (±5.25)	6.08 (±4.14)
	Time Delay (s)	15.33 (±5.91)	15.52 (±3.70)	12.67 (±5.66)
	Time Constant (s)	24.91 (±4.25)	24.19 (±6.08)	21.29 (±6.18)
$\dot{Q}$				
	Baseline (L/min)	7.37 (±1.37)	7.37 (±1.16)	6.66 (±1.60)
	Amplitude (L/min)	8.16 (±1.89)	8.41 (±2.85)	8.22 (±2.96)
	Time Delay (s)	4.59 (±7.16)	5.61 (±9.43)	3.02 (±6.77)
	Time Constant (s)	31.40 (±10.52)	34.74 (±8.00)	38.93 (±12.74)
SV (mL)				
	Baseline (mL)	91.47 (±10.75)	87.42 (±15.25)	91.89 (±15.95)
	Amplitude (mL)	27.34 (±8.43)	25.84 (±10.99)	23.77 (±15.18)
	Time Delay (s)	9.59 (±6.06)	11.71 (±10.13)	11.79 (±8.68)
	Time Constant (s)	33.27 (±13.21)	26.26 (±11.40)	29.60 (±11.30)
HR (bpm)				
	Baseline (bpm)	80.47 (±12.86)	85.55 (±9.07)	74.07 (±14.53)
	Amplitude (bpm)	52.05 (±10.91)	53.96 (±13.34)	56.26 (±12.66)
	Time Delay (s)	-0.36 (±5.12)	-0.71 (±6.34)	-6.54 (±7.52)
	Time Constant (s)	32.45 (±8.17)	33.12 (±6.97)	36.91 (±11.00)

5.2.1 Pulmonary oxygen consumption - VO_{2p}

A significant difference in baseline values between conditions ($p = 0.031$) was identified. However, post-hoc analysis revealed no differences between conditions. WBC (8.58 ± 3.60 $mL \cdot kg^{-1} \cdot min^{-1}$) was higher than TN (5.20 ± 0.55 $mL \cdot kg^{-1} \cdot min^{-1}$, $p = 0.092$) but not sufficient enough to reach significance. LC (5.48 ± 1.10 $mL \cdot kg^{-1} \cdot min^{-1}$) was not different from TN ($p = 1.000$) and although lower, was not significantly different from WBC ($p = 0.129$) either. For Amp, TD and τ, there were no significant differences between conditions ($p = 0.730$, $p = 0.171$ and $p = 0.643$ respectively).

5.2.2 Muscle oxygen consumption - VO_{2m}

A significant difference in baseline values between conditions ($p = 0.013$) was identified. However, the post-hoc analysis revealed no differences between conditions. WBC (16.04 ± 5.08 µm) was higher than TN (12.65 ± 3.43 µm, $p = 0.114$) and LC (12.33 ± 3.04 µm, $p = 0.107$) but not enough to reach significance. For Amp, TD and τ, no significant differences were indicated between conditions ($p = 0.249$, $p= 0.248$ and $p = 0.258$ respectively).

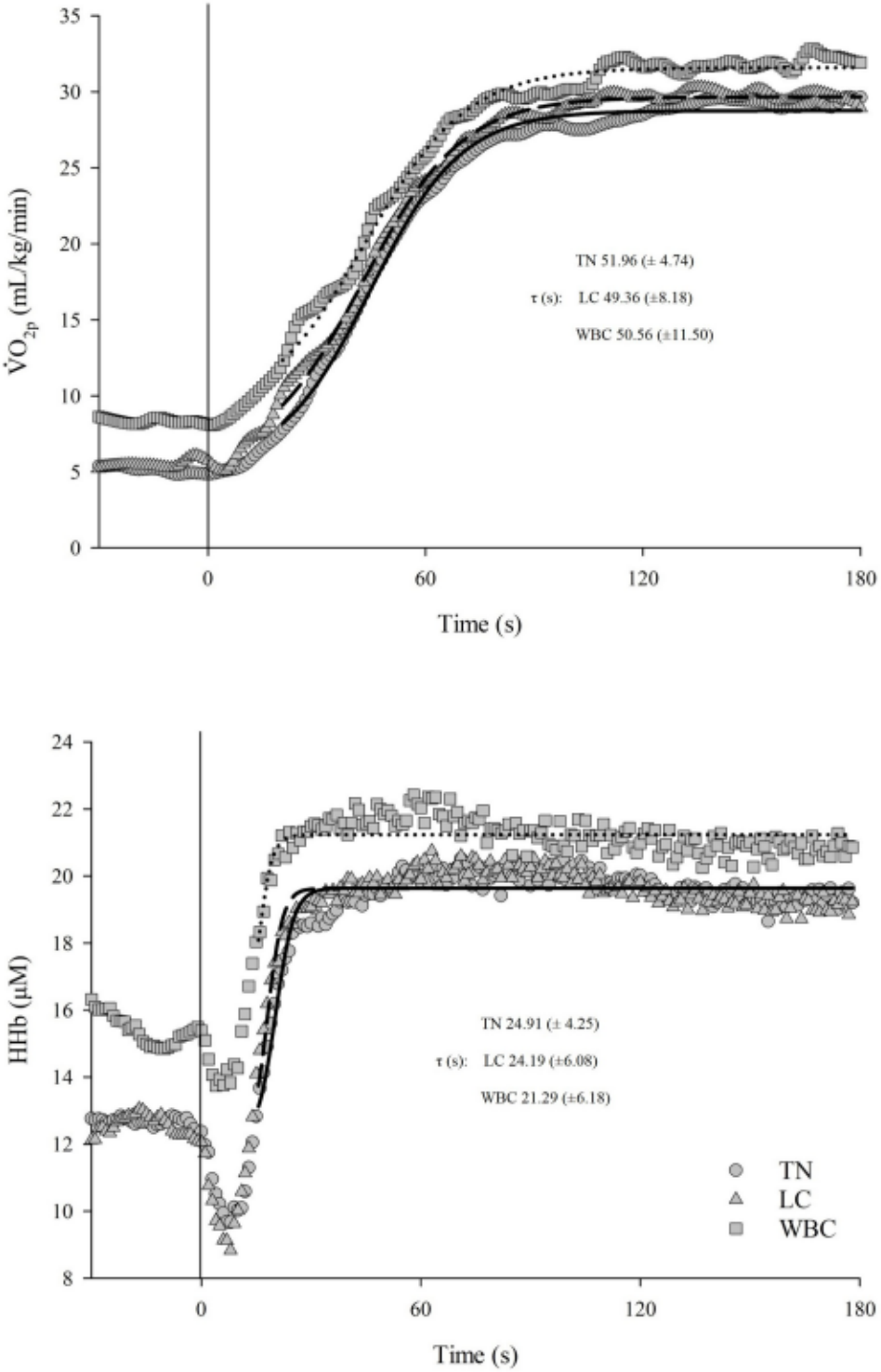

Figure 7. Pulmonary O_2 kinetics (Top); muscle O_2 kinetics (Bottom) – continuous mean response for each condition with a 4-parameter curve fit.

5.2.3 Cardiac output – $\dot{Q}$

No significant differences between conditions were indicated for BL, Amp, TD and τ ($p = 0.288$, $p = 0.846$, $p = 0.833$ and $p = 0.348$ respectively).

5.2.4 Stroke volume - SV

For BL, Amp, TD and τ no significant differences between conditions were indicated ($p = 0.703$, $p = 0.668$, $p = 0.827$ and $p = 0.309$).

5.2.5 Heart rate - HR

A significant difference between conditions was indicated for BL ($p = 0.008$). Upon post hoc analysis, no significant differences were observed. LC BL (85.55 ± 9.07 bpm) was higher than WBC (74.07 ± 14.53 bpm; $p = 0.052$), but not enough to reach significance. TN (80.47 ± 12.86 bpm) was not different from LC ($p = 0.269$) or WBC ($p = 0.171$). For Amp, TD and τ, no significant differences between conditions were indicated ($p = 0.179$, $p = 0.077$ and $p = 0.546$ respectively).

