EFFECTS OF TRAINING IN THE COLD ON OXYGEN UPTAKE KINETICS

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ABSTRACT

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The kinetics of oxygen uptake (VO_2) describe how fast VO_2 reaches steady state during transition to exercise. VO_2 kinetics is an important determinant of aerobic performance because fast VO_2 kinetics will lead to less O_2 deficit at the onset of exercise. Pre-cooling of working muscles prior to exercise has been shown to slow the VO_2 kinetic response to exercise but is not clear if cold environment without muscle pre-cooling has an effect on VO_2 kinetics. There is currently only one study examining the effects of training in the cold on aerobic performance and mitochondrial biogenesis. Therefore, the aim of this study was to examine the effects of training in the cold on oxygen uptake and cardiovascular hemodynamics kinetics as well as aerobic performance.

37 untrained individuals aged 20-35 (51 % female) took part in a training intervention. 18 participants were randomly assigned to train in a climatic chamber set at 0 °C and 19 participants were randomly assigned to train in room temperature (~ 22°C). 15 participants form cold group and 19 participants from thermoneutral completed the 7-week high-intensity interval training intervention (3 training sessions/week). Before and after the training intervention, kinetics of VO_2, heart rate (HR), stroke volume (SV), and cardiac output (CO) during moderate exercise as well as muscle blood flow and peak oxygen consumption (VO_2peak) were assessed. The effects of training temperature on training adaptations were assessed comparing differences between the groups after training. The effects of the training intervention were studied comparing pre and post measurements and correlations between kinetic variables and changes in these variables were assessed to see what factors affected aerobic performance and adaptations to training.

The results of this study showed that training in the cold did not affect training-induced adaptations of maximal aerobic performance (VO_2peak and maximal cycling power), muscle blood flow or kinetics of VO_2, HR, SV, and CO. However, the training intervention improved maximal aerobic performance and the rate of VO_2 and HR kinetics. HR and CO kinetics correlated with maximal aerobic performance before and after training but VO_2 kinetics did not. Also, the change in VO_2 kinetics did not correlate with the change in maximal aerobic performance. The lack of difference between the groups indicates that training in the cold does not deteriorate training adaptations and there likely were no differences in muscle temperature between the groups during training. VO_2 kinetics were faster after training and the improvement in the rate of VO_2 kinetics is likely attributable to changes in activation of oxidative metabolism.

Key words: cold exposure, thermoregulation, oxygen uptake kinetics, exercise

ABBREVIATIONS

amp	amplitude
AO	arterial occlusion
BL	baseline
C	cold training group
CO	cardiac output
diffHb	difference between oxy – deoxyhemoglobin
Hb	hemoglobin
HHb	deoxygenated hemoglobin
HR	heart rate
ICG	impedance cardiography
LT	lactate threshold
Mb	myoglobin
NIRS	near-infrared spectroscopy
O_2Hb	oxygenated hemoglobin
Q_m	muscle blood flow
SV	stroke volume
τ	time constant
TD	time delay
tHb	total hemoglobin
T_m	muscle temperature
TN	thermoneutral training group
VCO_2	carbon dioxide production
VO	venous occlusion
VO_2	oxygen consumption
VO_{2p}	pulmonary oxygen consumption
VO_{2m}	muscle oxygen consumption
VO_{2max}	maximal oxygen consumption
VO_{2peak}	peak oxygen consumption
Wmax	maximal cycling power

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1 INTRODUCTION

Kinetics is a scientific discipline examining the dynamic profiles of respiratory, cardiovascular, and muscular systems in response to transitions from rest to exercise or increments in workload. Kinetic research aims at discovering the sites and mechanisms of control in human metabolism during exercise. The rate at which oxygen consumption (VO_2) increases to the level required to sustain a certain workload, i.e., VO_2 kinetics, is important for aerobic performance because faster VO_2 kinetics will lead to a smaller O_2 deficit at the onset of exercise (or a workload) and consequently to less metabolic perturbation. (Poole & Jones 2012.) The rate of VO_2 kinetics is dependent mostly on exercise intensity and physical fitness status but some disease states and medications may affect the rate of VO_2 kinetics as well (Hughson 2009).

Cold environmental temperatures cause changes to occur in human thermoregulatory pathways. When exposed to cold temperatures, the production of heat increases and heat loss to environment is decreased. Metabolic heat production is increased via shivering of skeletal muscles and non-shivering thermogenesis in mitochondria. (Castellani & Tipton 2016.) Heat loss to environment is decreased by vasoconstriction of blood vessels in the skin which decreases the amount of heat transfer from the body core to the skin and then to its surroundings (Hall & Guyton 2011, 868).

Exercising in the cold has been shown to affect various physiological systems. However, there is next to no research on long-term effects of training in the cold. This information would be useful to especially winter sports athletes who are frequently training in cold environments and people working in cold conditions. It is yet unknown if cold environment deteriorates the training-induced adaptations of the cardiorespiratory system and therefore, the aim of this study was to investigate the effects of training in the cold on VO_2 and cardiovascular hemodynamics (heart rate, stroke volume, and cardiac output) kinetics as well as aerobic performance.

2 OXYGEN UPTAKE KINETICS

2.1 Oxygen uptake kinetics at the onset of exercise

Oxygen consumption (VO_2) increases exponentially at the onset of exercise. This rise in VO_2 plateaus within about 3 minutes, that is when steady state is achieved. The time required to reach steady state depends on exercise intensity but also on how fast VO_2 responds to the increased workload i.e., VO_2 kinetics. (Burnley & Jones 2007.) The difference between the steady state value of oxygen consumption and measured VO_2 at the onset of exercise is termed O_2 deficit. Any improvement in the rate of VO_2 kinetics will result in smaller O_2 deficit and smaller change in intramuscular H^+ and lactate concentrations as well as smaller depletion of phosphocreatine and glycogen storages at the onset of exercise or workload increment (Poole & Jones 2012).

Pulmonary oxygen uptake response to increased workload can be divided into three different phases (figure 1). Phase I of pulmonary VO_2 has been called the cardiodynamic phase. When measuring oxygen uptake at the pulmonary level, there is a 10-20 s delay before deoxygenated blood from working muscles reaches the lungs for gas exchange. That is why the rapid rise in pulmonary VO_2 in phase I results mainly from increased venous return and right ventricular output leading to increased pulmonary blood flow. Phase II, the primary, fundamental, or fast component, reflects muscle oxygen uptake kinetics and the rise in pulmonary VO_2 responds to the increased oxygen demand in working muscles. Third phase of pulmonary VO_2 reflects the steady state at intensities below the lactate threshold (LT) but at intensities above LT the third phase is called the slow component. At power outputs above LT, VO_2 keeps increasing after the primary component but at a slower rate. During the slow component VO_2 rises steadily above the anticipated steady state VO_2 value. (Burnley & Jones 2007.)

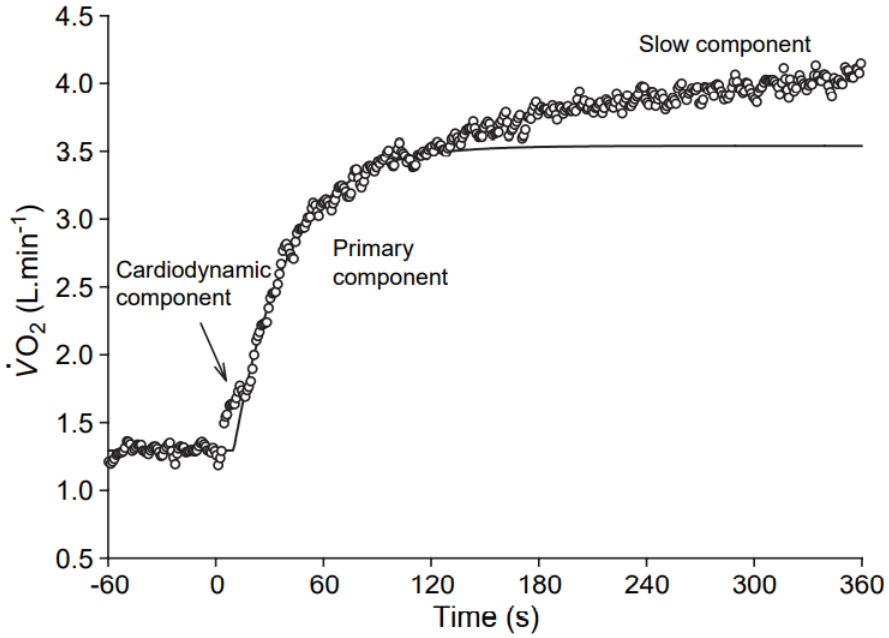

FIGURE 1. Three phases of pulmonary oxygen uptake kinetics at power outputs above lactate threshold. The line represents the expected steady state oxygen uptake (VO_2). (Burnley & Jones 2007.)

There are many factors affecting the kinetics of oxygen uptake. Exercise intensity is one main determinant of VO_2 kinetics. For VO_2 kinetics, exercise intensity can be divided into three domains: moderate, heavy, and severe. Moderate intensity describes exercise intensities below LT where steady state VO_2 can be reached and there is little or no change in blood lactate concentration. Intensities above LT where VO_2 slow component is evident and blood lactate level is elevated but stabilizes over time are classified as heavy. Severe intensity includes exercise intensities where VO_2 slow component and blood lactate levels do not stabilize but continue to increase with time. (Burnley & Jones 2007.) In addition to exercise intensity, other important factors affecting VO_2 kinetics are physical fitness status with higher fitness level leading to more rapid VO_2 response while some disease states and medications are related to slower VO_2 kinetics (Hughson 2009). Cooling of muscle tissue has also been associated with slower VO_2 kinetics (Ferretti et al. 1995; Shiojiri et al. 1997; Wakabayashi et al. 2018) which has led to the question whether environmental temperature affects VO_2 kinetics or not.

2.2 Effects of exercise training on oxygen uptake kinetics

Aerobic training has been found to have a positive effect on the rate of VO_2 kinetics. In cross-sectional studies trained people have been observed to have faster VO_2 kinetics than untrained in young, middle-aged, and older people (Caputo et al. 2003; George et al. 2018; Grey et al. 2015; Siline et al. 2020; Unnithan et al. 2015). Age has been associated with slower VO_2 kinetics, but physical fitness status seems to be a more important determinant of VO_2 kinetics than age and exercise training seems to eliminate the age-related slowing of VO_2 kinetics (George et al. 2018; Grey et al. 2015; Siline et al. 2020). In experimental studies both lower intensity continuous endurance training and high-intensity interval training have been found to increase the rate of pulmonary VO_2 kinetics (Berger et al. 2006; Christensen et al. 2016; McKay et al. 2009; Murias et al. 2011; Schaumberg et al. 2020; Zoladz et al. 2013) and McKay et al. (2016) observed faster VO_2 kinetics even after two training sessions.

