The role of heat as a conditioning stimulus in endurance athletes

by

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ABSTRACT

Objective: The purpose of this study was to investigate the effects of regular post-exercise hot water immersion (HWI) on selected physiological adaptations and on exercise performance in a temperate environment in trained road cyclists. **Methods:** Fourteen male cyclists were assigned to either an HWI ($n = 7$) group or a control (CON, $n = 7$) group. Both groups completed 9 high-intensity interval training sessions (over 3 weeks), with each training session followed by sitting in a water tub for 30 min. Participants from the HWI group were immersed in 42°C water, whereas a thermoneutral water temperature of 34°C was used for the CON group. Core and intramuscular temperature were continuously recorded during the first water immersion session and for 30 min post-session. Before and after the intervention, the cyclists performed a 20-km time trial test and an incremental test to exhaustion to determine lactate turn point, maximal oxygen consumption and peak power output. Venous blood at rest was sampled pre- and post-intervention to assess changes in plasma volume. Muscles biopsies were obtained from the *vastus lateralis* pre- and post-intervention to assess changes in mitochondrial function. Variables were analysed using *t*-test and two-way repeated measures analysis of variance. **Results:** Intramuscular temperature was significantly higher in the HWI than in the CON group at the end of the water immersion treatment (37.8 ± 0.4 vs 36.2 ± 0.5 °C, $p=0.001$) and 30 min post-immersion (36.7 ± 0.4 vs 35.1 ± 1.2 °C, $p=0.01$). In addition, HWI group had significantly higher core temperature immediately post-immersion than the CON group (37.8 ± 0.4 vs 37.1 ± 0.2 °C, $p=0.01$). However, all other measures were not significantly different between the groups. Nevertheless, there was a significant improvement in 20-km time trial performance in both the HWI (2009.8 ± 147.3 vs 1977.5 ± 134.5 seconds, $p=0.01$) and the CON group (2010.4 ± 182.3 vs 1974.2 ± 185.7 seconds, $p=0.04$). **Conclusion:** Three weeks of high intensity interval training led to an improved 20-km time trial performance, but the post-exercise HWI protocol used in this study did not provide additional performance benefits.

STUDENT DECLARATION

"I, Metodija Kjertakov, declare that the Master by Research thesis entitled „ The role of heat as a conditioning stimulus in endurance athletes" is no more than 60,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature ███████████████ Date: 16·08·2019

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

acetyl CoA	acetyl coenzyme A
ADP	adenosine diphosphate
AMPK	5' AMP-activated protein kinase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BIA	bioelectrical impedance analysis
BL	blood lactate
BM	body mass
CaMK	calmodulin kinase
BIOPS	biopsy preserving solution
CS	citrate synthase
CI	leak respiration through Complex I
CI_P	maximal oxidative phosphorylation capacity through CI
$CI+CII_P$	oxidative phosphorylation with parallel electron supply form CI and Complex II
$CI+CII_E$	noncoupled electron transport system capacity through CI and CII
CII_E	electron transport system capacity through CII
CK	creatine kinase
CON	control
CO_2	carbon dioxide
CP	creatine phosphate
C_T	body core temperature
Cyt-c	cytochrome c
Dmax	maximal deviation method
DXA	dual-energy X-ray absorptiometry
EHS	exertional heat stroke
ETC	electron transport chain
FAD	flavin adenine dinucleotide
FFA	free fatty acids
FT	fast twitch
GE	gross efficiency
GXT	incremental test protocol