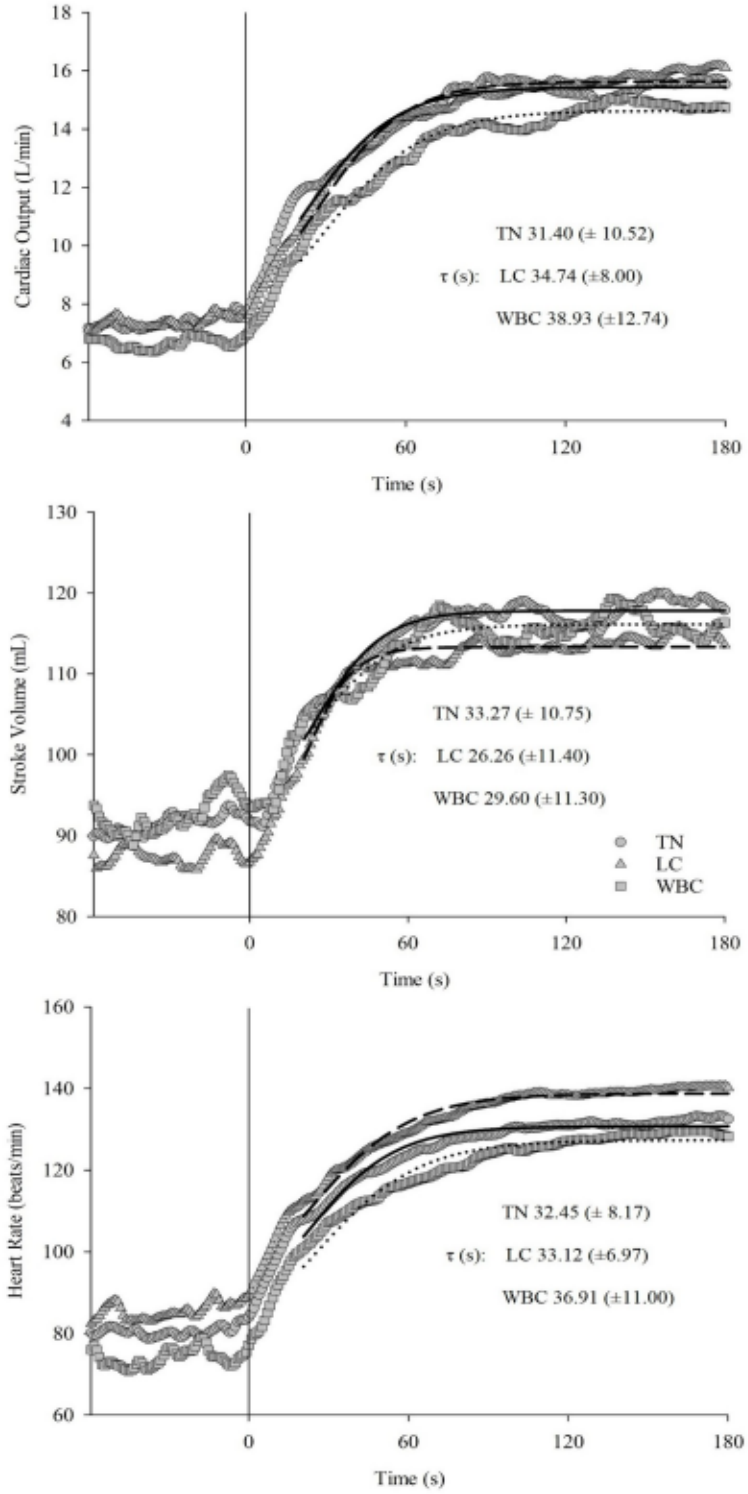

Figure 8. Cardiac output (Top); stroke volume (Middle); heart rate kinetics (Bottom) – continuous mean response for each condition with a 4-parameter curve fit.

5.3 Oxygen Consumption and Cardiovascular Hemodynamics

Table 3. Mean oxygen consumption and hemodynamics.

Mean (±SD)		*Thermoneutral*	*Muscle Cooling*	*Whole-Body Cooling*
$\dot{V}O_{2p}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)				*
	Pre-Exercise	5.11 (±0.48)	5.45 (±0.86)	8.32 (±3.44)
	Post-Exercise #	29.39 (±5.29)	29.71 (±5.02)	32.07 (±5.86) †
$\dot{V}O_{2m}$ ($mLO_2 \cdot min^{-1} \cdot 100g^{-1}$)				
	Pre-Exercise	0.29 (±0.15)	0.24 (±0.15)	0.26 (±0.16)
	Post-Exercise #	0.56 (±0.28)	0.65 (±0.39)	0.63 (±0.38)
$\dot{Q}_m$ ($mL \cdot min^{-1} \cdot 100mL^{-1}$)		*		
	Pre-Exercise	1.91 (±0.54)	1.48 (±0.41)	1.04 (±0.48)
	Post-Exercise #	3.84 (±1.45)	2.75 (±1.05)	2.18 (±0.88)
$\dot{Q}$ (L/min)				*
	Pre-Exercise	7.40 (±1.60)	7.81 (±0.88)	6.72 (±1.69)
	Post-Exercise #	15.45 (±2.82)	16.32 (±3.19)	14.88 (±3.19)
SV (mL)				
	Pre-Exercise	93.63 (±12.16)	92.76 (±8.78)	92.13 (±16.27)
	Post-Exercise #	118.51 (±20.01)	118.56 (±21.04)	115.94 (±20.38)
HR (bpm)				
	Pre-Exercise	80.24 (±14.19)	85.91 (±8.16)	74.17 (±14.33)
	Post-Exercise #	131.75 (±19.07)	137.41 (±14.71)	128.25 (±18.17)

(* significant difference from the other conditions, # significant difference from the other time points)

5.3.1 Pulmonary oxygen consumption – $\dot{V}O_{2p}$

There was a significant difference between conditions ($p = 0.009$). WBC (15.24 ± 12.6 $mL \cdot kg^{-1} \cdot min^{-1}$) was significantly higher than TN (13.28 ± 11.81 $mL \cdot kg^{-1} \cdot min^{-1}$, $p = 0.023$). LC (13.50 ± 11.84 $mL \cdot kg^{-1} \cdot min^{-1}$) was not significantly different from TN ($p = 0.744$) and although lower, not different from WBC ($p = 0.056$) either. There was also a significant difference between time

points ($p = 0.000$). Post (30.39 ± 5.54 mL·kg^{-1}·min^{-1}) exercise values were significantly higher than BL (5.33 ± 0.72 mL·kg^{-1}·min^{-1}, $p = 0.000$) and Pre exercise (6.29 ± 2.52 mL·kg^{-1}·min^{-1}, $p = 0.000$). BL and Pre were not different ($p = 0.106$). The was also a significant difference between the interaction of conditions and time points ($p = 0.037$). TN BL (5.33 ± 0.72 mL·kg^{-1}·min^{-1}) was not different from Pre (5.11 ± 0.48 mL·kg^{-1}·min^{-1}, $p = 0.909$). LC BL (5.33 ± 0.72 mL·kg^{-1}·min^{-1}) was not different from Pre (5.45 ± 0.86 mL·kg^{-1}·min^{-1}, $p = 1.000$). WBC BL (5.33 ± 0.72 mL·kg^{-1}·min^{-1}) was not different from pre (8.32 ± 3.44 mL·kg^{-1}·min^{-1}, $p = 0.093$). All other time points in each condition were significantly different ($p = 0.000$). Time points within conditions showed that Post WBC (32.07 ± 5.86 mL·kg^{-1}·min^{-1}) was significantly higher than TN (29.39 ± 5.29 mL·kg^{-1}·min^{-1}, $p = 0.016$). Post LC (29.71 ± 5.02 mL·kg^{-1}·min^{-1}) was not different from TN ($p = 0.871$) and although lower, not statistically significantly different from WBC ($p = 0.053$) either.

5.3.2 Muscle oxygen consumption – VO_{2m}

No significance between conditions ($p = 0.930$) was indicated. There was a significant difference between time points ($p = 0.034$); Post (0.61 ± 0.35 mLO_2·min^{-1}·100g^{-1}) significantly higher than Pre (0.26 ± 0.15 mLO·min^{1}·100g^{1}, $p = 0.028$). BL was not different from Pre ($p = 1.000$) or Post ($p = 0.347$). No differences of the interaction between conditions and time points was identified ($p = 0.276$).

5.3.3 Muscle blood flow – $\dot{Q}_m$

A significant difference between conditions ($p = 0.011$) was indicated. TN (2.21 ± 1.56 mL·min^{-1}·100mL^{-1}) was significantly higher than LC (1.70 ± 1.08 mL·min^{-1}·100mL^{-1}, $p = 0.031$) and WBC (1.364 ± 0.89 mL·min^{-1}·100mL^{-1}, $p = 0.040$). LC was not significantly higher than WBC ($p = 0.160$). A significant difference between time points ($p = 0.000$) was also indicated. BL (0.88 ± 0.62 mL·min^{-1}·100mL^{-1}) was not significantly lower than Pre (1.47 ± 0.60 mL·min^{-1}·100mL^{-1}, p

= 0.054), but significantly lower than and Post (2.92 ± 1.34 $mL \cdot min^{-1} \cdot 100mL^{-1}$, p = 0.001). Pre was also significantly lower than Post (p = 0.002). A significant difference between the interaction of conditions and time points (p = 0.044) was indicated. TN BL was different from Pre (p=0.009) and Post (p=0.001), and Pre was different from Post (p=0.008). LC Post was different from BL (p=0.006) and Pre (p=0.025). WBC Post was different from BL (p=0.047) and Pre (p=0.008). Differences for time points within conditions were also indicated. Pre TN was different from LC (p=0.005) and WBC (p=0.007), and LC different from WBC (p=0.017).