The rate of VO_2 kinetics depends on O_2 delivery to the working muscles via pulmonary gas exchange and muscle blood flow and the rate of O_2 utilization in the working muscles. It is still debated what is the rate-limiting step in VO_2 kinetics at the onset of exercise (Hughson 2009), but according to Poole and Jones' (2012) review, O_2 delivery does not limit VO_2 kinetics in healthy subjects. The rate of oxidative metabolism is mainly determined by the activation of enzymes and the amount of oxidative substrates in tricarboxylic acid cycle and electron transport chain (Grassi 2001; McKay et al. 2009). Christensen et al. (2016) witnessed a faster primary VO_2 kinetic response to moderate-intensity exercise after 2 weeks of high intensity training together with increased fatty acid oxidation and electron transport system capacity. However, maximal activity of citrate synthase enzyme or cytochrome c oxidase (markers of mitochondrial density) remained unchanged after the training period. In support of this finding, Zoladz et al. (2013) observed that 5 weeks of moderate-intensity exercise training also accelerated the primary component of VO_2 kinetics during moderate-intensity exercise, but they found no change in muscle fiber capillarization or markers of mitochondrial biogenesis. Thus, faster VO_2 kinetics following a short period of training is likely more attributable to increase in activation of oxidative phosphorylation via enzymes and not mitochondrial biogenesis (Zoladz et al. 2013). Longer training interventions should also induce mitochondrial biogenesis but

following shorter interventions, faster VO_2 kinetics is likely a result of activation of oxidative enzymes.

In Murias' et al. (2011) study, 3 weeks of endurance training on a cycle ergometer (45 min at 70 % VO_2peak, three times a week) resulted in faster VO_2 kinetics in both young and old women and after 9 weeks the decrease in the time to reach steady state VO_2 was even greater. There was no further acceleration of VO_2 kinetics after 12 weeks of training. They found no significant changes in local muscle oxygenation profiles following training, however, the change in local muscle deoxygenation (deoxygenated hemoglobin concentration) normalized to change in VO_2 time constant decreased significantly following training. This suggested that training resulted in better matching of O_2 distribution and O_2 utilization resulting in smaller O_2 extraction and arteriovenous O_2 difference (a-vO_{2diff}) at a given VO_2. Faster VO_2 kinetics with improved microvascular blood flow distribution may imply that O_2 distribution may be a rate-limiting factor in VO_2 kinetics at least for those with slow VO_2 kinetics. (Murias et al. 2011.)

2.3 Measurement of pulmonary and muscle oxygen uptake kinetics

Pulmonary VO_2 (VO_{2p}) kinetics is measured using a breath-by-breath gas analyzer. In open-circuit spirometry, the gas concentrations of inspired air are constant (20.93 % oxygen, 0.03 % carbon dioxide and 79.04 % nitrogen) and by measuring the volume of inspired and expired air and gas concentrations of expired air, it is possible to measure oxygen consumption (VO_2) and carbon dioxide production (VCO_2) (McArdle et al. 2010, 181). Ventilation is measured by a flow sensor or a turbine measuring air flow and volume through a mouthpiece. Measurement of CO_2 concentrations from expired air are typically based on infrared light absorption and measurement of O_2 concentrations are made by paramagnetic or electrochemical analyzers. Mass spectrometry allows for analysis of all gases at the same time and is considered the 'gold standard' in analyzing gas concentrations. (Ward 2018.) When measuring kinetics, breath gases are measured from every respiratory cycle i.e., breath-by-breath. Breath-by-breath measurement allows for analysis of rapid changes in VO_{2p}.

There is a large amount of variation and noise in breath-by-breath VO_2 signal due to variation between respiratory cycles. To improve signal-to-noise ratio, a common practice in measuring VO_2 kinetics is to perform multiple transitions (rest to exercise or workload increases) and averaging the data from two or more repetitions. Measured VO_2 from multiple transitions is then time-aligned (zero presents the onset of exercise) and usually linearly interpolated to 1-s intervals. (Keir et al. 2014.)

VO_2 kinetics are analyzed by fitting the data to a mathematical monoexponential or two or three component function. The function used in fitting the VO_2 response depends on the exercise intensity domain (moderate, heavy, or severe). Variables analyzed from VO_2 kinetic response at the onset of moderate-load exercise are usually time constant (τ), amplitude (amp) and time delay (TD) from the onset of exercise to phase II of VO_2 kinetics (corresponding to the cardiopulmonary component). (Poole & Jones 2012.) The time constant is a parameter derived from the fitted exponential function and it represents the time it takes to reach 63 % of the steady state VO_2 response. In other words, smaller time constant means faster VO_2 kinetics. Amplitude is the difference between baseline and steady-state VO_2. (Burnley & Jones 2007.) When studying VO_2 kinetics from moderate intensity exercise, a monoexponential function can be used after a time delay of about 20 seconds:

$$VO_2(t) = VO_{2baseline} + VO_{2amplitude}(1 - e^{-t-TD/\tau})$$

When studying VO_2 kinetics from severe or heavy intensity exercise domains where phase III slow component is evident, two or three component functions are needed. Amplitude and time constant can also be analyzed for the slow component. (Poole & Jones 2012.)

Pulmonary VO_2 kinetics closely reflect muscle tissue VO_2 kinetics during exercise except for phase I (cardiopulmonary component) of VO_2 kinetics, where the rise muscle VO_2 has a delay compared to pulmonary VO_2 (Poole & Jones 2012). However, the application of near-infrared spectroscopy (NIRS) allows for simultaneous measurement of pulmonary and muscle VO_2 kinetics and it is possible to measure muscle tissue oxygenation and deoxygenation curves in

addition to pulmonary VO_2 kinetics. NIRS is a non-invasive and direct method to measure muscle tissue blood flow and oxygenation (van Beekvelt et al. 2001b).

NIRS technology is based on near-infrared light (NIR) transmitted into a biological tissue. Near-infrared light has a spectrum of 700-2000 nm and it can penetrate biological tissues because of small amount of scattering. NIR spectrum of ~ 700-900 nm is used in NIRS measurements because at wavelengths above 900 nm, light absorption to water in tissues increases. A continuous wave NIRS device has a light source transmitting continuous near-infrared light and a light detector. A multi-distance NIRS device has multiple distances between light sources and detectors (e.g., multiple light sources) that affect the penetration depth of light into the tissue (figure 2). The penetration depth of light into the tissue is approximately half of the distance between the source and the detector. (Hamaoka & McCully 2019.)

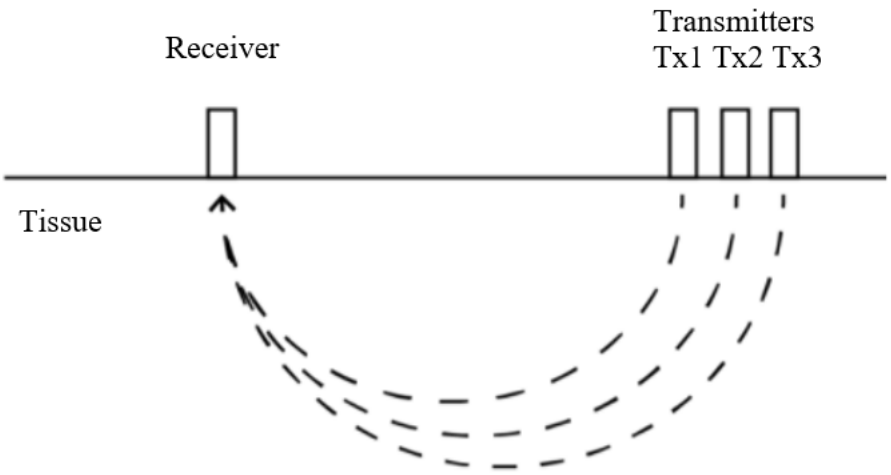

FIGURE 2. A multi-distance near-infrared spectroscopy and penetration depth of near-infrared light. (Picture source: Artinis Medical Systems, https://www.artinis.com/theory-of-nirs)

In biological tissues NIR is mainly absorbed by hemoglobin (Hb) and myoglobin (Mb). The amount of NIR light absorption by Hb and Mb depends on whether or not oxygen is bound to its iron core. Based on differences in light absorption at different NIR wavelengths, NIRS technology is able to measure oxygenated hemo/myoglobin (O_2Hb), deoxygenated

hemo/myoglobin (HHb) and from those total hemo/myoglobin (tHb) can be calculated (O_2Hb + HHb = tHb). (Barstow 2019.) It is not possible to differentiate Hb and Mb from NIRS signal, because Hb and Mb have identical light spectral characteristics (van Beekvelt et al. 2001b). However, for clarity, the terms O_2Hb, HHb and tHb that are widely used in research papers are used throughout this thesis.

To quantitatively measure muscle VO_2 (VO_{2m}) and muscle blood flow via NIRS, blood flow occlusion is applied to control blood flow in muscle tissue by inflating a cuff around the limb. Muscle blood flow (Q_m) can be measured during venous occlusion and muscle VO_2 can be measured during venous or arterial occlusion. Venous occlusion (VO) is performed by rapidly inflating a cuff to 60-80 mmHg, blocking blood flowing out of the limb but not arterial blood flowing in. Arterial occlusion (AO) blocks both venous and arterial blood flow from the occluded limb, creating an ischemic condition. Q_m can be calculated from the increase in tHb during venous occlusion and VO_{2m} can be calculated during venous occlusion from the increase in HHb or during arterial occlusion from the decrease in difference between O_2Hb and HHB (diffHb) (O_2Hb - HHb = diffHb). (Barstow 2019.)

Because NIRS is non-invasive method of measuring muscle tissue oxygenation, there are some limitations to its use. One limitation is adipose tissue thickness between the NIRS device and muscle tissue. Adipose tissue thickness does not affect measurement of muscle tissue oxygenation in populations with low adipose tissue thickness (van Beekvelt et al. 2001b) but needs to be considered when measuring obese patients or patients with muscle atrophy (Grassi & Quaresima 2016). In addition to adipose tissue thickness, changes in skin blood flow, for example due to thermoregulation during exercise, can affect results of NIRS measurements (Grassi & Quaresima 2016).

3 CARDIOVASCULAR HEMODYNAMICS

3.1 Kinetics of heart rate and stroke volume

Cardiac output (CO) is the product of heart rate (HR) and stroke volume (SV). Heart rate is the beating frequency of the heart controlled by the sinoatrial node and the autonomic nervous system and stroke volume is the amount of blood ejected from the heart with each stroke. Typical resting cardiac output of a male weighing 70 kg is 5 L/min (heart rate 70 bpm and stroke volume 0.7 ml/min). Resting cardiac output in endurance trained individuals is similar to untrained individuals but increased blood volume, myocardial contractility and compliance of the left ventricle lead to greater stroke volume at rest. Greater stroke volume together with increased parasympathetic and decreased sympathetic stimulation cause lower resting heart rate in endurance trained individuals. Cardiac output also depends on the arterial pressure and peripheral resistance and can be calculated by following formula:

$$CO = \frac{\text{Mean arterial pressure}}{\text{Total peripheral resistance}}$$

Mean arterial pressure is calculated as follows: diastolic blood pressure + (⅓(systolic – diastolic blood pressure)). Thus, a decrease in peripheral resistance with no change in arterial pressure leads to increased cardiac output. For example, during exercise vasodilation decreases peripheral resistance and increase in systolic blood pressure increases arterial pressure leading to increased cardiac output. (McArdle et al. 2010, 325-343.)