HA	heat acclimation
Hb	haemoglobin
Hct	haematocrit
HIT	high-intensity interval training
HR_{DP}	heart rate deflection point
HR	heart rate
HSP72	heat shock protein 72
HWI	hot water immersion
H^+	hydrogen
H_2O	water
LT	lactate threshold
LTP	lactate turn point
MAPK	p38 mitogen-activated protein kinase
MiR05	mitochondrial respiration medium
MLSS	maximal lactate steady-state
M_T	intramuscular temperature
mtDNA	mitochondrial DNA
NAD	adenine dinucleotide
NRF	nuclear respiratory factor
OBLA	onset of blood lactate accumulation
OXPHOS	maximal oxidative phosphorylation
O_2	oxygen
PGC-1α	peroxisome proliferator-activated receptor gamma co-activator 1-alpha
P_i	inorganic phosphate
PPO	peak power output
PV	plasma volume
RER	respiratory exchange ratio
RV	running velocity
ST	slow twitch
SUIT	substrate-uncoupler-inhibitor titration protocol
TFAM	transcription factor A
TT	individual time trial

CHAPTER ONE – REVIEW OF LITERATURE

1.1. Main characteristics of road cycling

Competitive road cycling is a physiologically demanding sport that takes place on paved roads. The sport is governed by the Union Cycliste Internationale (UCI), which is based in Aigle, Switzerland. According to the UCI database, in December, 2018 there were 18 cycling teams classified as WorldTour teams, 27 as Pro Continental Teams and 176 as Continental teams. The average annual WorldTour team budget composed of 25 to 33 riders is $US20 million. About 80% of the budget is spent on riders' salaries. The minimal wage for a professional cyclist is $US30 000 a year, but a star like Chris Froome earns about $US4 million.

On average, 30,000 to 35,000 km are covered each year by professional cyclists in training and competition (Faria et al., 2005a; Lucía et al., 2001; Mujika & Padilla, 2001). The typical racing season starts in early-January and finishes at the end of October. During the racing season a professional cyclist will enter between 80 – 100 races including: numerous one-day races, several 1-week stage races and at least one 3-week stage race (Atkinson et al., 2003; Jeukendrup et al., 2000; Lucía et al., 2001; Mujika & Padilla, 2001). In contrast, amateur cyclists ride, on average, no more than 25,000 km a year and participate in fewer than 50 races (Atkinson et al., 2003). Due to the broad spectrum of road cycling events, each event is discussed separately.

Individual time trials

An individual time trial (TT) is a race in which cyclists try to cover a set distance as fast as possible. This race can be a single event (up to 100 km long) or part of a stage race. For example, the grand tours typically include one short (5-10 km) TT and 1-2 long TTs (40-60 km). Time trials are also known as "the race of truth" because the winning depends only on each rider's physical capabilities. In addition, aerodynamics can be an important determinant of TT performance given the fact that the air resistance is the main resistance experienced by cyclists during these races (Faria et al., 2005b; Lucía et al., 2003; Santalla et al., 2012). Although considerable data has been accumulated from simulated TT under laboratory conditions during the past decades, limited information is currently available on official TT competitions. In one of the available studies, Padilla et al., (2000) investigated the exercise intensity during

TTs of various distances in a group of professional cyclists, by relating their racing heart rate (HR) values with those previously obtained during an incremental test protocol (GXT). The study revealed that prologue (i.e., short) TTs were raced at highest HRs (88-90% of HR_{max}). The TTs of 27 – 36.5 km were raced at 82-88% of HR_{max} and slightly lower HRs (79-84% of HR_{max}) were recorded during longer (49 km) TTs. In another study, Lucía et al., (1999) documented that during a 63 km TT in the Tour de France, riders spent 95% (approximately 70 min) of race time at HR between 88% and 100% of HR_{max}. The investigators also estimated that the winner of the TT maintained an intensity of >90% of $\dot{V}O_{2max}$ almost during the entire race, achieving an average speed of 50 km/h. However, not all TTs within stage races are raced at maximal level. It appears that the role of an individual cyclist within a team determines the intensity at which a TT is raced. Indeed, Padilla et al., (2000) reported that cyclists who strived for a high overall ranking rode the TTs at slightly higher intensity (81-88% of HR_{max}) than the cyclist who had a strategic role (75-81% of HR_{max}). It also has been estimated that TT specialists can maintain >400 watts (W) in long (>40 km) TTs, whereas average professional riders maintain an average power of about 350 W. The highest estimated average power output for a long (58 km) TT during the Tour de France was 458 W (Padilla et al., 2000).