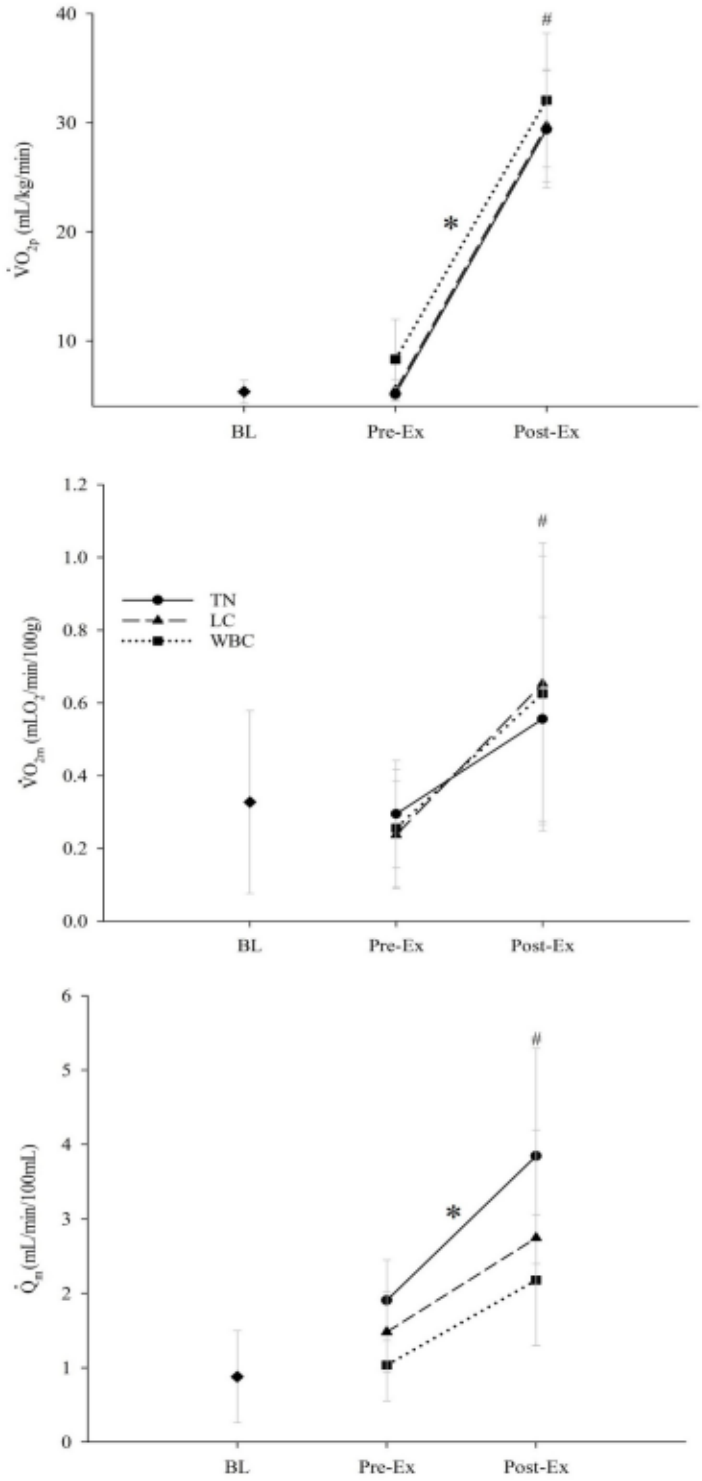

Figure 9. $\dot{V}O_{2p}$ (Top); $\dot{V}O_{2m}$ (Middle); mean $\dot{Q}_m$ (Bottom) – baseline, pre- and post-exercise. * Significant difference between conditions of WBC *vs.* TN and LC for $\dot{V}O_{2p}$, and TN *vs.* LC and WBC for $\dot{Q}_m$. # Significant difference between time points pre- and post-exercise for $\dot{V}O_{2p}$, $\dot{V}O_{2m}$, and $\dot{Q}_m$.

5.3.4 Cardiac output – Q˙

A significant difference between conditions (p = 0.007) was indicated. WBC (9.35 ± 4.53 L/min) was significantly lower than LC (10.56 ± 4.53 L/min; p =0.039). TN (10.10 ± 4.31 L/min) was not different from LC (p = 0.338) or WBC (p = 0.220). A significant difference of between time points (p = 0.000) was indicated. Post (15.55 ± 3.13 L/min) was significantly higher than BL (7.14 ± 1.43 L/min; p = 0.000) and Pre (7.31 ± 1.51 L/min; p = 0.001) exercise. BL and Pre were not different (p = 0.659). A significant difference between the interaction of conditions and time points was identified (p = 0.021). All conditions had no difference BL to pre, but the remainder (BL to Post, Pre to Post) were different.

5.3.5 Stroke volume - SV

No significant difference between conditions (p = 0.682) was indicated. There was a significant difference between time points (p = 0.001). Post (117.67 ± 20.09 mL) was significantly higher than BL (91.86 ± 12.83 mL; p = 0.001) and Pre (91.84 ± 12.54 mL; p = 0.006). No significant difference between the interaction of conditions and time points was identified (p = 0.882).

5.3.6 Heart rate - HR

A significant difference between conditions (p = 0.009) was indicated. There was a significant difference between LC (101.15 ± 4.59 bpm) and WBC (94.18 ± 5.74 bpm; p = 0.049). A significant difference between time points (p = 0.000) was indicated. Post (132.47 ± 17.82 bpm) was significantly higher than BL (78.15 ± 14.49 bpm; p = 0.000) and Pre (80.11 ± 13.45 bpm; p = 0.000) exercise. A significant difference between the interaction of conditions and time points was identified (p = 0.003). All conditions had no difference BL to pre, but the remainder (BL to Post, Pre to Post) were different.

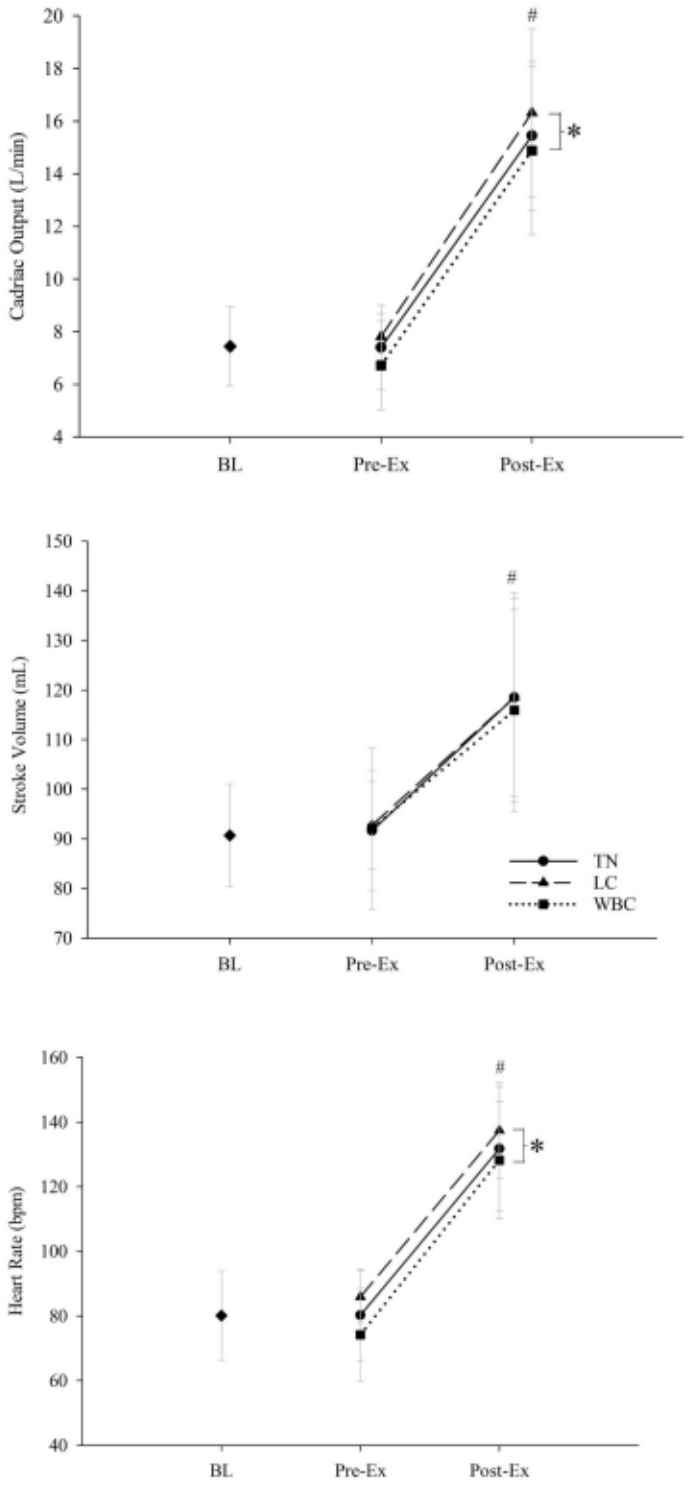

Figure 10. Mean cardiac output (CO) (Top); mean stroke volume (SV) (Middle); mean heart rate (HR) (Bottom) – pre- and post-exercise. * Significant difference between conditions of LC *vs.* WBC for CO, and LC *vs.* WBC for HR. # Significant difference between time points pre- and post-exercise for CO, SV, and HR.

5.4 A-VO_2Difference and Total Peripheral Resistance

Table 4. Arteriovenous oxygen difference and total peripheral resistance.

Mean (±SD)	*Thermoneutral*	*Muscle Cooling*	*Whole-Body Cooling*
WB A-VO_2Diff (mLO_2/L)			*
Pre-Exercise	49.20 (±14.33)	50.22 (±10.53)	88.16 (±29.75)
Post-Exercise #	145.02 (±14.42)	133.90 (±13.18)	151.05 (±26.12)
Leg A-VO_2Diff (mLO_2/L)	*		
Pre-Exercise	*0.16 (±0.07)*	0.16 (±0.10)	0.29 (±0.17)
Post-Exercise	*0.17 (±0.09)*	0.24 (±0.10)	0.27 (±0.10)
WB TPR (mmHg/L/min)			
Pre-Exercise	*14.91 (±3.29)*	14.81 (±2.97)	18.34 (±4.94)
Post-Exercise #	*6.81 (±2.14)*	6.77 (±1.48)	7.18 (±2.34)

(* significant difference from the other conditions, # significant difference from the other time points)

5.4.1 Whole-body A-VO_2difference

A significant difference between conditions was indicated ($p = 0.023$). WBC (98.07 ± 46.31 mLO_2/L) was significantly higher than LC (79.71 ± 40.03 mLO_2/L, $p = 0.045$). TN (83.08 ± 45.80 mLO_2/L) was not different from LC ($p = 0.325$) and WBC ($p = 0.146$). There was a significant difference between time points ($p = 0.000$). Post (143.32 ± 20.13 mLO_2/L) was significantly higher than BL (64.96 ± 25.03m mLO_2/L, $p = 0.000$) and Pre (62.53 ± 27.00 mLO_2/L, $p = 0.000$). BL was not different from Pre ($p = 1.000$). A significant difference in interaction between conditions and time points was also indicated ($p = 0.006$). All conditions had no difference BL to pre, but the remainder (BL to Post, Pre to Post) were different.