Transition from rest to exercise causes an increase in metabolic demand in working muscles. As stated before, cardiac output increases during exercise in order for the blood flow to deliver oxygen and other nutrients to working muscles. With increased cardiac output, stroke volume increases linearly at first but reaches a maximum at about halfway of cardiac output maximum. Heart rate increases quite linearly with cardiac output and after the plateau in stroke volume, any increase in cardiac output is due to an increase in heart rate. (Hall & Guyton 2011, 1038-1039.) In sedentary males, cardiac output increases about four times above the resting value to

20-22 L/min but in trained endurance athletes cardiac output may reach 35-40 L/min. (McArdle et al. 2010, 343.) Increase in maximal cardiac output following endurance training relies solely on increased stroke volume, because maximal heart rate does not change or slightly decreases in response to endurance training. At submaximal intensities endurance trained individuals have similar or lower cardiac outputs than their sedentary counterparts, with lower heart rate and greater stroke volume. (McArdle et al. 2010, 465-466.)

Unlike VO_2 kinetics, HR, SV, and CO exhibit an almost immediate response to exercise. Increase in cardiac output at the onset of exercise is driven by vagal withdrawal (reduction in parasympathetic stimulation) and at heavy and severe exercise intensity domains increased sympathetic stimulation, which causes the kinetic response of HR and CO to be biphasic at higher intensities. (Poole & Jones 2012.) Time constant of cardiac output kinetics has been found to be considerably shorter than τ of VO_2 kinetics (Davies et al. 1972; Francescato et al. 2013; Lador et al. 2006), meaning blood flow reaches steady state more rapidly compared to oxygen consumption. Considering heart rate and stroke volume kinetics, Grucza et al. (1990) observed τHR to be two-fold greater than τSV (44.4 ± 22.2 s vs. 15.2 ± 2.9 s, respectively) during transitions from rest to cycling at 50 % of maximal oxygen consumption (VO_2max). τSV was similar to τCO (15.2 ± 2.9 s vs. 16.1 ± 4.1 s, respectively). The heart rate response seemed to be biphasic in nature, which they hypothesized might have been a result of delayed vasodilation response. This was also observed in a study by Izem et al. (2019) where HR increased rapidly at the start of cycling (rest to exercise transitions, HR target 125 bpm) but reached steady state value only after 150 seconds. SV on the other hand increased to steady state value after 45 s of exercise, due to increased venous return and improved left ventricle relaxation. Much like VO_2 kinetics, training has been shown to reduce τHR (McKay et al. 2009; Murias et al. 2011; Schaeumberg et al. 2020), end-exercise HR (McKay et al. 2009; Schaumberg et al. 2020) and amplitude and time constant of CO (Schaumberg et al. 2020).

3.2 Measurement of cardiovascular hemodynamics

Measuring HR is relatively easy by measuring electrical currents of the heart with electrodes placed on opposite sides of the heart. This measurement is known as an electrocardiogram.

(Hall & Guyton 2011, 121.) Measurement of cardiac output and/or stroke volume is not as straight-forward and has previously required invasive or laborious measurements. Methods for measuring cardiac output include direct Fick method, indicator dilution method and CO_2 rebreathing method. Direct Fick method is based on the Fick equation:

$$\text{CO (ml/ min)} = \frac{\text{VO}_2 \text{ (ml/min)}}{\text{a-vO}_2 \text{ difference (ml/100 ml blood)}}$$

When oxygen consumption in one minute and difference in O_2 concentration between arterial and venous blood (a-vO_2 difference) is known, it is possible to calculate how much blood circulates during the minute to account for the consumed O_2. Measuring a-vO_2 difference is difficult because it requires blood collection via catheters from an artery and from a vein close to the right atrium for mixed venous sample. Indicator dilution method is based on injecting a dye and measuring its dilution in circulation via blood collection. CO_2 rebreathing method substitutes O_2 values in Fick equation with CO_2 and can be measured without blood collection from breath-by-breath open-circuit spirometry. However, CO_2 rebreathing method can only be used during steady state but not maximal exercise. (McArdle et al. 2010, 341-342.)

In the beginning of the 21st century, impedance cardiography devices started to emerge. In short, impedance cardiography (ICG) measures changes in bioelectrical impedance in the thorax during cardiac ejection to calculate stroke volume. The device emits a high-frequency (75 kHz) and low-amperage (1.8 mA) voltage via two transmitting electrodes and impedance is measured via two sensing electrodes. (Charloux et al. 2000.) Bioelectrical impedance decreases during systole when aortic blood volume and flow velocity increase and conversely, impedance increases during diastole when blood volume and flow decrease (Tonelli et al. 2013). The placement of the electrodes is as follows: two electrodes are placed on the left side of the subject's neck, above the subclavicular fossa and two electrodes on the subject's back, at the height of the xiphoid process. Two additional electrodes are placed in places of V1 and V6 of traditional 12-lead ECG measurement to monitor HR. (Charloux et al. 2000.) The PhysioFlow ICG device has previously been validated against the direct Fick method at rest and during maximal and submaximal exercise. (Charloux et al. 2000; Richard et al. 2001).

4 EXERCISE PHYSIOLOGY IN THE COLD

4.1 Human thermoregulation in the cold

Human core temperature varies between individuals and it may range from under 36 °C to over 37.5 °C. Core temperature is very strictly regulated and remains within ± 0.6 °C even in varying environmental conditions. Temperature of the skin, on the other hand, varies greatly with environmental temperature. (Hall & Guyton 2011, 867.) Core temperature of the body changes due to changes in heat storage. If heat production is greater than dissipated heat, core temperature rises and on the contrary if heat loss to environment is greater than heat production, core temperature will fall. Body's heat storage can be presented as an equation:

$$S = M – (+ \text{Work}) – E \pm R \pm C \pm K$$

where S = heat storage, M = metabolic heat production, E = evaporation, R = radiation, C = convection, K = conduction. (Castellani & Tipton 2016.) Evaporation, radiation, convection, and conduction are all heat exchange pathways of the body. Evaporation means loss of heat from the body through evaporating water from the skin or lungs and radiation dissipates heat from the body through infrared heat rays. Conduction can transfer heat to solid objects or to air (or water) surrounding the body that can then be carried away by convection currents. (Hall & Guyton 2011, 868-869.)

When a person is exposed to cold environmental temperatures, temperature of the skin begins to decrease. To maintain core temperature, the body needs to increase heat production and/or decrease heat loss. In humans, the primary pathway of temperature regulation in cold environment is regulation of blood flow. During cold exposure, the capillaries in peripheral regions of the body and arterio-venous anastomoses constrict, which decreases the rate of skin blood flow in the periphery and consequently decreases the amount of heat dissipation from the body to its surroundings. (Hall & Guyton, 2011, 867-868.) Arterio-venous anastomoses (AVAs) directly connect small arteries to small veins and are most abundant in regions that are most exposed to changing environmental temperatures (hands, feet, ears). Due to dense

innervation by the sympathetic nervous system and thick muscular walls, AVAs can effectively regulate skin blood flow. (Walløe 2015.) Cold exposure also increases the secretion of catecholamines (epinephrine and norepinephrine) as well as cortisol (Wilkerson et al. 1974). Norepinephrine is the primary vasoconstrictive agent in the cold together with neuropeptide Y (Castellani & Tipton 2016).

In addition to cutaneous vasoconstriction, cold exposure increases body's metabolic heat production. Even mild cold exposure (6 °C temperature decrease) has been observed to increase energy expenditure significantly (Dauncey 1981; van Marken Lichtenbelt et al. 2002; Wijers et al. 2008). Metabolic heat production can be increased via increasing voluntary movement or involuntarily by shivering and non-shivering thermogenesis. Shivering is rhythmic contractions of muscles where most of the produced energy is liberated as heat and little mechanical work is performed. Thus, mechanical efficiency is close to zero. The intensity and extent of shivering increase as environmental temperature decreases. Shivering usually begins in the torso and expands to the limbs. Non-shivering thermogenesis produces heat without muscle contraction in brown and beige adipose tissue through uncoupling protein 1 (UCP1). (Castellani & Tipton 2016.) Brown adipose tissue activates in cold environment and metabolic activity of brown adipose tissue is greater during winter (Au-Yong et al. 2009). Wijers et al. (2008) observed that non-shivering thermogenesis was evident also in mitochondria of skeletal muscles during mild cold exposure (22 °C vs 16 °C).

4.2 Exercise responses to cold environmental temperatures

4.2.1 The effects of cold exposure on cardiovascular hemodynamics

Cold exposure causes cutaneous vasoconstriction that results in redirection of blood flow from the periphery to the core. This leads to a rise in systolic and diastolic blood pressure as well as a decrease in heart rate via baroreceptor reflex at rest (Korhonen 2006). During submaximal exercise in the cold, blood pressure has been found to be higher (González-Alonso et al. 2000) and heart rate lower (González-Alonso et al. 2000; Gagnon et al. 2013; McArdle et al. 1976; Sink et al. 1989; Therminarias et al. 1989) compared to thermoneutral environment. In addition

to the baroreceptor reflex, facial cooling stimulates trigeminal nerve in the face leading to bradycardia which may be another mechanism leading to lower heart rate in the cold (Gagnon et al. 2013).

Despite lower heart rate during cold exposure, cardiac output remains unchanged during exercise in the cold due to greater stroke volume (McArdle et al. 1976; Stevens et al. 2015). Greater stroke volume in the cold has also been attributed to greater central blood volume leading to increased central blood pressure (Castellani & Tipton 2016).

In addition to decreased cutaneous blood flow, blood flow to working muscles during exercise has also been found to decrease during cold exposure (Castellani & Tipton 2016). In a study by Ishii et al. (1992), muscle cooling (cold water immersion) decreased the temperature of the vastus lateralis muscle by 7.5 °C and the drop in muscle temperature caused a 37 % decrease in muscle blood flow during cycling at 70 W and a 27 % decrease at 125 W.

4.2.2 The effects of cold exposure on oxygen uptake kinetics

Reduced muscle temperature (T_m) has been shown affect VO_2 kinetics. In a study by Shiojiri et al. (1997), six male participants performed four 2-minute cycling bouts at 50 W in normal temperature conditions and after muscle cooling (cold water immersion for one hour or until T_m decrease of 6 °C). Time constant of VO_2 was greater after muscle cooling (36.0 ± 7.7 vs. 27.5 ± 4.4 s) but there were no differences in time constant of cardiac output between conditions indicating that slower VO_2 kinetics was a result of decreased O_2 extraction and/or oxidative metabolism in working muscles. Wakabayashi et al. (2018) found VO_2 kinetics to be slower during 30-minute cycling at LT after lower-body cold water immersion for 30 min in 12 °C water (mean response time 45.6 ± 7.8 s with reduced T_m vs. 36.1 ± 7.7 s with normal T_m). Muscle cooling was continued during cycling with water-circulating pad. The relative change in tissue oxygen saturation of the vastus lateralis muscle was lower and relative change in HHb concentration was greater with reduced T_m while blood lactate concentration was also higher. According to Wakabayashi et al. (2018) slower VO_2 kinetics and greater glycolytic metabolism might be a result of lower oxygen delivery to pre-cooled muscles.

Ferretti et al. (1995) showed VO_2 kinetics to be slower with reduced T_m also in the severe exercise intensity domain. In their study, six participants cycled for three minutes at the lowest power eliciting VO_2max with and without thigh muscle cooling. The power eliciting VO_2max was lower with reduced T_m, and VO_2 kinetics were slower with greater O_2 deficit and blood lactate accumulation with lower T_m. Muscle blood flow was lower in pre-cooled muscles at the same relative power (power eliciting VO_2max with reduced T_m vs. power eliciting VO_2max with normal T_m) but at the same absolute power (both tests done at the power eliciting VO_2max with muscle cooling) Q_m was similar between conditions. Slower VO_2 kinetics were attributed to lower O_2 delivery and decreased O_2 extraction in working muscles as well as greater glycolytic and alactic metabolism at the workload eliciting VO_2max.