Team time trials

Team time trials (TTT) are usually integrated into stage races. During TTTs, teams of 4-9 cyclists attempt to cover a set distance as fast as possible. In comparison to TT, TTT has a more intermittent nature because of the longer periods of drafting and recovery. Unfortunately, there is lack of information on the physiological demands during TTTs. Padilla et al., (2000) compared the differences in physiological responses between TTs and TTTs. Despite the fact that riders can draft behind other riders during TTTs, the investigators found that these races were more physically demanding than TTs.

Riding in a pack of riders can reduce the oxygen cost of cycling by 50-90% (Blocken et al., 2018). In track racing, in comparison to the lead rider, power output within a pace line is lower in the second, third and fourth positions of riding by 29, 36 and 36%, respectively (Broker et al., 1999). The biggest advantage of drafting is observed at higher speeds when a rider is surrounded by other riders (Blocken et al., 2018). This

makes drafting a very important skill in road racing. However, it seems that drafting in TTT does not result in the same drafting effect that a cyclist might gain when riding in the peloton (Jeukendrup et al., 2000).

Road races

Road races typically range in duration from 60 km (i.e., criteriums) to 300 km and are characterised by a mass-start. Although some races are as long as 600 km (e.g., Paris-Brest-Paris) most professional classic races such as the Paris-Roubaix, Milan-San Remo and the Tour of Flanders are usually 250-300 km. These races start with a large number of riders (e.g., 174 riders started the 2018 Milan-San Remo) and the first rider who crosses the finish line is the race winner. Unlike TT during which riders can't draft behind other riders to conserve energy, drafting is an extremely important aspect of road racing. As already discussed, the rider drafting usually does considerably less work than the pulling rider. Another important factor that can affect the race outcome is team tactics. Indeed, road races are largely influenced by team tactics of the top teams and nowadays it is virtually impossible to win a big race without the support of the team. Jeukendrup et al., (2000) reported that average power output during road races > 200 km may vary between 150 and 300 W. Finally, criteriums are short races that take place on a small, looped course. The most important factors in these events are sprint speed and bike handling skills.

Stage races

Stage races may last between 2 to 21 days, and sometimes more than one stage can be held in one day. The winner of any stage race is the rider who completes all the stages in the lowest overall time. The length of the race influences the effort exerted by riders (Rodríguez-Marroyo et al., 2009). In that study, the researchers determined the effort exerted by professional cyclists in 5-day, 8-day and 21-day stage races by monitoring HR. It was found that the average time spent in the highest HR zone was significantly higher in 5-day (~31 min) and 8-day (~28 min) races than in 21-day races (~14 min). These findings suggest that riders adopt more aggressive racing strategies during shorter stage races. The tactics during a stage race are dictated by the race characteristics and the team ambitions for the given race. For example, teams may target the overall rankings and not focus on single stage wins within a stage race. On the other hand, a cyclist may intentionally conserve their energy throughout the

individual stage in order to get to the final sprint in the best position for the win. Some riders will also launch a breakaway in an attempt to eventually win a stage, gain individual or team media exposure or scoring intermediate sprint/mountain points. However, due to race dynamics, team tactics and fatigue, the majority of the breakaways are often doomed to fail.

The Grand Tours of cycling, which include Tour de France, Giro d'Italia and Vuelta a España, are without question the most popular and prestigious cycling races in the UCI road racing calendar. From a physiological point of view, these races are extreme endurance sporting events during which riders must race daily for 21 days with only 1 to 2 days of rest. Historically, the Tour de France was >5000 km long, but the distance has gradually decreased over the years (Santalla et al., 2012). In the last few decades, the competitors cover an average distance of 3650 ± 208 km (Santalla et al., 2012). The Grand Tours are comprised of TT, flat stages, medium and high mountain stages. Sometimes a TTT stage is included. Performance in the TT stages and mountain stages is of crucial importance to the overall final rankings of the race. All riders in the Grand Tours are members of sponsored trade teams (the 2018 Tour de France started with 176 riders from 22 teams) and each of these teams enters the event with different objectives.