5.4.2 Leg A-VO_2difference

A significant difference indicated between conditions ($p=0.001$). Post hoc Wilcoxon Signed Rank test indicated that TN BL was different from TN Pre ($p = 0.017$), LC BL was different

from LC Pre ($p = 0.025$), and LC Pre was different from LC Post ($p = 0.036$). WBC Pre was different from TN Pre ($p = 0.012$) and LC Pre ($p – 0.012$). WBC Post was also different from TN Post ($p = 0.050$), therefore suggesting that TN and WBC conditions are different.

5.4.3 Total peripheral resistance

There was no significant difference between conditions ($p = 0.088$). There was a significant difference between time points ($p = 0.000$). Post (6.92 ± 203 mmHg/L/min) was significantly lower than BL (14.34 ± 3.23 mmHg/L/min, $p = 0.000$) and Pre (16.02 ± 4.17 mmHg/L/min, $p = 0.000$). No differences were observed between BL and Pre ($p = 0.545$). There was also a significant difference between the interaction of conditions and time points ($p = 0.047$). All conditions had no difference BL to pre, but the remainder (BL to Post, Pre to Post) were significantly different.

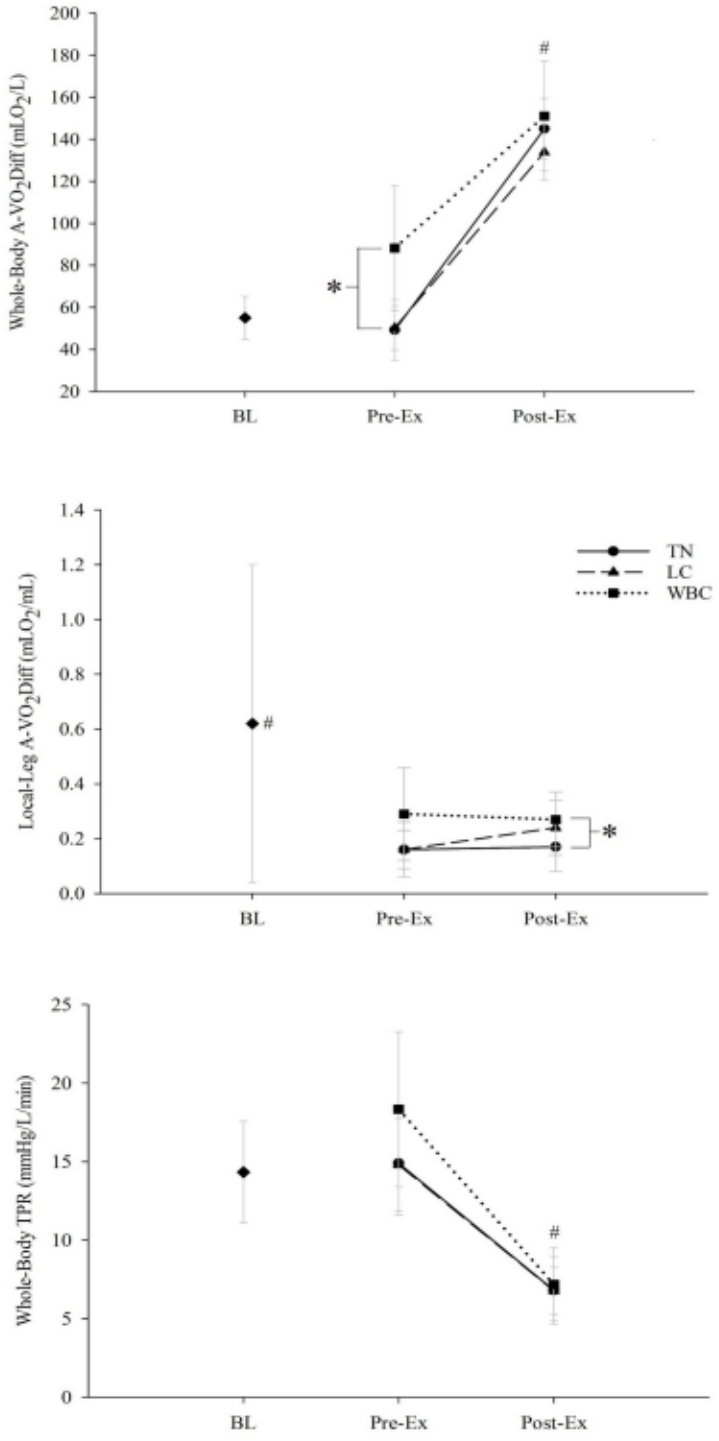

Figure 11. Mean pre- and post-exercise whole-body A-VO_2Diff (Top); local-leg A-VO_2diff (Middle); whole-body TPR (Bottom). * Significant difference between conditions of WBC *vs.* LC for whole-body A-VO_2Diff, and for WBC *vs.* TN for local-leg A-VO_2Diff. # Significant difference between time points pre- and post-exercise for whole-body A-VO_2Diff and whole-body TPR.

5.5 Venous Blood Gas and Metabolites

5.5.1 pH

There was a significant difference between conditions ($p = 0.008$). WBC (7.34 ± 0.05) was significantly lower than LC (7.38 ± 0.02, $p = 0.013$). TN (7.37 ± 0.03) was not different from LC ($p = 0.164$) or WBC ($p = 0.064$). There was also a significant difference between time points ($p = 0.000$). Post (7.33 ± 0.04) was significantly lower than BL (7.38 ± 0.02, $p = 0.009$) and Pre (7.38 ± 0.03, $p = 0.000$). BL and Pre were not different ($p = 1.000$). A significant difference between the interaction of conditions and time points ($p = 0.002$) was also indicated. LC Pre (7.40 ± 0.01) was significantly higher than LC Post (7.36 ± 0.02, $p = 0.009$). WBC BL (7.38 ± 0.02) was significantly higher than WBC Pre (7.34 ± 0.03, $p = 0.020$) and WBC Post (7.30 ± 0.04, $p = 0.004$), while WBC Pre was also significantly higher than WBC Post ($p = 0.005$). Time points within conditions also showed differences. Pre TN was different from Pre WBC ($p = 0.036$), while Pre WBC was also different from Pre LC ($p = 0.001$). No differences were observed between Post and the conditions.

5.5.2 Lactate

There was no significant difference between conditions ($p = 0.212$). There was a significant difference between time points ($p = 0.000$). Post (2.91 ± 0.80 mmol/L) was significantly higher than BL (0.91 ± 0.26 mmol/L, $p = 0.000$) and Pre (1.17 ± 0.36 mmol/L, $p = 0.000$). No significant difference between the interaction of conditions and time points was indicated ($p = 0.336$).

5.5.3 Partial pressure of oxygen

There was a significant difference between conditions ($p = 0.001$). TN (35.05 ± 13.99 mmHg) was significantly higher than WBC (19.67 ± 7.83 mmHg, $p = 0.012$). There was also a significant difference between time points indicated ($p = 0.026$). However, the post hoc test did not indicate any statistically significant differences. A significant difference between the interaction of conditions and time points was also indicated ($p = 0.000$). TN BL (22.57 ± 3.66 mmHg) was significantly lower than TN Pre (41.57 ± 12.56 mmHg, $p = 0.025$) and TN Post (41.00 ± 13.49 mmHg, $p = 0.027$). No other differences were observed. Time points within conditions also had differences. Pre TN was different from Pre WBC ($p = 0.006$) and post TN was different from Post WBC ($p = 0.034$).

5.5.4 Partial pressure of carbon dioxide

There was a significant difference between conditions ($p = 0.000$). WBC (55.04 ± 6.43 mmHg) was significantly higher than TN (48.01 ± 4.72, $p = 0.020$) and LC (47.34 ± 4.44 mmHg, $p = 0.004$). No significant differences between time points was indicated ($p = 0.088$). There was a significant difference between the interaction of conditions and time points ($p = 0.004$). TN Pre (44.26 ± 3.25 mmHg) was significantly lower than TN BL (49.60 ± 2.19 mmHg, $p = 0.044$) and TN Post (50.16 ± 5.49 mmHg, $p = 0.030$). No differences were observed between time points for LC. WBC BL (49.60 ± 2.19 mmHg) was significantly lower than WBC Pre (55.22 ± 1.79 mmHg, $p = 0.028$) and WBC Post (59.34 ± 2.83 mmHg, $p = 0.027$). For time points within conditions, Pre WBC (56.13 ± 4.67 mmHg) was significantly higher than Pre TN (44.26 ± 3.25 mmHg, $p = 0.002$) and Pre LC (45.19 ± 4.34 mmHg, $p = 0.001$), along with Post LC (47.24 ± 5.08 mmHg) being significantly lower than Post WBC (59.34 ± 6.93 mmHg, $p = 0.015$).

5.5.5 Base excess

No differences between conditions was identified (p = 0.227). There was a significant difference between time points (p = 0.003). Post (1.81 ± 02.28 mmol/L) was significantly lower than BL (4.14 ± 1.46 mmol/L, p = 0.045) and Pre (3.17 ± 1.78 mmol/L, p = 0.011) with no differences between BL and Pre (p = 0.374). There was no significant difference between the interaction of conditions and time points (p = 0.215).