However, there are also conflicting results showing similar VO_2 kinetics with reduced muscle temperature. In a study by Ishii et al. (1992), six untrained male participants cycled at 75 and 125 W for 5 minutes with and without muscle cooling (T_m 28.0 ± 1.6 °C and 35.5 ± 0.9 °C, respectively). Blood lactate accumulation was greater with reduced T_m but there were no significant differences in kinetics of VO_2 or Q_m at neither workload between conditions, although the kinetics tended to be slower and Q_m decreased and O_2 deficit increased with reduced T_m.

The reason for slower VO_2 kinetics with reduced T_m is likely a result of lower oxygen delivery to working muscles because of reduced muscle blood flow (Ferretti et al. 1995; Wakabayashi et al. 2018) but temperature also affects hemoglobin's affinity to oxygen. Lowered temperature in tissues induces a leftward shift in oxyhemoglobin dissociation curve, meaning less oxygen is released to the tissue from hemoglobin when temperature is reduced (McArdle et al. 2010, 278-279).

Based on current knowledge, reduced T_m causes slower VO_2 kinetics but there are no studies examining the effects of whole-body cold exposure on VO_2 kinetics. Therefore, it is not known if exercising in cold environment affects the rate of VO_2 kinetics. Exercising in cold environmental temperature without muscle pre-cooling is not likely to reduce T_m at temperatures close to 0 °C. This was evident in a study by Parkin et al. (1999) where participants

cycled to exhaustion at a power corresponding to 70 % VO_2max in 3 °C and 20 °C. There was no difference in rectal temperature between conditions during exercise or between temperature of the vastus lateralis muscle at fatigue. This is supported by a study by Gagnon et al. (2017) where skin cooling (exercising at 0 °C) did not affect oxygenation of vastus lateralis during running and walking but core cooling (sitting in 0 °C until core cooling was achieved) induced greater muscle deoxygenation during exercise. Because exercising in cold environmental temperatures is not likely to reduce T_m, it is also likely that cold exposure does not affect the rate of VO_2 kinetics. Endo et al. (2003) also showed that stimulation of the face with cold air induced bradycardia but did not subsequently affect the rate of VO_2 kinetics.

4.2.3 The effects of cold exposure on aerobic performance

The economy of human locomotion can be assessed by measuring VO_2 required to sustain a specific workload during exercise. Greater oxygen consumption at a given workload means greater energy expenditure and inferior economy of locomotion. Most studies have associated exercising in the cold (air temperature -10-5 °C or water temperature 18-25 °C) with greater oxygen consumption during exercise compared to thermoneutral environment (air temperature 20-24 °C or water temperature 33-34 °C) (Galloway & Maughan 1997; Holmér & Bergh 1974; McArdle et al. 1976; Stevens et al. 1987; Therminarias et al. 1989; Timmons et al. 1985), although some studies have shown equal oxygen consumption in cold (0-5 °C) and thermoneutral (20 °C) temperatures (Dolny & Lemon 1988; Febbraio et al. 1996; Layden et al. 2002). Increased oxygen consumption during cold exposure might be a result of increased metabolic thermogenesis and shivering in non-working muscles but in addition, Oksa et al. (2002) found that electromyographic (EMG) activity in agonist and antagonist muscles was greater in 5 °C compared to 25 °C. Greater energy expenditure in the cold is likely a combination of increased metabolic thermogenesis and increased antagonist coactivation and energy expenditure in working muscles, leading to inferior mechanical efficiency at the same workload.

Inferior economy of locomotion could lead to inferior aerobic performance in the cold. Very cold environmental temperatures (-20 °C) have been shown to shorten time to exhaustion (TTE)

(Oksa et al. 2004; Patton & Vogel 1984; Quirion et al. 1989) and result in lower maximal oxygen consumption (VO_2max) (Oksa et al. 2004; Quirion et al. 1989) but results of aerobic performance during milder cold exposure (-5-5 °) have not been as unanimous.

Parkin et al. (1999) witnessed significantly longer TTE (42 %) in 4 °C compared to thermoneutral environment when cycling at 70 % of VO_2max, but in Galloway and Maughan's (1997) study there was no difference in TTE with the same protocol. In Galloway and Maughan's (1997) study, TTE was longest in 11 °C. Therminarias et al. (1989) also did not find a difference in workload in an incremental test in -2 °C vs. 24 °C but intensity at LT was 27 % higher in the cold. In a study by Sandsund et al. (2012), endurance athletes did a VO_2max test running in various temperatures (-14°C, -9 °C, -4 °C, 1 °C, 10 °C and 20 °C) while wearing a cross-country skiing suit. There were no differences in VO_2max between temperatures, but TTE was longest in -4 °C and 1 °C and running speed at LT was greatest in -4 °C. Oksa et al. (2004) and Quirion et al. (1989) also did an incremental VO_2max test in 0 and 20 °C and Oksa et al. (2004) observed no differences in VO_2max or duration of the test between temperatures while Quirion et al. (1989) observed a lower VO_2max and shorter test duration in the cold.

Improvements in aerobic performance observed in some studies with no differences in VO_2max can be explained by many factors. Possible explanations for inferior performance in thermoneutral environmental temperatures may be increased energy expenditure from increased pulmonary respiration and circulation, greater loss of fluids during exercise or greater rise in core temperature (heat stress) during exercise (Sandsund et al. 2012). In addition, Febbraio et al. (1996) and Fink et al. (1975) found that muscle glycogen utilization during exercise was lower in the cold compared to thermoneutral or hot environmental temperatures and Fink et al. (1975) also observed greater depletion of intramuscular triglycerides in 9 °C vs. 41 °C. Reduced rate of muscle glycogenolysis and increased use of fat as an energy substrate in the cold may be another reason behind increased submaximal but not maximal endurance observed in some studies.

4.3 The effects of cold exposure on training adaptations

Humans adapt to repeated cold exposure and changes in thermogenesis can be observed even after short cold exposures. In Silami-Garcia and Haymes' (1989) study, participants were exposed to cold environment (10 °C) 10 times in two weeks and the duration of cold exposure was only 60 minutes at a time. After the cold exposure protocol, the start of shivering in 10 °C was delayed from 26.2 to 55.6 minutes and metabolic thermogenesis was lowered from 14.78 kcal/h to -2.64 kcal/h (compared to resting metabolic rate in thermoneutral). These changes were apparent already after five cold exposures. Wakabayashi et al. (2017) also showed that repeated muscle cooling resulted in changes in oxidative metabolism during submaximal isometric exercise. Resting muscle oxygen consumption was not altered but VO_{2m} during isometric contraction was greater in the experimental group after the intervention indicating that repeated muscle cooling may facilitate muscle oxidative metabolism.

Unfortunately, there are very few studies about exercise training in the cold, and it is yet not clear if cold environmental temperature affects training adaptations. A recent study by Shute et al. (2020) examined the effects of training temperature on training adaptations and aerobic performance. 24 participants were divided into cold and control groups and they trained for 3 weeks in 7 °C or 20 °C. The training period included 14 one-hour training sessions cycling on a cycle ergometer at intensity corresponding to RPE (rating of perceived exertion) 15, five days a week. Before and after the training period, muscle biopsies were taken from vastus lateralis and analyzed for protein content and gene expression. Participants also completed a VO_2max test (in thermoneutral environment) and an absolute intensity trial at 50 % VO_2max (in 7 or 20 °C).

Although PGC-1α (a marker of mitochondrial biogenesis) has been observed to increase more in 7 °C after one training session (Slivka et al. 2012), which could lead to more substantial improvements in aerobic performance, the difference was not evident after the 3-week training intervention. There were no differences in training-induced changes in VO_2max or mRNA expression of markers of mitochondrial biogenesis. The acute responses of PGC-1α and VEGF (vascular endothelial growth factor) mRNA expression adapted to cold environmental

temperature following the training period and acute increases in mRNA expression of these markers were not evident after training. There were no differences in heart rate or workload in the absolute intensity trials following the training intervention. Fat-free mass increased after training in the thermoneutral group but not in the cold group. This was explained by previously documented blunting of muscle growth response after strength training with local muscle cooling (Zak et al. 2018).

At the moment, there are no studies about training-induced differences in VO_2 kinetics following training in the cold. Local muscle cooling has been shown to slower VO_2 kinetics (Ferretti et al. 1995; Shiojiri et al. 1997; Wakabayashi et al. 2018), which could result in slower VO_2 kinetics following training. However, muscle temperature is not likely to be reduced during training in cold environmental temperatures compared to thermoneutral, because circulation increases in working muscles resulting in similar muscle and core temperatures during exercise (Parkin et al. 1999). Based on these studies, it is hard to form a hypothesis about training-induced adaptations in VO_2 kinetics following training in the cold. It is likely, however, that training in the cold does not result in slower VO_2 kinetics compared to training in thermoneutral environment since muscle temperature in working muscles is not likely to be altered without a muscle cooling protocol.

5 RESEARCH QUESTIONS AND HYPOTHESES

The purpose of this study was to investigate the effects of training in the cold (0 °C) on kinetics of oxygen uptake and cardiovascular hemodynamics as well as aerobic performance.

1. Does training in the cold affect training-induced differences in pulmonary O_2 kinetics compared to thermoneutral environment?

   Hypothesis: No.
   Although muscle cooling has been found to decrease the rate VO_2 kinetics and increase glycolytic metabolism (Ferretti et al. 1995; Shiojiri et al. 1997; Wakabayashi et al. 2018), it is likely that training in the cold without muscle pre-cooling does not reduce muscle temperature (Parkin et al. 1999) and therefore does not affect VO_2 kinetics during or following training.

2. Does training in the cold affect training-induced differences in aerobic performance and kinetics of cardiovascular hemodynamics compared to thermoneutral environment?

   Hypothesis: No
   Previous research has not observed differences in VO_2max or heart rate after three weeks of training in 7 °C vs. 20 °C (Shute et al. 2020).

3. Is the change in VO_2 kinetics associated with the change in aerobic performance?

   Hypothesis: Yes
   Faster O_2 kinetics should improve aerobic performance by creating smaller O_2 deficit and sparing muscle glycogen and phosphocreatine storages (Poole & Jones 2012). In a study by Berger et al. (2006), the improvement in the rate of VO_2 kinetics was associated with an improvement in peak oxygen consumption (VO_2peak).

6 METHODS

6.1 Participants

37 participants volunteered to participate in this study and were randomly assigned into cold group (C, n=18) and thermoneutral group (TN, n=19). 15 participants from C (8 females and 7 males) and 19 participants from TN (10 females and 9 males) completed the whole training intervention. Three participants from the cold group dropped out during the training period because of personal reasons. All participants were healthy and did not take part in regular moderate to vigorous aerobic exercise. Participants' characteristics are listed in table 1. All participants provided written consent and were screened for any underlying health conditions with Get Active and health screening -questionnaires. The study was approved by Ethics Committee of the Northern Ostrobothnia Hospital District.

TABLE 1. Participants' characteristics. Values are presented as mean ± SD.