To date, a number of reports have described the physiological demands of the Grand Tours. In one study, Lucía et al., (1999) monitored the HR response of eight professional cyclists during all stages of Tour de France. The results showed that riders spent 71, 23 and 7 hours in the low, medium and high-intensity zones, respectively based on pre-race ventilatory measures. In a subsequent study, Padilla et al., (2001) recorded the HR in seventeen professional cyclists during Grand Tours. Average percentages of HR_{max} in high-mountain, semi-mountain and flat stages were 61 ± 5, 58 ± 6 and 51 ± 7, respectively. Subsequently, the advancement in the power meters allowed researchers to directly measure power output during actual races. Vogt et al., (2007) monitored power output in 15 professional cyclists during the 2005 Tour de France. The researchers reported that the mean power output was the highest in mountainous stages (234 ± 13 W), followed by semi-mountainous stages (228 ± 22 W) and flat stages (218 ± 21 W). They also found that mean cadence was lower on mountainous (81 ± 15 rpm), compared to flat (87 ± 14 rpm) and semi-mountainous

stages (86 ± 14 rpm). Vogt et al., (2007) also have published power output data collected from one professional cyclist during the 2005 Giro d'Italia. This case study reported a mean power output of 132 ± 26 W during the flat stages and a mean value of 235 ± 30 W in high-mountain stages.

The high power outputs sustained by the riders during the Grand Tours are associated with very high energy expenditures. Indeed, a study conducted during Tour de France (Saris et al., 1989), found a mean daily energy expenditure of 6066 kcal, which is perhaps the highest value that has ever been reported for a period longer than one week. Similar energy expenditure (5700 kcal) was recorded a decade later by Jeukendrup et al., (2000) during a stage of the same race. However, both studies also revealed that the energy requirements were met with sufficient food intake, which allowed the riders to maintain their body weight throughout the race. Furthermore, Jeukendrup et al., (2000) point out that the riders who lose weight are at risk of not finishing the race. This is not surprising knowing that losing body weight is a clear sign that catabolism predominates (Neumann, 2000), which has been associated with poor exercise performance (Fogelholm, 1994; Hall & Lane, 2001; Horswill et al., 1990). The importance of adequate nutrition for cycling performance is covered in section 1.3.7.1.

1.2. Energy for cycling

During cycling, contracting elements in active muscles require a constant supply of energy. The only form of energy that can be directly utilized by active muscle fibres is adenosine triphosphate (ATP), a compound composed of one molecule of adenosine and three inorganic phosphate (P_i) groups. When acted on by the enzyme adenosine triphosphatase (ATPase), ATP gets broken down to a molecule of adenosine diphosphate (ADP) and an inorganic phosphate (P_i) molecule, releasing a large quantity of energy that powers skeletal muscles contractions. However, muscle fibres store very small quantities of ATP and must therefore continually resynthesize it in order to sustain muscular contractions. There are three different energy-producing systems that the body uses to regenerate ATP: the ATP-CP system, glycolytic system, and oxidative system. The first two of these energy systems do not require oxygen to generate ATP and are also referred to as anaerobic. In contrast, the third energy system requires oxygen to produce ATP and is also known as aerobic. These

processes will be described only briefly. For more detailed information in this area, the reader is referred to McArdle et al., (2006).

ATP-CP system

The first system is the least complicated of the three energy systems. This system uses a compound called creatine phosphate (CP) for ATP resynthesis. CP is made up of a P_i group attached to a creatine molecule. When ATP gets broken down to ADP, the enzyme creatine kinase (CK) triggers the transfer of the P_i group from CP to ADP to rebuild ATP. Since this transfer is very rapid, depletion of ATP stores is prevented. However, the CP level declines rapidly.