5.5.6 Bicarbonate

A significant difference between conditions was indicated (p = 0.022). However, upon analysis of the post hoc tests, the results revealed no differences observed between conditions. There was a significant difference between time points (p = 0.029). However, analysis of the post hoc tests again revealed no differences observed between time points. A significant difference between conditions within time points was indicated (p = 0.014). Post hoc tests revealed no differences in conditions within time points. However, there were significant differences observed with the interaction of conditions and time points. Pre WBC (30.18 ± 0.95 mmol/L) was significantly higher than Pre TN (26.96 ± 1.76 mmol/L, p = 0.016) and Pre LC (27.67 ± 1.84 mmol/L, p = 0.033). No other differences were observed.

5.5.7 Total carbon dioxide

There was a significant difference between conditions (p = 0.016). However, upon analysis of the post hoc tests, the results revealed no differences observed between conditions. There was no difference between time points (p = 0.061). The was a significant difference between the interaction of conditions and time points (p = 0.020). Upon further analysis, there no differences in conditions within time points; however, there were significant differences observed with time points within conditions. Pre TN (28.36 ± 1.96 mmol/L) was significantly lower than Pre WBC

(31.93 ± 01.02 mmol/L, p = 0.016) and Pre LC (29.07 ± 01.99 mmol/L, p = 0.019). No other differences were observed.

5.5.8 Saturation of oxygen

A significant difference between conditions was identified (p = 0.001). TN (57.41 ± 23.88 %) was significantly higher than WBC (28.67 ± 18.25 %, p = 0.010). There was no difference between time points (p = 0.077). A significant difference between the interaction of conditions and time points was identified (p = 0.000). TN BL (36.86 ± 9.05 %) was significantly lower than TN Pre (69.14 ± 20.18 %, p = 0.022) and TN Post (66.23 ± 24.17 %, p = 0.039). Time points within conditions also showed significant differences. Pre WBC (22.93 ± 20.67 %) was significantly lower than TN (69.14 ± 20.18 %, p = 0.008) and Pre LC (53.21 ± 23.59%, p = 0.039). Post TN (66.23 ± 24.17 %) was significantly higher than Post WBC (26.21 ± 19.60 %, p = 0.033).

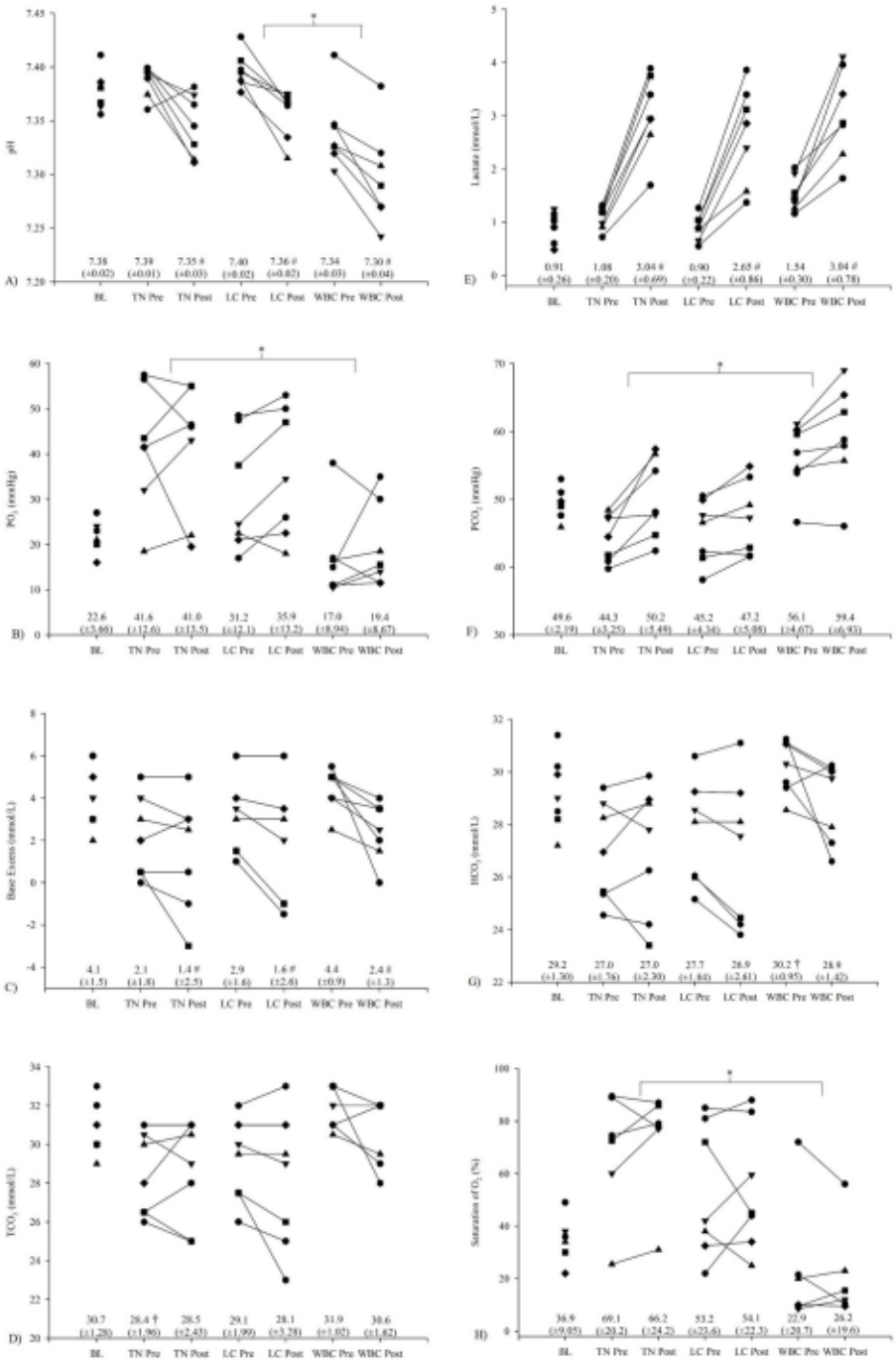

Figure 12. Individual averaged response for pH (A); PO_2 (B); base excess (C); TCO_2 (D); lactate (E); PCO_2 (F); HCO_3 (G); saturation of O_2 (H); with overall mean ± standard deviation. * Significant difference between conditions of LC *vs*. WBC for (A), TN *vs.* WBC for (B), TN *vs.* WBC for (F), and TN *vs*. WBC for (H). # Significant difference in time points between pre- and post-exercise for (A), (C), and (E). † Significant difference in the interaction between condition and time where TN pre-exercise was lower than LC and WBC pre-exercise for (D), and WBC pre-exercise was higher than TN and LC pre-exercise for (G).

6 Discussion

This is the first study to compare whole-body and local muscle cooling on $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ kinetics at the onset of moderate-intensity exercise. Subjects performed two cycling transitions after the anterior portion of the leg was cooled to reduce T_m by 6 °C, assessed intramuscularly from the *vastus lateralis* (VL), and after both core and anterior leg cooling to lower T_m by 6 °C and T_c by 1 °C. We expected core cooling to result in slower phase II $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ kinetics, along with lower $\dot{Q}$ and $\dot{Q}_m$. We also expected that muscle cooling would result in slower $\dot{V}O_{2m}$ kinetics as a result of a lower $\dot{Q}_m$. The key findings in this study were that neither $\dot{V}O_{2p}$ nor $\dot{V}O_{2m}$ kinetics were influenced by whole-body or muscle cooling during transitions from rest to moderate-intensity exercise. This was observed despite an increase in $\dot{V}O_{2p}$, whole-body A-VO_2Diff, local-leg A-VO_2Diff and PCO_2, and a decrease in $\dot{Q}$, HR, $\dot{Q}_m$, pH, PO_2 and sO_2 with core cooling, as well as, a decrease in $\dot{Q}_m$ with muscle cooling only.

The cycling protocol used in this study was similar to that by Ferretti et al. (1995), where 3-minute moderate-intensity cycling transitions were used to prevent any drastic increases in the cooled T_m as Ishii et al. (1992) reported that T_m stays very constant during the first minutes exercise but increases rapidly thereafter. The method used to establish the individualized workload was crucial to standardizing the moderate-intensity work for all participants. Similar to methods used by (Scheuermann, Bell, Paterson, Barstow, & Kowalchuk, 2002), we used a ramp exercise test to determine the VT, then calculated the moderate work rate corresponding to 80% of the VT. The ambient whole-body cooling and local muscle cooling in this study used validated methods (Cotter, Sleivert, Roberts, & Febbraio, 2001; O'Hanlon & Hovarth, 1970; Raven et al., 1970; Wakabayashi et al., 2017; Yanagisawa, Niitsu, Takahashi, Goto, & Itai, 2003). Similar to other studies, we

used ice water (5 °C) perfused cuffs as the method to locally cool the muscle. It was effective in decreasing T_m, and the magnitude of change falls in line with previous research (Bergh & Ekblom, 1979; Blomstrand et al., 1986; Ishii et al., 1992; Shiojiri et al., 1997). However, unlike other studies that have used cold-water immersion methods to reduce T_m and subsequently T_c as well, our protocol reduced only the T_m of the intended muscle (VL) and surrounding agonist muscles. The disadvantage to cold-water immersion protocols is that they can result in a decreased T_m, but also a decrease in the antagonist muscle groups, the hips, knees, and T_c, which may, independently or combined, influence the outcome of the results if only a change in muscle temperature is desired.

6.1 Temperature Responses

During WBC, T_c and $\overline{T}_{sk}$ were cooler compared to TN and LC, indicating that the cooling method was highly effective in reducing the skin and core tissue temperature. Our target decrease in T_c was -1.0 °C; however, by the time participants began exercising the average change in T_c was -0.83 ± 0.31 °C. The VL cooling method also resulted in significant decreases in T_m and T_{leg} during the LC and WBC conditions. Our target decrease in T_m was -6.0 °C; however, by the time participants began exercising the average change in T_m was -5.75 ± 0.87 °C for LC and -6.11 ± 1.21 °C for WBC. Shiojiri et al. (1997) used a lower-body cold-water immersion protocol and observed a decrease in T_m by -6.6 °C and T_c by -1.8 °C. In our study, the change in T_m was comparable, but the change in T_c was not as significant.