	COLD	TN	ALL
n	15	19	34
Age	24.7 ± 3.2	24.4 ± 3.5	24.6 ± 3.3
Height (cm)	177.0 ± 12.1	172.0 ± 10.3	174.2 ± 11.2
Body mass (kg)	80.3 ± 17.9	77.4 ± 12.2	78.7 ± 14.8
BMI (kg/cm^2)	25.4 ± 3.8	26.2 ± 3.4	25.8 ± 3.5
BF %	27.3 ± 10.0	30.3 ± 10.7	28.9 ± 10.3
FM (kg)	22.4 ± 11.9	23.5 ± 9.3	23.0 ± 10.4
FFM (kg)	57.9 ± 12.9	53.9 ± 11.2	55.7 ± 12.0
VO_2peak (ml/kg/min)	42.8 ± 9.4	40.5 ± 9.5	41.3 ± 9.4
Wmax (W)	265 ± 60	246 ± 60	254 ± 60

TN = thermoneutral training group, BMI = body mass index, BF % = body fat percent, FM = fat mass, FFM = fat free mass, VO_2peak = peak oxygen uptake, Wmax = maximal cycling power in the VO_2peak test.

6.2 Experimental protocol

Participants attended the laboratory for pre- and post-training measurements prior to the training intervention and 48-96 hours after the last training session. The participants were asked to abstain from alcohol, nicotine products, caffeine, and vigorous exercise 24 hours before their measurement sessions. Participants recorded their dietary and fluid intake for 24 hours before pre-training measurements and were asked to replicate the same diet for 24 hours prior to post-training measurements. Anthropometrics assessments (height and body mass) were done in the morning in a fasted state in conjunction with other measurements (not included in this thesis) and exercise measurements (VO_2 kinetics and VO_2peak testing protocols) in the afternoon after lunch. Lunch was also recorded and replicated for post-training measurements. Pre- and post-training measurements were done at the same time of day ± 2 hours. Body fat percent was estimated by a bioimpedance analysis scale (Omron HBF-514C, Omron Healthcare Co., Ltd., Kyoto, Japan). Body composition assessment was done on a different day, before the first training session and in connection with the last training session.

Duration of the training intervention was seven weeks and included three high-intensity interval training sessions per week for a total of 21 training sessions. Cold group completed their training sessions in a climate chamber (Arctest Oy, Espoo, Finland) set at 0 °C and 50 % relative humidity and thermoneutral group trained in room temperature (approximately 22 °C). Training sessions were done on a cycle ergometer. Each week participants completed one short, one medium and one long training session and the order of the training sessions was self-selected. Each training session was done on a different day and there was at least one rest day between every training session. Intensity, duration and/or number of repetitions increased every two weeks. Training protocol is presented in table 2. If participants were not able to finish the training sessions with the prescribed intensity, intensity was decreased so that participant was able to complete the session.

TABLE 2. The 7-week training protocol for cold and thermoneutral groups.

	Session	Intensity (% Wmax)	Duration	Rest	Repetitions
Weeks 1-2	Long	80 %	5 min	2.5 min	3
	Medium	90 %	2 min	2 min	4
	Short	100 %	30 s	30 s	6
Weeks 3-4	Long	80 %	6 min	3 min	3
	Medium	90 %	2 min	2 min	6
	Short	105 %	30 s	30 s	8
Weeks 5-6	Long	85 %	6 min	3 min	4
	Medium	95 %	2 min	2 min	8
	Short	120 %	40 s	20 s	10
Week 7	Long	85 %	7 min	3.5 min	4
	Medium	95 %	2 min	2 min	10
	Short	130 %	40 s	20 s	12

Wmax = maximal cycling power cycling power in the VO_2peak test.

6.3 Data Collection

6.3.1 VO_2 kinetics protocol and muscle blood flow

Pulmonary VO_2 and cardiovascular hemodynamics kinetics were assessed during transitions to moderate intensity exercise. Oxygen consumption was measured breath-by-breath using a breath gas analyzer (Oxycon Pro, Jaeger, Wuerzburg, Germany). The breath gas analyzer was calibrated for air flow and O_2 and CO_2 concentrations according to manufacturer's instructions before every measurement. Kinetics of cardiovascular hemodynamics (heart rate, stroke volume and cardiac output) were measured beat-by-beat with an impedance cardiography device (PhysioFlow PF-05 Lab1, Manatec Biomedical, Poissy, France). Six Ag/AgCl electrodes (PhysioFlow HTFS50PF, Manatec Biomedical, Poissy, France) were placed on the participant, two on the left side of the subject's neck, one in the middle of the sternum, one on the rib closest to V6, and two along the center of the spine. Conducting gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories Inc., NJ, USA) was applied to the tip of the electrodes to improve conductance. Prior to placing the electrodes, the skin was shaved and

cleaned with 70 % ethanol solution. The electrodes and base of the cord was secured in place with surgical tape (Transpore™, 3M, MN, USA). Muscle blood flow was measured with a wireless near-infrared spectrometer (Portamon, Artinis Medical Systems, Zetten, the Netherlands). Q_m was measured from the vastus lateralis muscle and the NIRS device was placed on the skin on top of the belly of the muscle, 10 cm above and 5 cm lateral from the patella. The NIRS device was covered with a cloth to block any external light and attached to the skin with surgical tape. The distance between light-emitting and receiving optodes were 30, 35 and 40 mm. The optical wavelengths emitted were 760 and 850 nm and the sampling rate was recorded at 5 Hz. The NIRS device measured O_2Hb, HHb, tHb and diffHb.

VO_2 kinetics protocol is presented in figure 3. After instrumentation, participant was seated on a cycle ergometer (Monark 839E, Monark Exercise AB, Vansbro, Sweden). In the end of a 3-minute baseline measurement, two 15-second venous occlusions with 10-second break in-between, were applied. For venous occlusions, an inflatable cuff (E20, Hokanson Inc., Bellevue, WA, USA) was placed on the participants' thigh proximal to the NIRS device. Venous occlusions were applied with 80 mmHg of pressure. After 1-minute baseline measurement, participant started pedaling with the load set at 0 W (unloaded pedaling). Unloaded pedaling was continued for one minute and then load was increased to 100 W for three minutes. The transitions were done on an electronically braked cycle ergometer and it took about 5-6 seconds to reach 100 W. After three minutes, participant ceased pedaling and two venous occlusions were repeated. VO_2 kinetics protocol was followed by 20-minute resting period and after the resting period the VO_2 kinetics protocol was repeated.

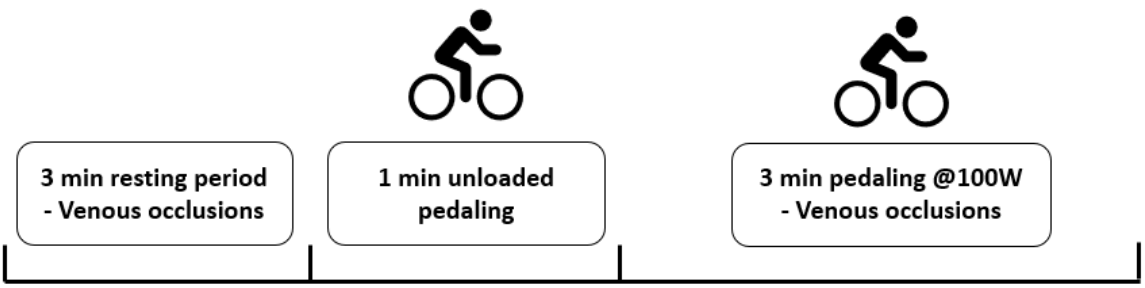

FIGURE 3. VO_2 kinetics measurement protocol.

6.3.2 VO_2peak testing protocol

VO_2peak testing was done immediately following theVO_2 kinetics protocol. Pulmonary gas exchange and cardiovascular hemodynamics were measured as described previously. The starting load was 20 W for females and 30 W for males. Load was increased 20 W every three minutes, until respiratory exchange ratio (RER) 1.0 was achieved. After RER exceeded 1.0, load was increased 30 W every minute until exhaustion. Peak values for oxygen uptake (ml/kg/min and l/min) were calculated as highest 30 s averages. Maximal cycling power (Wmax) was the last load that participant could sustain for at least 45 seconds.

6.4 Data Analysis

6.4.1 Oxygen uptake and cardiovascular hemodynamics kinetics

Baseline values of all kinetics variables (VO_{2p}, SV, HR, and CO) were calculated as 30-second averages from the end of unloaded pedaling. For all kinetic variables, the data from two transitions were linearly interpolated to 1-second intervals, time-aligned and averaged between two transitions. Responses to the increase in workload from 0 W to 100 W were fitted using a mono-exponential function:

$$Y(t) = Y_{BL} + Amp\ (1 - e^{-(t-TD)/\tau})$$

where Y presents the variable at any time (t), Y_{BL} is the baseline value measured before onset of exercise, TD is time delay from the start of exercise to the end of phase I of VO_{2p}, Amp is the amplitude of Y above baseline and τ is the time constant of the response corresponding to the time it takes to reach 63 % of the difference between baseline and steady-state value. For VO_{2p} kinetics the fitting was done from 20 sec into the workload until the end of the three-minute workload. (Bell et al. 2001.) For cardiovascular hemodynamics, the fitting was done from the start of the 100 W workload until the end of the workload (Murias et al. 2011).

In the pre-training measurements 5 of the participants for VO_2peak and 4 of the participants for VO_2 kinetics were recorded with a different breath gas analyzer and their results were excluded from the analyses. For VO_2peak the final number of participants was 30 (11 in cold and 19 in thermoneutral) and for VO_2 kinetics 29 (10 in C and 19 in TN). Some of the participants had to be excluded for cardiovascular hemodynamics analyses because of missing measurements and poor data quality. Final number of participants was 22 for heart rate (C = 11, TN = 11), 16 for stroke volume (C = 7, TN = 9) and 20 for cardiac output (C = 9, TN = 11).

6.4.2 Muscle blood flow

Vastus lateralis muscle blood flow was calculated from the rise in tHb during the venous occlusions. The slope of tHb was calculated and averaged from seconds 5-15 of each occlusion. The following equation was used to calculate Q_m in ml/min/100 ml:

$$Q_m = \left(\frac{\left(\Delta tHb * 60 \Big/ \frac{([Hb] * 1000)}{4} \right) * 1000}{10} \right)$$

where ΔtHb was the average change in one second and [Hb] is the hemoglobin concentration. (van Beekvelt et al. 2001a; van Beekvelt et al. 2001b.) A value of 8.5 mmol/l was used for [Hb] for males and 7.5 mmol/l for females (van Beekvelt et al. 2001a). For some of the participants muscle blood flow could not be calculated because of missing measurements or poor data quality and the final number of measurements analyzed for Q_m was 27 (C = 12, TN = 15). The aim was also to quantitatively measure VO_{2m} via arterial occlusions, but the arterial occlusions were not successful due to too low produced pressures of the occlusion cuff.

6.4.3 Statistical analyses

The results are presented as mean ± SD (standard deviation). The statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA).