Although the CP concentration in muscles can vary from athlete to athlete, the supply of this compound is exhausted within 30 seconds. Thus, the capacity to maintain ATP levels using the ATP-CP system is limited. Nevertheless, this energy system plays a crucial role during high-intensity, short-duration exercises, such as sprints or an attempt to close a gap in cycling (Cheung, 2017; Gregor & Conconi, 2000).

Glycolytic system

The second energy system generates ATP via the breakdown of glucose, a process known as glycolysis. The glucose metabolised during this process originates from the blood through the digestion of carbohydrate and the breakdown of the glycogen stored in the liver. Glucose can also come from muscle glycogen. The process by which the glycogen is broken down into glucose is called glycogenolysis.

The process of glycolysis begins with the conversion of either glucose or glycogen into a molecule called glucose-6-phosphate. Conversion of a molecule of glucose to glucose-6-phosphate necessitates one molecule of ATP. On the other hand, the conversion of glycogen to glucose-6-phosphate occurs without this energy expenditure. Glycolysis of one molecule of glycogen yields three molecules of ATP, whereas one molecule of glucose burned results in only one molecule of ATP because one molecule of ATP is used for the conversion of glucose to glucose-6-phosphate. In addition, this process results in the formation of pyruvic acid, which, in the absence of oxygen, is converted to lactic acid.

The glycolytic system can produce a larger quantity of ATP than the ATP-CP system. However, it produces less power than the ATP-CP system. Glycolysis is the dominant energy system for high-intensity, moderate-duration exercise lasting 1-3 min, such as prologues and breakaways in cycling (Cheung, 2017; Gregor & Conconi, 2000).

Oxidative system

The final energy system provides energy for ATP resynthesis mainly from the oxidation of carbohydrate and fat. This is a very complex process that occurs within the cell organelles called mitochondria, which are very often referred to as the "powerhouse" of the cell. Unlike the other two energy systems, the oxidative system provides a constant supply of ATP. However, the power of this system is half of that in the glycolytic system, thus it cannot support highly intense activity. Athletes who partake in endurance-based events rely heavily on the oxidative system (Cheung, 2017; Gregor & Conconi, 2000).

The process of ATP resynthesis from oxidative breakdown of carbohydrate starts in the same way as glycolysis, i.e., conversion of either glucose or glycogen to pyruvic acid. However, because oxygen is present, the pyruvic acid is not converted to lactic acid as in the glycolytic system. Rather, pyruvic acid is converted into a compound called acetyl coenzyme A (acetyl CoA) and it then passes through a series of biochemical reactions called the Krebs cycle and the electron transport chain (ETC). The Krebs cycle generates carbon dioxide (CO_2) and hydrogen (H^+) from the oxidation of acetyl CoA. Whereas the CO_2 is expired via the lungs, H combines with the coenzymes flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) and transports them to the mitochondria where they enter the ETC. Subsequently, the H^+ atoms are split into protons and electrons, and while the protons combine with oxygen (O_2) to form water (H_2O), the electrons pass through a series of reactions that liberate energy to generate ATP from ADP.

As mentioned above, fat also supplies energy for ATP production. Triglycerides stored in adipose cells and skeletal muscle fibres are major energy sources. The process of energy release from fat begins with the breakdown of a triglyceride molecule to one molecule of glycerol and three molecules of free fatty acids (FFA). After splitting from glycerol, FFA transforms to acetyl CoA in the mitochondria during a reaction called β-oxidation. Acetyl CoA then enters the Krebs cycle and it is oxidized in the same way

as the acetyl unit formed by glucose catabolism. As in carbohydrate metabolism, the Krebs cycle generates H that is transported to the ETC where ATP resynthesis occurs.

Compared with the glycolytic system, the oxidative system is far more efficient in terms of ATP gain. Oxidative breakdown of one molecule of glycogen allows for the resynthesis of 39 molecules of ATP. When the process begins with glucose, the net gain is 38 molecules of ATP, because, as previously mentioned, one molecule of ATP is used for conversion of glucose to glucose-6-phosphate prior to the start of the process of glycolysis (McArdle et al., 2006). Furthermore, oxidation of one molecule of fat yields 129 ATP molecules (McArdle et al., 2006). The key determinant in the selection of which substrate will be the main contributor to ATP production is the intensity of exercise. Generally, the utilization of carbohydrate for ATP production increases with intensity (Romijn et al., 1993). During periods of carbohydrate depletion, protein can also be used to resynthesise ATP (Lemon & Mullin, 1980).