A study conducted by Ishii et al. (1992), investigated the effects of reduced leg T_m on pulmonary O_2 kinetics and $\dot{Q}_m$ during cycling at low (75 W) and moderate (125 W) work rates. The participants were immersed in cold water (20 °C) up to their abdomen for 90-120 minutes. This resulted in T_m decreasing by -7.5 °C and T_c decreasing by -1.6 °C. Similar to the results in our study under the temperature range examined, they reported that pulmonary $\dot{V}O_2$ kinetics at the onset of submaximal exercise is not influenced by lower T_m, despite a reduction in $\dot{Q}_m$. The magnitude of cooling can play a significant role in the effect observed within the tissues. A review by Racinais & Oksa (2010) on *Temperature and Neuromuscular Function*, highlighted the impact that lowered muscle temperatures have on exercise performance, specifically on the aspects of neuromuscular function (mechanical, biochemical and neural), where there is a decline at reduced muscle temperatures. Thus, further decreases in T_m, say -9 °C, may further slow cellular metabolism and elicit a more prominent kinetics response.

6.2 Pulmonary and Muscle Oxygen Uptake and Kinetics

Baseline $\dot{V}O_{2p}$ demand was higher in the WBC condition compared to TN and LC. This finding was not unusual as $\dot{V}O_{2p}$ is known to be elevated in the cold due to shivering and non-shivering thermogenesis (Beelen & Sargeant, 1991; Doubt, 1991; Hanna et al., 1975; Ishii et al., 1992; O'Hanlon & Hovarth, 1970). Although shivering was not directly measured, it is known to be intiated shortly after the onset of whole-body cold exposure (Hovarth, 1981), and visually the participants were shivering durign WBC. A recent study compared the reliability and validity of various methods ($\dot{V}O_2$, electromyography, mechanomyography and the bedside shivering assessment scale) to assess cold-induced shivering thermogenesis, suggesting that they all have

good-to-excellent reliability but are better when used ini tandem. This included the use of the bedside shivering assessment scale wich is a visual identification of shivering (Arnold, Hemsley, Hodder, Havenith, & Lloyd, 2020). If the rate of an individual's heat loss is increased, then non-exercising thermogenic mechanisms (shivering) would be initiated, contributing to an increase in VO_{2p}, which can even persist during exercise in some cases (Hoar, Raymond, Langworthy, Johnsonbaugh, & Sode, 1976; McArdle, Magel, Lesmes, & Pechar, 1976). The stimulation of brown adipose tissue can result in the activation of non-shivering thermogenesis, which can increase VO_2 (Cannon & Nedergaard, 2004). However, it is important to note that it has an inverse relationship to shivering in terms of contribution to heat production (Ouellet et al., 2012). As shivering increases, brown adipose tissue heat production decreases. This supports the results observed in our study where VO_{2p} was higher at the onset of exercise but not substantially different at the end.

Shivering might also involve co-activation of muscle agonist and antagonist groups, which would result in an increase in activity from muscles that oppose movement (Pozos, 1981). The cold decreases net mechanical efficiency, and thus the activation of greater muscle mass may be needed to compensate for the reduced rate of oxidative reactions at cold muscle temperatures. This increased co-activation of muscle mass may contribute to a greater VO_{2p} while no change would be observed in local VO_{2m} because it only reflects oxygenation of a subset of fibers (Ishii et al., 1992). A study by Sargeant (1987) looked at reduced leg temperature (from lower body cold-water immersion) and found a decrease in maximal peak force compared to control (non-cooled leg muscles). With reduced contractile force per cross-sectional area in cooled muscles, it has been proposed that more motor units may be required to perform the same work output and thus, raising the overall VO_{2p} demand (Blomstrand et al., 1986; Doubt, 1991;

Ishii et al., 1992). Recently, this has been supported by Mallette, Green, Gabriel, & Cheung (2018), where they used surface electromyography (EMG) to compare the effect that muscle cooling had on contractile properties. They found that the muscle contractility is impaired with muscle cooling, suggesting that there is a compensatory increase in the number of active motor units and firing rates to produce the same amount of force as non-cooled muscles.

At the local muscle level, Gagnon et al. (2017) examined the effect that skin and core cooling prior to exercise had on muscle deoxygenation. They found that with core cooling prior to exercise, there was greater deoxygenation of the VL during the early stages of exercise. They suggested that the mechanisms behind their results may be related to the muscle fiber type distribution. Pringle et al. (2003), demonstrated that fiber type is significantly related to both speed and the amplitude of the VO_{2p} kinetics response at the onset of constant-load sub-maximal exercise (during moderate cycling exercise at 80% of VT). The key findings in their study were the differences in contraction efficiency and oxidative enzyme activity between type I and type II muscle fibers. They suggested this may be responsible for the differences observed, relating their results to the potential switch from aerobic to anaerobic metabolism during exercise in the cold. Reduced temperatures have also been shown to decrease the rate of ATP hydrolysis and calcium reuptake/release, therefore reflecting a decrease in cellular metabolism (Ferretti, 1992). With lowered T_m (-4 °C), Bergh & Ekblom (1979) found a 15% decrease in maximal O_2 consumption, which was compatible with a Q_{10} of approximately 1.4 (normal being approximately 2). They suggested that at subnormal temperatures HR decreases linearly with body temperature. Thus, a lower HR would limit the convective O_2 transport via blood flow to the working muscles.

Furthermore, the initiation of shivering mechanisms results in the recruitment of large muscle groups. Although we did not directly measure shivering, it can be assumed that it

occurred with the onset of whole-body cooling and the subsequent decrease in T_c. Hovarth (1981) explained that shivering is initiated immediately or shortly after the onset of whole-body cold exposure. The quadriceps and surrounding leg muscles contribute to an estimated 32% of the overall shivering response (Bell, Tikuisis, & Jacobs, 1992). In this case, the already activated muscle groups from shivering could have resulted in a priming response. Priming is the result of prior muscle activation that increases blood flow, oxygenation, oxidative enzyme activity and electromyographic activity, which accelerates the overall $\dot{V}O_2$ response of a subsequent exercise bout (Jones, Koppo, & Burnley, 2003). This may explain why no differences were observed for $\dot{V}O_{2m}$ or its kinetics because shivering may have primed the oxidative enzyme pathways which resulted in rapid O_2 uptake kinetics. In thermoneutral conditions, priming exercise has been used in situations where muscle O_2 supply may be limited (during arm exercise, supine leg exercise, sedentary individuals, etc.). Evidence suggests that a prior bout of exercise will increase muscle blood flow and O_2 supply via vasodilation and a rightward shift in the oxyhemoglobin dissociation curve and thus, speeding up O_2 kinetics (Fukuba et al., 2004; A. M. Jones, Berger, Wilkerson, & Roberts, 2006; Koppo & Bouckaert, 2005; Poole & Jones, 2012; Scheuermann et al., 2002). With core and muscle cooling, Shiojiri et al. (1997) observed a slower τ, due to what they suggested was from either a reduced $\dot{Q}$ or reduced A-$\dot{V}O_2$Diff. The results of our study showed a more prominent effect with core and muscle cooling compared to muscle cooling alone, suggesting that lower O_2 transport via a reduced $\dot{Q}$ may not be limiting the kinetics of $\dot{V}O_2$.

Similar to the results observed in our study, Beelen & Sargeant (1991) recorded a higher $\dot{V}O_{2p}$ but no differences in pulmonary O_2 kinetics at the onset of exercise after 45 minutes of lower-body cold-water immersion. They reported higher blood lactate

following cooling at the start of exercise, but also a faster removal of blood lactate during exercise. They suggested that it indicated a higher level of muscle hypoxia due to cold-induced vasoconstriction. This could also result in an elevated $\dot{V}O_{2p}$, due to the energetic costs for removal of extra lactate released into the blood at initial tissue hypoxia. A more recent study by Wakabayashi et al. (2018), found that at the onset of exercise, transient peak muscle deoxygenation was observed during exercise in the cold. This was associated with the delay of pulmonary O_2 kinetics compared to TN. They also observed a higher lactate concentration with reduced T_m, suggesting that there would be an increase in the recruitment of glycolytic metabolism during exercise (greater anaerobic contribution in the cold). This supports lower tissue oxygenation from reduced O_2 delivery caused by cold-induced vasoconstriction. Ferguson, Eves, Roy, Hodges, & Cheung (2018) examined the effects of mild whole-body hypothermia on self-paced exercise performance. Comparing the results of 15 km time trials in thermoneutral, hypothermic, and hypothermic with hyperoxia, they found that the hyperoxia condition restored performance in the hypothermic condition. This was suggested to be due to the maintenance of oxygen availability by maintain levels of arterial saturation and cerebral and muscle oxygenation. Biochemical changes, including reduced ATP utilization/availability and decreased calcium release/uptake in the muscle, due to impaired sarcoplasmic reticulum ATPase, may also hinder muscular performance (Edwards et al., 1972; Halder & Gao, 2014; Oksa, Rintamäki, Mäkinen, Martikkala, & Rusko, 1996).