Normal distribution was assessed with Shapiro-Wilk test. Independent samples t-test was used for normally distributed variables and Mann-Whitney U test for the variables that failed the test of normality to see if there were any differences between the groups before training. For normally distributed variables a two-way repeated measures ANOVA (time x temperature) was used to study the effects of training temperature. For the variables that were not normally distributed, the means between the groups after training were compared with Mann Whitney U test. The effects of training within the groups and within the whole study population were studied with paired samples t-test for normally distributed variables and with Wilcoxon signed rank test for not normally distributed variables. Correlations between the absolute and relative changes in VO_2peak, Wmax, τVO_2, τHR, τSV, and τCO following training were studied with Spearman's rho and the same test was applied for VO_2peak, Wmax, τVO_2, τHR, τSV, and τCO before and after training. Correlations were also studied between the pre-measurement value of VO_2, HR, SV, and CO time constant and VO_2peak and the absolute and relative changes in these variables. Level of statistical significance was set at $p < 0.05$.

7 RESULTS

7.1 Participants' characteristics

Participants' characteristics before and after the training intervention are listed in table 3. 31 participants completed all 21 training sessions and only 3 participants completed 20 sessions. No participant completed less than 20 sessions. There were no differences between the groups in any variable before training. Training temperature did not have an effect on any of the characteristic variables following training ($p > 0.05$). When looking at all participants, body mass reduced from 78.7 ± 14.8 to 78.1 ± 14.6 kg ($p < 0.05$), fat mass reduced from 23.0 ± 10.4 to 22.6 ± 10.1 kg ($p < 0.01$), VO_2peak increased from 41.4 ± 9.4 to 44.5 ± 8.1 ml/kg/min ($p < 0.001$) and maximal cycling power increased from 254 ± 60 to 289 ± 65 W ($p < 0.001$) following the training intervention. Training intervention reduced fat mass significantly in TN (23.5 ± 9.3 vs. 22.8 ± 8.9 kg, $p < 0.05$) but not in C. When looking at both groups combined, no change in BF % or FFM was observed, although there was a small but significant reduction in body fat percent in the thermoneutral group (30.3 ± 10.7 to 29.6 ± 10.8 %, $p < 0.05$).

TABLE 3. Participants' characteristics before and after training in cold and thermoneutral groups (n = 15 and n = 19, respectively) and both groups combined.

	COLD		TN		ALL	
	Pre	Post	Pre	Post	Pre	Post
Body mass (kg)	80.3 ± 17.9	79.7 ± 17.8	77.4 ± 12.2	76.9 ± 11.7	78.7 ± 14.8	78.1 ± 14.6*
BF %	27.3 ± 10.0	27.4 ± 9.8	30.3 ± 10.7	29.6 ± 10.8*	28.9 ± 10.3	28.3 ± 10.3
FM (kg)	22.4 ± 11.9	22.3 ± 11.7	23.5 ± 9.3	22.8 ± 8.9*	23.0 ± 10.4	22.6 ± 10.1**
FFM (kg)	57.9 ± 12.9	57.4 ± 12.9	53.9 ± 11.2	54.1 ± 11.5	55.7 ± 12.0	55.6 ± 12.1
VO_2peak (L/min)	3.4 ± 0.9	3.6 ± 0.9**	3.1 ± 0.8	3.3 ± 0.7**	3.2 ± 0.8	3.5 ± 0.8***
VO_2peak (ml/kg/min)	42.0 ± 9.5	46.0 ± 7.9**	40.5 ± 9.5	43.3 ± 8.3***	41.4 ± 9.4	44.5 ± 8.1***
Wmax (W)	265 ± 60	299 ± 73***	246 ± 60	282 ± 59***	254 ± 60	289 ± 65***

TN = thermoneutral training group, Pre = before training, Post = after training, BF % = body fat percent, FM = fat mass, FFM = fat free mass, VO_2peak = peak oxygen uptake, Wmax =

maximal cycling power, * = statistically significant difference from before training, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2 Oxygen consumption and cardiovascular hemodynamics kinetics

VO_2, HR, SV, and CO baseline values, amplitudes, time constants and time delays of the primary component before and after training are presented in table 4 and continuous mean responses for VO_2, HR, SV, and CO are presented in figure 4. There were no differences between the groups in any kinetic variable before training and training temperature did not have an effect on the kinetics of VO_2, HR, SV, and CO ($p > 0.05$). When looking at all participants, VO_2 baseline (BL) decreased from 10.0 ± 1.3 to 9.5 ± 1.2 ml/kg/min ($p < 0.01$), amplitude increased from 11.1 ± 2.2 to 11.8 ± 2.4 ml/kg/min ($p < 0.05$), time constant decreased from 28.5 ± 7.6 to 21.0 ± 5.1 s ($p < 0.001$) and time delay increased from 14.9 ± 4.1 to 18.2 ± 2.6 s ($p < 0.001$) following training. Similar effects were observed for HR with baseline decreasing from 110 ± 14 to 103 ± 10 bpm ($p < 0.001$), time constant decreasing from 40.7 ±18.2 to 28.4 ± 10.8 s ($p < 0.001$) and time delay increasing from 3.5 ± 7.8 to 5.6 ± 4.6 s ($p < 0.05$), but there was no change in HR amplitude. There were no statistically significant differences in BL, amp, τ, or TD for SV or CO following training, although τCO decreased from 43.2 ± 15.7 to 29.8 ± 12.1 s in TN ($p < 0.05$).

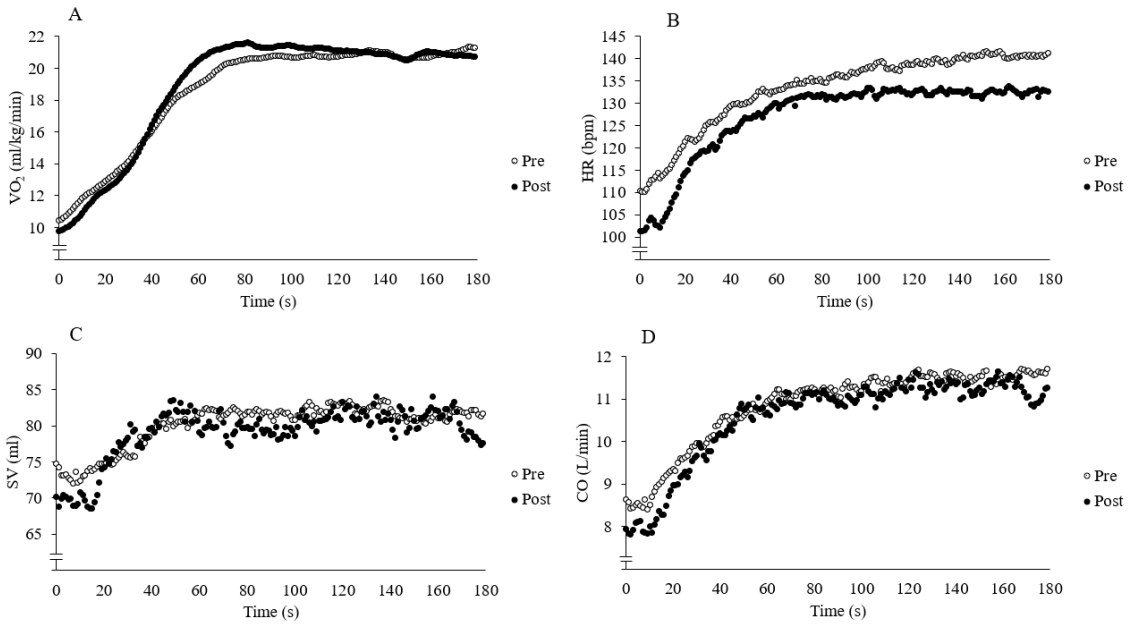

FIGURE 4. Continuous mean VO_2 and cardiovascular hemodynamics response to moderate cycling exercise. A = oxygen consumption (VO_2), B = heart rate (HR), C = stroke volume (SV), D = cardiac output (CO), pre = before training, post = after training

TABLE 4. Oxygen consumption and cardiovascular hemodynamics kinetics before and after training.

	COLD		TN		ALL	
	Pre	Post	Pre	Post	Pre	Post
VO_2 BL (ml/kg/min)	10.2 ± 1.6	10.2 ± 1.6	10.0 ± 1.7	9.2 ± 0.8**	10.0 ± 1.3	9.5 ± 1.2**
VO_2 amp (ml/kg/min)	10.5 ± 2.2	11.4 ± 2.6	11.5 ± 2.1	12.1 ± 2.3*	11.1 ± 2.2	11.8 ± 2.4*
VO_2 τ (s)	29.2 ± 7.0	22.7 ± 6.7*	28.2 ± 8.0	20.0 ± 4.0***	28.5 ± 7.6	21.0 ± 5.1***
VO_2 TD (s)	15.6 ± 4.0	18.3 ± 3.6	14.5 ± 4.2	18.2 ± 1.9**	14.9 ± 4.1	18.2 ± 2.6***
HR BL (bpm)	109 ± 13	103 ± 11*	112 ± 155	102 ± 9**	110 ± 14	103 ± 10***
HR amp (bpm)	27 ± 10	28 ± 8	35 ± 20	34 ± 11	31 ± 16	31 ± 10
HR τ (s)	37.5 ± 14.3	28.2 ± 7.6*	43.8 ± 21.7	28.6 ± 13.7**	40.7 ± 18.2	28.4 ± 10.8***
HR TD (s)	2.9 ± 4.1	6.4 ± 4.6*	4.1 ± 10.4	5.5 ± 4.8	3.5 ± 7.8	5.6 ± 4.6*
SV BL (ml)	75.9 ± 22.8	68.8 ± 13.6	71.5 ± 9.6	73.6 ± 8.6	73.4 ± 16.2	71.5 ± 10.9
SV amp (ml)	10.1 ± 3.2	10.7 ± 6.5	9.9 ± 5.4	11.5 ± 5.2	10 ± 4.4	11.2 ± 5.6
SV τ (s)	35.5 ± 14.1	33.5 ± 23.8	31.5 ± 16.3	27.2 ± 11.7	33.3 ± 15.0	30 ± 17.6
SV TD (s)	10.0 ± 4.0	11.0 ± 11.0	7 ± 11.8	12.1 ± 17.8	8.3 ± 9.1	11.6 ± 14.7
CO BL (L/min)	8.8 ± 2.3	8.1 ± 2.4	8.4 ± 1.6	8.1 ± 1.3	8.6 ± 1.9	8.1 ± 1.8
CO amp (L/min)	3.1 ± 1.0	3.6 ± 1.5	3.2 ± 1.5	3.5 ± 1.0	3.2 ± 1.3	3.6 ± 1.2
CO τ (s)	34.8 ± 12.0	36.6 ± 19.0	43.2 ± 15.7	29.8 ± 12.1*	39.4 ± 14.4	32.8 ± 15.5
CO TD (s)	6.6 ± 3.8	11.6 ± 17.8	7.3 ± 15.9	2.1 ± 10.8	7.0 ± 11.8	6.4 ± 14.8

TN = thermoneutral training group, Pre = before training, Post = after training, VO_2 = oxygen consumption, BL = baseline, amp = amplitude, τ = time constant, TD = time delay, HR = heart rate, SV = stroke volume, CO = cardiac output, * = statistically significant difference from before training, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.3 Muscle blood flow

Muscle blood flow in the vastus lateralis muscle before and after exercise is presented in table 5. There was a significant increase of about 0.3 ml/min/100 ml in muscle blood flow following

exercise but there were no changes in muscle blood flow following the training intervention. There were no differences in muscle blood flow between the groups before training and training temperature did not affect muscle blood flow.

TABLE 5. Muscle blood flow in the vastus lateralis muscle before and after exercise.