Although it is common to classify sporting events as either anaerobic or aerobic, it must be recognized that none of these energy systems works in isolation. Indeed, all three energy systems provide a portion of needed energy for ATP resynthesis at all times. The contribution of each system depends on the intensity and duration of the exercise. For example, in the shorter track cycling events, such as 200 m sprint lasting ~ 10 seconds, the estimated contribution of ATP-CP, glycolytic and oxidative system to the total energy requirement was 40, 55 and 5%, respectively (Jeukendrup et al., 2000). Events lasting ~4 min, such as the 4000 m team pursuit, requires a 75% contribution from the oxidative system, but only 1% from the ATP-CP system (Jeukendrup et al., 2000). In the longest track event, i.e., the 1-hour record, >95% of the energy requirement is provided by the oxidative system (Jeukendrup et al., 2000). Similarly, long-distance road racing relies heavily on the oxidative breakdown of fats and carbohydrate for energy. The proportion of anaerobic energy supply during a Grand Tour has been estimated to be only 7% (Lucía et al., 1999). Nevertheless, the contribution of anaerobic metabolism to the overall result could be considerable given the fact that this energy system plays an important role during the stages of the tour that usually determine the result (i.e., mountain stages and TTs) (Faria et al., 2005b; Lucía et al., 1999).

A good indicator of which energy system dominates during exercise is the concentration of lactate in the blood. Low concentrations of lactic acid in the blood indicate that the oxidative system was the main contributor to ATP resynthesis, whereas high concentrations of lactic acid in the blood indicate that significant portion of the energy utilized for ATP resynthesis was derived from the anaerobic glycolytic energy systems.

1.3. Determinants of cycling performance

Over the past several decades, sport scientists and exercise physiologists have been able to identify a number of physiological factors that are related to road cycling performance. These include maximal oxygen uptake ($\dot{V}O_{2max}$), maximal lactate steady-state (MLSS), peak power output (PPO), gross efficiency (GE), muscle fibre type and body composition. Each of these factors is discussed below.

1.3.1. Maximal oxygen uptake ($\dot{V}O_{2max}$)

Maximal oxygen uptake is defined as the highest rate at which oxygen can be taken up and used by the body during severe exercise to aerobically resynthesize ATP, and it is probably the most commonly used measure to describe athletes' endurance potential. $\dot{V}O_{2max}$ can be expressed in absolute units (i.e., liters of oxygen consumed per minute [$l \cdot min^{-1}$]) or relative units (i.e., milliliters of oxygen consumed per kilogram of body mass (BM) per minute [$ml \cdot kg^{-1} \cdot min^{-1}$]). The latter allows a more accurate comparison of different-sized athletes (Wilmore & Costill, 1994). High $\dot{V}O_{2max}$ values have been reported extensively in the scientific literature for elite endurance athletes, including cross-country skiers (Ingjer, 1991), distance runners (Legaz-Arrese et al., 2007), mountain bikers (Impellizzeri et al., 2005), road cyclists (Lucía et al., 2004) and triathletes (Sleivert & Rowlands, 1996). Values of 69 to 84 $ml \cdot kg^{-1} \cdot min^{-1}$ and 57 to 64 $ml \cdot kg^{-1} \cdot min^{-1}$ are commonly observed in highly-trained male (Lucía et al., 2001; Mujika & Padilla, 2001) and female (Martin et al., 2001) road cyclists, respectively. The sex difference in $\dot{V}O_{2max}$ values can be accounted for by a higher level of body fat and lower haemoglobin concentration in women compared to men. $\dot{V}O_{2max}$ values of ≥ 80 $ml \cdot kg^{-1} \cdot min^{-1}$ are typical for winners of the Grand Tours (Bell et al., 2017; Coyle, 2005; Lucía et al., 2003; Santalla et al., 2012). These values are 100% greater than those seen in sedentary individuals. The highest recorded $\dot{V}O_{2max}$ for a male road cyclist is 96 $ml \cdot kg^{-1} \cdot min^{-1}$ (Rønnestad et al., 2019).