Changes in $\dot{V}O_{2p}$ are also said to lag behind changes at the cellular level (Seeto, 2012). Wakabayashi et al. (2018) hypothesized that the differences might be exercise

intensity-dependent. While they examined an exercise intensity above the anaerobic threshold, Shiojiri et al. (1997) looked at exercise well below the anaerobic threshold. Our experimental design used 80% of VT as a moderate exercise intensity. Wakabayashi et al. (2018) suggested that the slower muscle O_2 kinetics in the cold could be caused by a limited supply of aerobic energy from lower perfusion of blood to the working muscles. A critical methodological consideration they highlighted was the use of the LT as a work rate. When participants were exercising at the LT in TN conditions, it corresponded to a moderate-intensity workload. However, in the cold conditions, they may have experienced a higher exercise intensity and exceeding the LT. They suggested that it may be appropriate to have participants exercising below the LT (80% of LT) to ensure they were in the moderate-intensity domain for both conditions. This could help resolve whether or not there is an elevated anaerobic contribution in the cold during moderate-intensity exercise. Keir, Murias, Paterson, & Kowalchuk, (2014) proposed that for moderate pulmonary O_2 kinetics, there is a strong relationship to baseline metabolic rate, rather than baseline work rate or the change in work rate. This may explain how intramuscular metabolic status, rather than recruited motor units, influences the O_2 kinetics.

6.3 Cardiovascular Hemodynamics and Muscle Blood Flow

Contrary to the results by Shiojiri et al. (1997) in previous research, our study had no differences in $\dot{Q}$, SV, and HR kinetics. Shiojiri et al. (1997) suggested that $\dot{Q}$ and SV responses during phase I are decreased under cold conditions, possibly due to a reduced rate of adjustment of blood flow, mainly in the working leg muscles. Vasoconstriction from whole-body and muscle cooling may

have resulted in lower-body blood pooling and a reduced effect of the muscle pump to increase venous return and recirculate pooling deoxygenated blood back to the core (Bruse, 1979). However, with studies using cold-water immersion, like the one conducted by Shiojiri et al. (1997) and many others, the hydrostatic pressure from water immersion compresses the peripheral veins, increasing the venous return, and resulting in less blood pooling in the legs (Beelen & Sargeant, 1991). An effect that does not occur with ambient air cooling. This may explain why studies using cold-water immersion as a cooling modality also had cardiovascular differences where ours did not. Although $\dot{Q}$ and HR were reduced during WBC exercise in our study compared to LC, it was not enough to provide a significant difference from TN.

Muscle blood flow decreased with cooling, following what would be expected with the above-mentioned vasoconstriction of superficial tissues. The cold conditions had significantly lower $\dot{Q}_m$ throughout exercise compared to TN. Ishii et al. (1992) observed a minimal change in T_m after 120 sec of exercise, despite sizeable differences in $\dot{Q}_m$ at exercise onset. Our results had a steady increase in T_m beginning almost immediately after exercise onset. Ferretti et al. (1995), reported a lower steady-state $\dot{Q}_m$ to be compatible with a reduction in O_2 supply to the working muscles, and that it may include a delay in the activation of oxidative pathways resulting in an increase of lactate accumulation in the cold.

Although our results showed a significant reduction in $\dot{Q}_m$ in the cold, VO_{2m} remained unchanged at exercise onset. This suggests that if cooling influences muscle O_2 kinetics, it may be irrespective of sizable changes in blood flow (Jones et al., 2012). The oxidative pathways may not have been affected, which is supported by the lack of differences in our pre and post-exercise lactate measurements and therefore, minimal

activation of anaerobic metabolism. Another possible explanation for why we did not see any differences in cardiovascular hemodynamics could be due to an intensity-dependent relationship with the muscle pump. Shiojiri et al. (1997) had participants exercising at a relatively light work rate and thus a minimal pump effect. With the increased intensity used in our study, the rhythmic exercise from cycling may have been powerful enough to overcome the initial limb vasoconstriction and provide adequate blood flow and tissue oxygenation during exercise (Gagnon, 2014).

6.4 Blood Gases and Metabolites

During cold stress, the accumulation of lactate (marked by a decrease in pH with PCO_2 being low or remaining normal), indicates reduced oxygenation or metabolic distress to some tissues (Ballinger, Vollenweider, Templeton, & Pierucci, 1961; Willford, Hill, & Moores, 1986). In our study, venous blood pH decreased with WBC. A pH lower than neutral (7.40), is associated with lactic acidosis and may impair muscle contractile function (Cooke et al. 1988; Lamb & Stephenon, 1994; Seeto, 2012; Xu & Rhodes, 1999). This is caused by anaerobic regeneration of ATP to supply the increased recruitment of type IIb muscle fibers to maintain constant power output in the cold (Seeto, 2012; Xu & Rhodes, 1999). An important consideration highlighted by Marsh & Sleivert (1999), is that muscle blood flow during exercise may aid in the removal of lactate through increased pH gradients. The decrease in PO_2 and sO_2 with the concordant increase in PCO_2 from WBC also reflects a leftward shift in the oxyhemoglobin dissociation curve and subsequently a higher O_2 affinity and lower O_2 availability.

Hayashi, Ishihara, Tanaka, & Yoshida (1999) demonstrated that hyperpnea can imped the unloading of O_2 to the muscle and delay the VO_2 response at exercise onset. Respiratory alkalosis is suggested to delay the diffusion of oxygen in active muscles due to a leftward shift in the oxyhemoglobin dissociation curve. However, with reduced circulatory perfusion to the muscles, but higher perfusion pressure, due to cold-induced vasoconstriction, the diffusion capacity of oxygen into the muscle may have been improved or restored. Perfusion pressure has been shown to play a role in the speed of oxygen kinetics, suggesting that increased pressure may improve the diffusion of oxygen into the muscle (Hughson et al., 1996; Koga et al., 1999). Therefore, despite the decreased PO_2 and sO_2 in the WBC condition, the pressure from the constricted arteries and veins may have facilitated an improved diffusion of oxygen to the working muscles.

Consequently, the increase of lactate in the blood may only last for a short period before it is quickly removed and oxidized (Astrand, Hultman, Juhlin-Dannfelt, & Reynolds, 1986), thus possibly explaining why no differences in lactate between conditions were observed in our study. Another alternative could be due to cold-induced vasoconstriction in the cooled muscles. Beelen & Sargeant (1991) found that there was a greater contribution of anaerobic glycolysis at the beginning of exercise (marked by higher blood lactate values) when exercise was performed with reduced muscle temperatures. This could be explained by cold-induced vasoconstriction which, was sustained at exercise onset despite the metabolic demand. Where we observed no differences after three minutes of exercise, Beelen & Sergeant also had identical blood lactate values for cold and thermoneutral exercise, with differences not arising until after 6 minutes of exercise.

6.5 Limitations and Methodological Considerations

An important limitation to address in this study was the small sample size (n = 8). However, despite a small sample size, there were still core differences between conditions. Methodological considerations include cooling the VL and surrounding muscles on both legs rather than only one side. By only cooling one side, we may have caused an imbalance that resulted in compensation from the opposite non-cooled leg during cycling, and thus may have blunted some of the whole-body kinetics responses. Since we observed no differences in local oxygen kinetics, we can presume that the one-legged model that we used was adequately reflected for whole-body kinetics as well. Another consideration would be to perform the BL NIRS measurement with participants seated upright on the cycle ergometer rather than laying down on the examination bed. With participants laying, the resting baseline values may be lower than if they were to be seated upright. It is important to recognize the potential for venous and arterial occlusions to cause subsequent circulatory system perturbations as well. However, with the study design using short 10 second occlusions times (the shortest duration for this commonly used practice), the impact on the subsequent bout of exercise is likely minimal.

6.6 Future Research

As the results in our study fall in line with that by (Beelen & Sargeant, 1991; Ishii et al., 1992), where no differences in O_2 kinetics are observed despite impaired O_2 transport from reductions in temperature, it may be beneficial to look at processes within the muscle (muscle cell and cell membrane) to examine cellular O_2 perfusion. Other areas

for future investigations should examine the complex role that exercise intensity has on oxidative reactions under systemic and local cooling. Possible avenues for future studies could explore EMG frequency and muscular contraction/recruitment during cooled muscular exercise at different exercise intensities and with measurements of lactate to help distinguish the anaerobic input. Greater recruitment of muscle mass may be observed because it has been reported that there is a shift in EMG to lower frequencies in cooled muscles, and thus, resulting in more muscle fibers, especially fast type, being recruited to maintain the given workload (Wakabayashi et al., 2015). This may fall in line with research by Blomstrand et al., (1986), where cooled muscled have a decreased force-generating capacity and may try to recruit more fast-twitch motor units to compensate for the increasing demand. This may also be reflected by a higher rate of glycolysis in cooled muscle and resulting in increased lactate levels and higher rates of muscle glycogen depletion. In the case that muscle priming due to shivering is also an area of interest, it is important to note that rectus femoris and the surrounding quadriceps muscles have varying fiber type composition between individuals. Therefore, differences in metabolic responses due to the different biochemical characteristics of fiber types is critical. Future studies may benefit from investigating this further by comparing trained and untrained individuals, as well as different sport-specific trained individuals (aerobic vs. anaerobic).