	COLD		TN		ALL	
	Pre	Post	Pre	Post	Pre	Post
Q_m (ml/min/100 ml)						
Pre-Exercise	0.8 ± 0.3	0.8 ± 0.4	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.2	0.8 ± 0.3
Post-Exercise	1.1 ± 0.3*	1.1 ± 0.2*	1.2 ± 0.3**	1.1 ± 0.2**	1.2 ± 0.3***	1.1 ± 0.2***

TN = thermoneutral training group, Q_m = muscle blood flow, Pre = before training, Post = after training, * = statistically significant difference from pre-exercise, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.4 Associations between kinetics and aerobic performance

Relative change in VO_2peak (ml/min/kg) correlated with relative change in maximal cycling power ($r = 0.45$, $p < 0.05$). There was no correlation between relative or absolute change in VO_2peak and relative or absolute change in τVO_2. Before training VO_2peak correlated with Wmax ($r = 0.75$, $p < 0.001$) and τHR ($r = -0.53$, $p < 0.05$). After training VO_2peak correlated with Wmax ($r = 0.63$, $p < 0.001$), τHR ($r = -0.67$, $p < 0.01$), and τCO ($r = -0.69$, $p < 0.01$). Maximal cycling power correlated negatively with τHR ($r = -0.70$, $p < 0.001$) and τCO ($r = -0.72$, $p < 0.001$) before training and with τHR after training ($r = -0.5$, $p < 0.05$). τVO_2 correlated with τCO after training ($r = 0.56$, $p < 0.05$). The associations between Wmax and HR and CO time constants before training as well as associations between VO_2peak and HR and CO time constants after training are shown in figure 5.

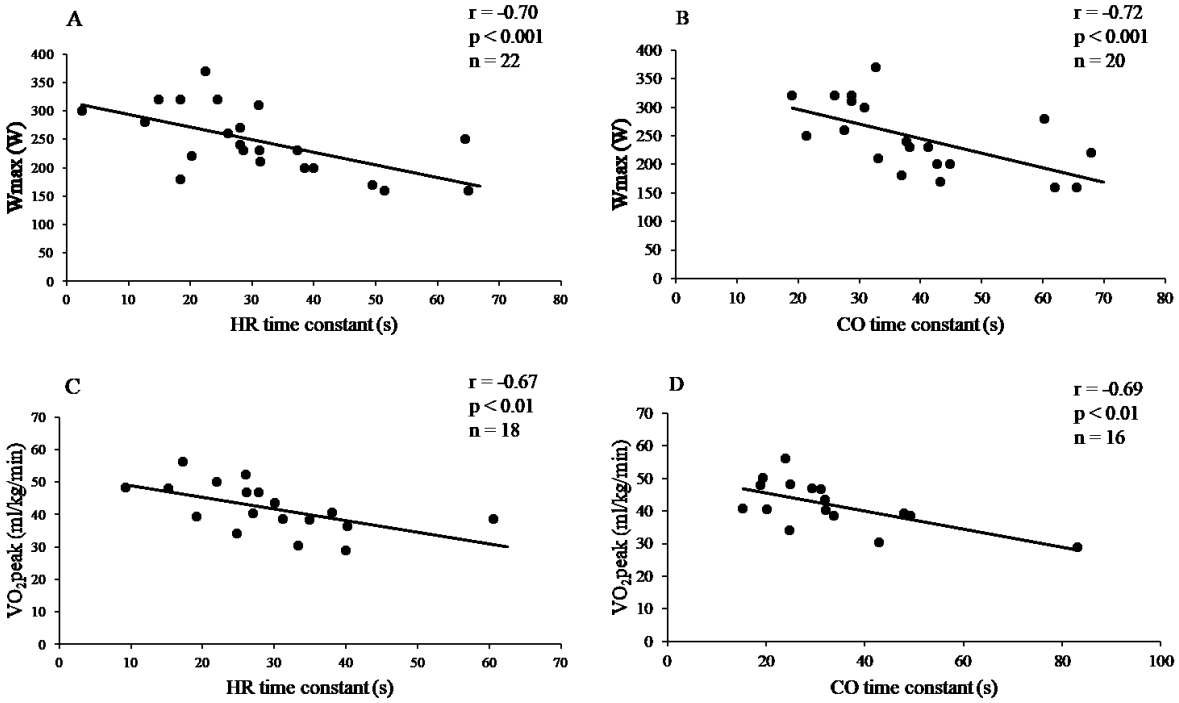

FIGURE 5. Associations between A = maximal cycling power (Wmax) and heart rate (HR) time constant before training, B = Wmax and cardiac output (CO) time constant before training, C = peak oxygen uptake (VO_2peak) and HR time constant after training, and D = VO_2peak and CO time constant after training

For all the variables the relative and absolute changes in time constant correlated negatively with the pre-measurement time constant meaning that greater time constant before training was associated with greater reduction in the time constant. The correlations for changes in absolute and relative values were VO_2: $r = -0.68$, $p < 0.001$ and $r = -0.56$, $p < 0.01$, HR: $r = -0.86$, $p < 0.001$ and $r = -0.69$, $p < 0.001$, SV: $r = -0.67$, $p < 0.01$ and $r = -0.67$, $p < 0.01$, and CO: $r = -0.71$, $p < 0.001$ and $r = -0.64$, $p < 0.01$, respectively. Pre-training VO_2peak (ml/min/kg) correlated with relative ($r = -0.53$, $p < 0.01$) but not absolute change in VO_2peak.

8 DISCUSSION

The main outcome of this study was that training temperature did not have an effect on VO_2 or cardiovascular hemodynamics kinetics or aerobic performance. The training intervention, however, did improve VO_2 and HR kinetics (smaller time constant) as well as aerobic performance (VO_2peak and Wmax). The change in aerobic performance after the training intervention did not correlate with changes in VO_2, HR, SV or CO kinetics, but HR time constant correlated with VO_2peak and Wmax before and after training.

8.1 Effects of training temperature

As was hypothesized, training temperature did not affect the training-induced changes in VO_2 kinetics. Previous studies have measured VO_2 kinetics after muscle cooling, but no studies have yet compared the effects of training in the cold vs. training in thermoneutral. Previous studies with muscle cooling have found that reduced T_m has resulted in slower VO_2 kinetics (Ferretti et al. 1995; Shiojiri et al. 1997; Wakabayashi et al. 2018). This has been thought to be a result of lower oxygen delivery to working muscles due to reduced muscle blood flow and reduced release of O_2 to tissues from Hb because of a leftward shift in oxyhemoglobin dissociation curve in colder temperatures. Training temperature did not affect muscle blood flow following training in this study.

It is likely that in this study the exercise protocol in C did not result in lowered T_m because no muscle pre-cooling was involved. Before starting a training session, the subjects only did a brief (5 minutes at 50 W) warm-up in the climate chamber and started their training session almost immediately after that. Exercise in the cold without muscle pre-cooling is not likely to reduce muscle or rectal temperature (Parkin et al. 1999). All the training sessions did involve resting periods between the repetitions, but these resting periods were only brief (from 20 s to 3.5 min) and most likely not long enough to allow T_m to decrease. There are currently no studies examining VO_2 kinetics in the cold without muscle pre-cooling, so it is not possible to comprehensively know what the effect of cold environmental temperatures on VO_2 kinetics is.

Still, it is plausible that cold environment alone would not affect VO_2 kinetics at least in working muscles because muscle blood flow and O_2 release in muscle tissue would not be compromised.

A number of studies have found that cold exposure increases oxygen consumption during exercise (Galloway & Maughan 1997; Holmér & Bergh 1974; McArdle et al. 1976; Stevens et al. 1987; Therminarias et al. 1989; Timmons et al. 1985) leading to inferior economy of locomotion. In the present study, oxygen consumption was not measured during the training sessions, but in the VO_2 kinetics protocol, neither baseline VO_2 nor amplitude of VO_2 response differed between the groups after training. This means that the training temperature did not have any long-term effects on exercise oxygen consumption at least at moderate-intensity exercise, but it is possible that oxygen consumption may still have differed between temperatures during training. It has also been shown that exercise metabolism is different in the cold with more emphasis on fat metabolism and less reliance on carbohydrates as an energy substrate (Febbraio et al. 1996; Fink et al. 1975). From only oxygen consumption, it is not possible to determine what energy substrate is being used for oxidative metabolism and substrate metabolism may have differed between the groups following training, but measurement of substrate oxidation is beyond the scope of this thesis.

It was also observed that training temperature did not affect cardiovascular hemodynamics (HR, SV, and CO) kinetics. It is well documented, that heart rate is lower during exercise in the cold (González-Alonso et al. 2000; Gagnon et al. 2013; McArdle et al. 1976; Sink et al. 1989; Therminarias et al. 1989) and the most plausible explanation for this is the baroreceptor reflex caused by elevated blood pressure from vasoconstriction in the periphery (Korhonen 2006). To maintain cardiac output at lower heart rate, stroke volume increases in the cold (McArdle et al. 1976; Stevens et al. 2015). This was also observed in a kinetics study by Shiojiri et al. (1997) where HR was lower at rest and at the end of exercise with pre-cooled muscles but there were no differences in CO between normal temperature and reduced T_m at rest or at the end of exercise nor time constants of HR and CO. Hence, it is likely that HR and SV may have differed between the groups during training, but this did not have any long-term effects or effect on HR, SV, or CO kinetics. Interestingly, τCO decreased significantly in the thermoneutral group but not in the cold group or when looking at both groups combined but there is no clear explanation for this since there were no differences between the groups in SV or HR time constants.

There were also no differences between the groups in VO_2peak and maximal cycling power after training. These results were in line with a training study conducted by Shute et al. (2020), where 3 weeks of training in 7 °C vs. 20 °C did not result in any differences in VO_2max. Although markers of mitochondrial biogenesis have been observed to be elevated after one training session in cold environment (Slivka et al. 2012), this response was adapted during the 3-week training period and the markers of mitochondrial biogenesis showed no differences between temperatures after training, explaining the lack of difference in VO_2max. Although aerobic performance may be enhanced in cold environment (Parkin et al. 1999; Sandsund et al. 2012; Therminarias et al. 1989), it did not translate to improved aerobic performance compared to thermoneutral training group after training in this study.

Another explanation for the lack of differences in aerobic performance might be the fact that the training protocol consisted of nearly maximal high-intensity interval training. Maximal aerobic performance (VO_2max) has not been found to be higher in the cold (Oksa et al. 2004; Quirion et al. 1989; Sandsund et al. 2012; Therminarias et al. 1989) but performance at lower intensities (TTE and workload at LT) has been improved in cold environments in previous studies (Parkin et al. 1999; Sandsund et al. 2012; Therminarias et al. 1989). Longer training sessions and lower intensities could have improved exercise tolerance in the cold during training due to less stress on thermoregulation and less dehydration (Sandsund et al. 2012) as well as greater fat oxidation (Febbraio et al. 1996; Fink et al. 1975). Better exercise tolerance at lower intensities in the cold could have translated to greater improvements in performance after training.