Although the magnitude of improvement of $\dot{V}O_{2max}$ is largely determined by genetics (Bouchard et al., 2011), it is undeniable that adequate endurance training will allow athletes to reach their trainable limit for $\dot{V}O_{2max}$ enhancement (Lundby et al., 2017; Midgley et al., 2006). The increased $\dot{V}O_{2max}$ in response to training is a result of two principal physiological changes: an increased oxygen delivery through an increased cardiac output and blood volume, and an increased oxygen extraction from the blood by the active muscles (Bassett & Howley, 2000; Lundby et al., 2017; Wagner, 1991; Wilmore & Costill, 1994). Adaptations contributing to oxygen extraction in muscles include increased capillary density and mitochondria density. However, whether $\dot{V}O_{2max}$ is primarily limited by central (cardiovascular) or peripheral (muscular) factors has been a subject of debate (Midgley et al., 2006). The classical view is that $\dot{V}O_{2max}$ is primarily limited by the ability of the cardiovascular system to deliver oxygen to the active muscles (Bassett & Howley, 2000; Wilmore & Costill, 1994). This is supported by research showing that artificially reducing (Ekblom et al., 1972; Pirnay et al., 1971) or increasing (Ekblom & Berglund, 1991; Gledhill, 1982) oxygen delivery alters $\dot{V}O_{2max}$.

It is widely accepted that high values of $\dot{V}O_{2max}$ are required for successful competition in endurance events. However, it must be noted that $\dot{V}O_{2max}$ on its own is not considered a valid performance indicator, particularly in groups of athletes with similar $\dot{V}O_{2max}$ values (Faria et al., 2005a). For example, Lucía et al., (2004) reported that laboratory-measured $\dot{V}O_{2max}$ was not related to performance in long TTs (>50 km) during the Tour de France in a group of 11 professional cyclists ($\dot{V}O_{2max}$ = 75.4 ± 6.2 ml·kg^{-1}·min^{-1}). Similarly, Impellizzeri et al., (2005) found that VO_{2max} was not correlated with performance during a 33.6 km mountain bike race in 12 high-level off-road cyclists ($\dot{V}O_{2max}$ = 76.9 ± 5.3 ml·kg^{-1}·min^{-1}). A poor correlation between $\dot{V}O_{2max}$ and exercise performance also has been observed in a study conducted on highly-trained distance runners (Conley & Krahenbuhl, 1980). Collectively, findings from these studies indicate that factors other than $\dot{V}O_{2max}$ play a more important role in determining endurance performance. Nevertheless, research has found that measurements of $\dot{V}O_{2max}$ have the ability to distinguish cyclists as being either trained or highly-trained (Faria et al., 2005a).

Maximal oxygen uptake is typically established using a GXT where the exercise intensity is gradually increased (every 60 seconds) until the individual reaches

voluntary exhaustion. Duration of the test is recommended to be in the range of 8 to 12 minutes to elicit $\dot{V}O_{2max}$. During the test, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) is measured via facemask and analysed in a metabolic cart. $\dot{V}O_{2max}$ is achieved when a plateau in $\dot{V}O_2$ has been reached despite an increase in exercise intensity.