7 Conclusions

In conclusion, despite an array of central (reduced $\dot{Q}$, $\dot{Q}_m$, HR, pH, PO_2, sO_2, and increased $\dot{V}O_{2p}$, whole-body A-VO_2Diff, local-leg A-VO_2Diff, PCO_2) and local changes (decreased $\dot{Q}_m$) induced by core and muscle cooling, limiting oxygen diffusion and perfusion, pulmonary and muscle O_2 kinetics remain unaltered during transitions from rest to moderate-intensity exercise. Our protocol was unique in that we induced local muscle cooling, without cooling cool antagonist muscles or leg joints. Where other studies employed low and moderate-heavy work rates (50W at 60 rpm, LT at 60rpm respectively), this study used an individualized moderate work rate that corresponded to 80% of the participant's VT (average 154W) at 80 rpm. It is possible that the effect cooling has on $\dot{V}O_{2p}$ and $\dot{V}O_{2m}$ and their kinetics becomes challenging to recognize or

disappears altogether at the exercise-intensity and cadence measured. Input from anaerobic metabolism or greater recruitment of muscle mass may help compensate for the impaired delivery and utilization of O_2 caused by core and muscle cooling. Alternatively, with the increase in $\dot{V}O_{2p}$ during WBC and the simultaneous decrease in $\dot{Q}$, HR, and $\dot{Q}_m$, it may be that reduced O_2 perfusion to working limbs does not substantially impact O_2 uptake kinetics under the temperature range examined. There may be a temperature-dependent relationship where higher levels of muscle cooling further impairs enzyme activation and substrate kinetics. Future research should examine different exercise intensities, muscle activation and fiber type, different ranges of muscle cooling, and the effect of different cooling modalities on O_2 uptake kinetics. In terms of real-world applications, cold exposure does not impede your body's ability to successfully carry oxygen from your lungs to your muscles. Furthermore, you can still successfully exercise in the cold despite reduced blood flow, as oxygen delivery is not compromised. This may be beneficial knowledge for occupational-health and safety situations, where the muscles can work effectively, even in the presence of some core and muscle cooling.

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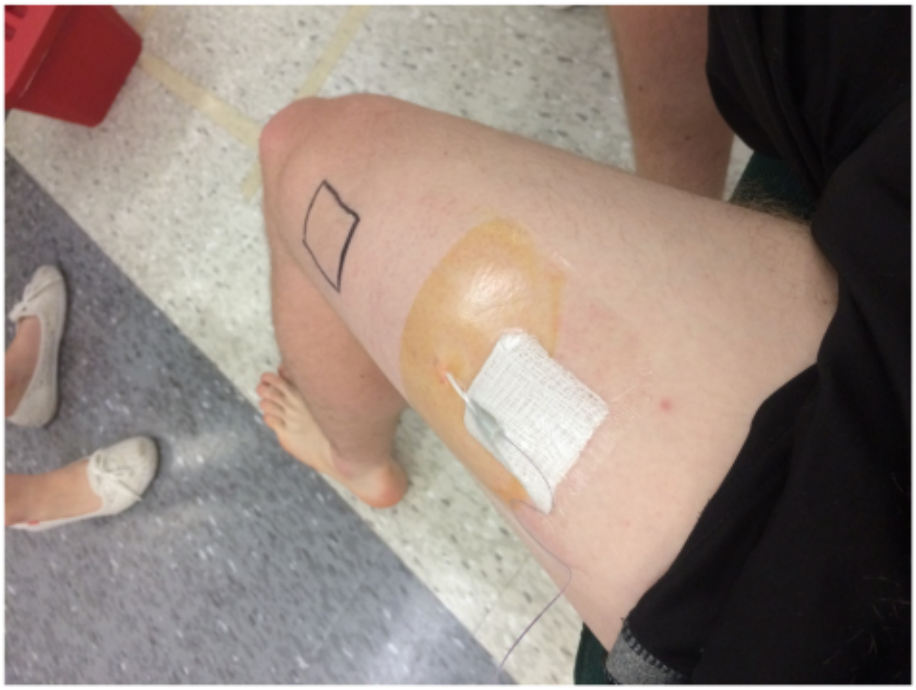
Appendix 1. Anthropometric measurements and descriptive statistics of participant sample.

Variable	*Mean (±SD)*
Age	25.25 (±5.45)
Height (cm)	175.38 (±6.91)
Weight (kg)	76.02 (±7.96)
Leg ATT (cm)	0.53 (±0.15)
Fat Mass (kg)	13.02 (±3.48)
Fat-Free Mass (kg)	63.00 (±5.31)
Body Fat (%)	16.93 (±3.02)
Waist-Hip Ratio	0.80 (±0.12)
Body Mass Index	24.74 (±2.32)
Body Surface Area (m^2)	1.92 (±0.12)
VO_{2max} *($mL \cdot kg^{-1} \cdot min^{-1}$)*	53.36 (±6.06)
$\dot{Q}_{max}$ *(mL/min)*	27.56 (±7.56)
SV_{max} *(mL)*	161.70 (±39.15)
HR_{max} *(bpm)*	191.63 (±5.52)
$\dot{Q}_{SFA}$ *(L/min)*	0.16 (±0.08)
Average Work Rate at 80% (W)	154 (31.43)

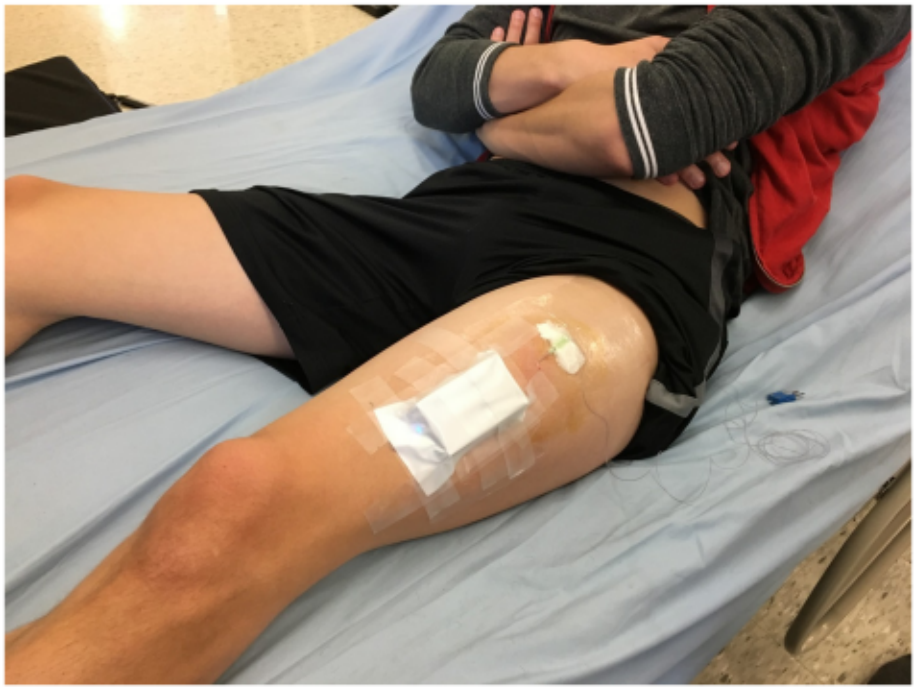
Appendix 2. Instrumentation and setup pictures

Testing set up in the environmental chamber.

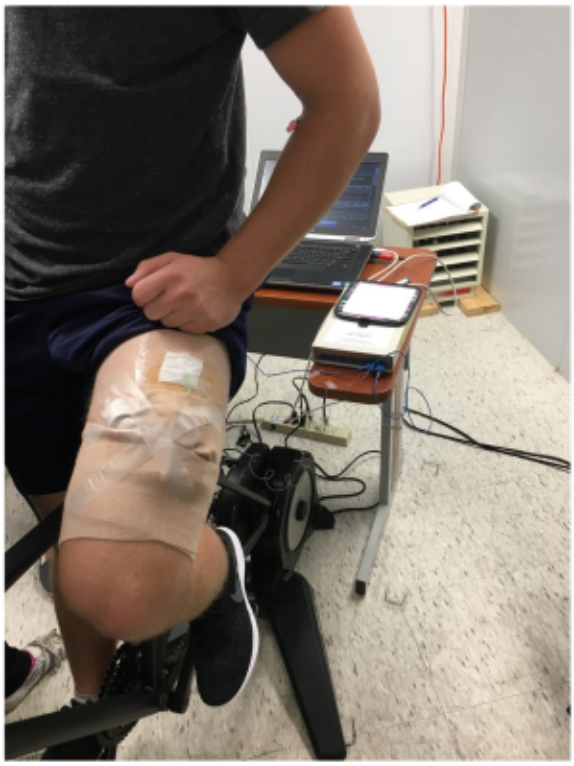
Intramuscular probe insertion view.



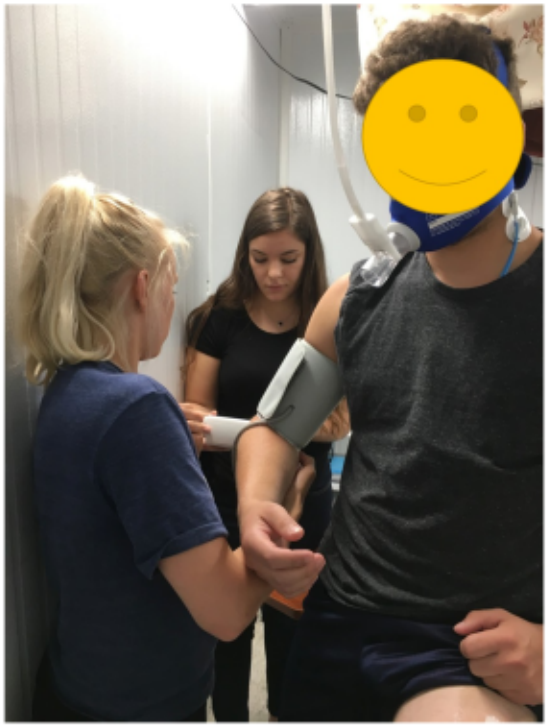
Intramuscular probe and NIRS configuration on the leg.



Participant ready to exercise on cycle trainer.



Pre-exercise blood pressure measurement.



Post-exercise blood pressure measurement.

Exercising subject.

Metabolic cart view of repeated exercise transitions for VO_2 and VCO_2.