An additional observation in this study was that body fat percent and fat mass decreased in the thermoneutral group but not in the cold group. In the study by Shute et al. (2020), fat free mass increased in thermoneutral training group but not in cold group and this was hypothesized to be a result of blunted muscle growth response in the cold. This was not the case in the present study, where fat free mass did not change in either of the groups following training. This is an interesting finding because cold exposure should result in increased energy expenditure due to greater metabolic heat production (shivering and non-shivering thermogenesis) and oxygen consumption has been found to be higher during exercise in the cold (Galloway & Maughan 1997; Holmér & Bergh 1974; McArdle et al. 1976; Stevens et al. 1987; Therminarias et al.

1989; Timmons et al. 1985). Frequent cold exposures may have led to more preservation of fat tissue for insulation from cold environmental temperatures.

8.2 Effects of training intervention

When looking at both groups combined, a 26 % reduction in τVO_2. Training also significantly improved maximal aerobic performance, seen as a 7 % improvement in VO_2peak (ml/kg/min) and 12 % improvement in maximal cycling power. Interval training has been previously shown to improve the rate of VO_2 kinetics (Berger et al. 2006; Christensen et al. 2016; McKay et al. 2009; Schaumberg et al. 2020). VO_2 kinetics is an important determinant of aerobic performance because faster VO_2 kinetics (smaller τ) means faster attainment of steady state and lower O_2 deficit and consequently smaller changes in H^+ and lactate concentrations and less depletion of phosphocreatine and glycogen storages (Poole & Jones 2012). Faster VO_2 kinetics can be a result of better O_2 delivery to working muscles and/or improved rate of O_2 utilization in mitochondria. It is usually thought that O_2 delivery is not the rate-limiting factor in VO_2 kinetics in healthy individuals (Grassi 2001; Poole & Jones 2021), but according to Murias' et al. (2011) study, O_2 delivery may limit VO_2 kinetics in those with slow VO_2 kinetics. However, training did not affect muscle blood flow in this study, so it is likely that improved rate of VO_2 kinetics was a result of better O_2 utilization in working muscles. After shorter training periods the speeding of VO_2 kinetics is likely more attributable to better activation of oxidative metabolism via enzymes (Christensen et al. 2016; Zoladz et al. 2013) but following long-term endurance training mitochondrial biogenesis and muscle fiber capillarization is expected (Zoladz et al. 2013).

In conjunction with a reduction of τVO_2, baseline value of VO_2 also declined and interestingly, the amplitude of VO_2 response increased. Previous studies have observed a reduction (McKay et al. 2009; Zoladz et al. 2013) or no change (Berger et al. 2006; Christensen et al. 2016; Schaumberg et al. 2020) in BL VO_2. For VO_2 response amplitude, a reduction (Schaumberg et al. 2020; Zoladz et al. 2013) or no difference (Berger et al. 2006; Christensen et al. 2016; McKay et al. 2009; Murias et al. 2011) has been observed following training at the same absolute intensity. Reduction in baseline or amplitude of VO_2 response would mean better

economy with lower VO_2 at the same absolute intensity. In this study however, despite lower BL VO_2, end-exercise VO2 was similar pre- and post-training which can be seen looking at the kinetic response curves (figure 4). With no difference between end-exercise VO_2, there is no difference in cycling economy. Cycling does not require weight-bearing and is not heavily dependent on technique which is why economy of movement during cycling is affected mostly by muscle fiber-type distribution i.e., individuals with larger percentage of type I fibers have better economy (McArdle et al. 2010, 208). In regard of this it makes sense that VO_2 was similar before and after training at the same absolute intensity because short interventions do not likely change the fiber type composition in muscles. Smaller baseline VO_2 may be a result of familiarization with the measurement situation and cycling with no resistance.

In addition to faster VO_2 kinetics, a 30 % reduction in τHR was observed. Previous studies have also showed speeding of HR kinetics following training (McKay et al. 2009; Murias et al. 2011; Schaumberg et al. 2020). τHR has been used as a proxy for central O_2 delivery to tissues and decreased τHR suggests adaptation of blood flow distribution and microvascular blood flow indicating enhanced endothelial response to exercise. (McKay et al. 2009; Murias et al. 2011.)

HR baseline was found to be reduced but there was no difference in amplitude after training. McKay et al. (2009) found no change in HR baseline or amplitude values, but end-exercise HR reduced significantly after training and Schaumberg et al. (2020) found a reduction in BL HR but no change in amplitude, leading to a reduced exercise HR. It is apparent from figure 4 that exercise HR was lower post-training for the whole duration of the kinetics protocol. Before training HR seemed to have a biphasic rise but after training HR stabilized after the primary component. This may indicate that the load used in the kinetics protocol (100 W) might have been too high for the HR to reach a steady state and stabilize but after training participants' improved fitness status allowed for the HR to stabilize at steady state level. It is well known that endurance training lowers HR at submaximal intensities because stroke volume increases due to better pumping capacity of the heart (increased left ventricle volume, reduced stiffness in cardiac muscle and arteries, increased diastolic filling time and improved cardiac contractility) (McArdle et al. 2010, 464).

Despite faster HR kinetics and lower exercise HR, no statistically different changes in any variables of SV and CO function were observed. This was probably due to large inter-individual variation in these variables and a small number of participants in the final analyses. The increase in SV in response to exercise was notably smaller than the increase in HR and for some participants the increase was so small that it was not possible to calculate the kinetics from transition to exercise. For these individuals, SV may have increased already during the unloaded pedaling and the increase in workload was accounted for only with an increase in HR. Also, there were some problems with signal quality especially those with more fat mass. Based on the kinetic curves in figure 4, there could be some exercise-induced changes in kinetics of SV and CO, but these differences were not statistically different. Training should have decreased τCO similarly to τHR and τVO_2 (Schaumberg et al. 2020), especially, when in this study τHR decreased as well.

Interesting observation in this study was that TD of VO_2 and HR kinetics increased after training. Christensen et al. (2016) also observed a longer TD in response to training but in most studies, there has been no change in TD (Berger et al. 2006; Christensen et al. 2016; McKay et al. 2009; Schaumberg et al. 2020). Time delay accounts for the phase I of VO_2 kinetics and is required to improve model fit in the calculations of VO_2 kinetics (Poole & Jones 2012). Based on these measurements, it is impossible to know if phase I of VO_2 kinetics was in fact longer or if this is just a result of model fitting to different types of curves. The kinetic curve post-training for all variables was steeper so the TD was more evident which might have resulted in the calculated TD to be longer.

8.3 Factors explaining aerobic performance

Our hypothesis was that τVO_2 would be an important determinant of aerobic performance and the change in τVO_2 would correlate with the change in aerobic performance following training. However, this was not the case as the change in τVO_2 did not correlate with the change in VO_2peak or Wmax and τVO_2 did not correlate with VO_2peak or Wmax before or after training. τHR and τCO seemed to be better explain aerobic performance since τHR correlated with

VO_2peak and Wmax before and after training and τCO correlated with VO_2peak after training and with Wmax before training.

These results contradict previous research from Berger et al. (2006), where the reduction in τVO_2 correlated with the improvement in VO_2peak during moderate intensity exercise. However, only maximal aerobic performance was measured and τVO_2 in moderate exercise could be a more important determinant of aerobic performance when measuring for example time to exhaustion. VO_2max in healthy individuals is mostly limited by the cardiovascular capacity for O_2 delivery, whereas VO_2 kinetics is more limited by O_2 utilization in working muscles (Poole & Jones 2012). This is supported by the fact that in the study by Christensen et al. (2016), a small τVO_2 was associated with greater fatty acid oxidation and electron transport system capacity. Also, in the present study τHR and τCO correlated with maximal aerobic performance before and after training. Given these facts, faster VO_2 kinetics and reduced O_2 deficit could translate to improved aerobic performance at submaximal, but not maximal intensities. In a study by Unnithan et al. (2015) τVO_2 was smaller for a group of trained compared to untrained adolescents but τVO_2 did not correlate with VO_2peak within the trained and untrained groups. VO_2 kinetics is a good determinant of aerobic performance between individuals with different training status but does not differentiate maximal aerobic performance within a group of similarly conditioned persons.

In the present study, slow kinetics for VO_2, HR, SV, and CO were associated with greater relative and absolute reduction in time constant which was evident by a negative correlation between pre-training τ and change in τ following training. Pre-training VO_2peak also correlated negatively with the relative change in VO_2peak. In other words, participants with inferior fitness improved their kinetics and maximal aerobic performance more. The same observation was found in a study by Berger et al. (2006) for τVO_2. Those with slow VO_2 and cardiovascular hemodynamics kinetics are more likely to improve the rate of kinetics more with training but those with already smaller time constants require more training volume and/or intensity to improve exercise on-kinetics.

8.4 Limitations and future research

One major limitation of this study was that measurements were only done before and after the training intervention. Measurement of ventilation and gas concentrations of inspired and expired air (especially VO_2), cardiovascular hemodynamics, rectal, skin and muscle temperatures, and for example rating of perceived exertion during training sessions would have given valuable information on the physiological differences occurring between training temperatures. Now it can only be speculated if there were differences for example in exercise VO_2, HR, SV, and T_m. This information could have been used to explain the results (and lack of differences) obtained in this study. In the present study the possible mechanisms behind improved aerobic performance and VO_2 kinetics were not measured, e.g., activation of oxidative metabolism and mitochondrial biogenesis.

In addition, unfortunately, good enough data quality on NIRS HHb kinetics measurements was not obtained to study kinetics of VO_{2m} so it can only be speculated if the improved rate of VO_{2p} kinetics meant a decrease in τVO_{2m} as well. The quality of NIRS data was especially low for those with less fat free mass and more fat mass so it is plausible that the amount of subcutaneous fat impaired the quality of NIRS measurements. Subcutaneous fat tissue thickness greater than 20 mm has been identified to interfere with NIRS signal (Grassi & Quaresima 2016).

Drop-out rate in the present study was only 8 % meaning that most of the participants completed the whole study from start to finish. However, due to problems with equipment and signal quality, number of participants in the final analyses was lower than 34 for most variables. This was especially apparent with stroke volume and cardiac output. Larger number of participants in the final analyses and equal sized groups would have added to the statistical power of the study. Especially for SV and CO increased number of good-quality measurements could have brought out statistical differences when comparing kinetic variables before and after training, which now failed to reach statistical significance.

As stated before, current research on the effects of training in the cold is very limited. Acute responses to exercise in cold environments are relatively well-known, but very little is known

about the long-term effects. Future research should focus on describing the effects of training in the cold on various physiological systems but also on measuring reasons for those results. These could include, for example, muscle biopsies to measure protein and gene expressions, mitochondrial content, and cell respiration and blood samples to measure concentrations of hormones and different metabolites. Focus should be also on longer training interventions and if their effects differ from shorter interventions.

8.5 Conclusions

Understanding the effects of training (or working) in the cold on cardiorespiratory fitness is important for example for winter sports athletes and people working in cold environments. This study showed that training temperature does not affect training-induced adaptations of the cardiorespiratory system. It is likely that there are some differences in acute responses to cold vs. thermoneutral environmental temperatures, but these responses are likely to be attenuated over time due to temperature acclimatization. In other words, training in the cold does not deteriorate the improvement in aerobic fitness or kinetics of oxygen consumption and cardiac output. In addition, 7 weeks of high-intensity interval training improved cardiorespiratory fitness and kinetics of VO_2 and cardiovascular hemodynamics in previously untrained individuals. VO_2 kinetics might not be the most important determinator of maximal aerobic performance but may be a more important factor of physical fitness in non-athletic populations, where maximal performance is less important than submaximal exercise capacity.

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