1.3.2. Maximal lactate steady-state (MLSS)

The ability to maintain the highest power output possible without a continual increase in blood lactate (BL) concentration appears to be a key characteristic of successful road cyclists (Atkinson et al., 2003; Faria et al., 2005a). In the literature, this intensity has been described as the maximal lactate steady-state (MLSS), which in professional cyclists can occur at ~ 90% of their $\dot{V}O_{2max}$ (Atkinson et al., 2003; Faria et al., 2005a). In contrast, Grossl et al., (2012) observed an MLSS at 78% of $\dot{V}O_{2max}$ in a group of 14 amateur cyclists ($\dot{V}O_{2max}$ = 60.5 ± 9.6 ml·kg^{-1}·min^{-1}). The physiological importance of MLSS lies in the fact that it demarcates the exercise intensity above which muscle glycogenolysis is markedly increased, leading to an exponential accumulation of lactate in the blood and rapid onset of fatigue (Faude et al., 2009). Unlike $\dot{V}O_{2max}$, MLSS has been demonstrated to be a strong predictor of endurance cycling performance. Indeed, Harnish et al., (2001) showed that mean cycling velocity (36.8 ± 1.0 km·1^{-1}), BL (6.7 ± 07 mmol·L^{-1}) and HR (174.7 ± 2.6 beats·min^{-1}) at MLSS were not significantly different than those elicited during the 40 km time trial (TT$_{40}$) (36.6 ± 0.9 km·1^{-1}, 6.1 ± 0.7 mmol·L^{-1}, 174.1 ± 2.1 beats·min^{-1}, respectively) in 9 (6 male and 3 female) trained cyclists. Furthermore, cycling velocity at MLSS was found to be highly related to TT$_{40}$ velocity (r = 0.84; p < 0.05).

Maximal lactate steady-state intensity is most commonly determined according to the protocol introduced by Beneke et al., (1996). Their approach involves blood sampling during several constant load tests of 30 min duration performed on different days over a range of intensities. The intention is to identify the highest exercise intensity where BL concentration increases by no more than 1.0 mmol·L^{-1} during the last 20 min of the test. The power output or HR established at MLSS is also used for training prescription (Faude et al., 2009; Philp et al., 2008). However, since the direct determination of the MLSS is time-consuming, researchers frequently predict the MLSS from the BL response to a single GXT.

Blood lactate data collected during a GXT are usually plotted against workload and the resulting curve is visually inspected for breakpoints (Bentley et al., 2007; Faude et al., 2009). Typically, two breakpoints can be observed along the curve. The first, defined as lactate threshold (LT), is where the BL begins to rise above pre-exercise levels. Exercise at this intensity can be sustained for >2 hours (Faude et al., 2009; Jones, 2006) providing that enough carbohydrates are consumed during exercise. Successful endurance athletes have been reported to spend the majority (~ 80%) of their training time at intensities below LT (Arne et al., 2009; Neumann, 2000; Seiler & Kjerland, 2006). In professional cyclists, LT is usually found between 300 and 400 W (Mujika & Padilla, 2001). The second breakpoint on the curve is characterized by a 'sudden and sustained' increase in BL (at around $2 - 4$ mmol·L^{-1}), which is a consequence of increased activation of glycolysis and reduced BL removal. This physiological event has been referred to as lactate turn point (LTP). Continuous exercise at an intensity between LT and LTP results in elevated but stable BL (Faria et al., 2005a; Jones, 2006). In contrast, exercise above LTP is associated with a continuous rise in BL and premature fatigue. For this reason, LTP has been accepted as a surrogate marker of MLSS (Faria et al., 2005a).

Often, researchers also use fixed BL concentrations of 2 and 4 mmol·L^{-1} as a reference for the first and second threshold, respectively (Figure 1.1) (Bentley et al., 2007; Bosquet et al., 2002; Faude et al., 2009). The 4 mmol·L^{-1} threshold, widely known as the onset of blood lactate accumulation (OBLA), corresponds well to cycling TT performance. In one study, McNaughton et al., (2006) found a significant and strong relationship ($r = 0.90$; $p < 0.01$) between mean power output at OBLA (289.4 ± 35.4 W) and mean power output (284.3 ± 42.1 W) during 30-min TT in 11 moderate- to well-trained ($\dot{V}O_{2max}$ = 62 ± 8 ml·kg^{-1}·min^{-1}) male cyclists. In another study, Amann et al., (2006) investigated the relationship between a number of physiological determinants and TT$_{40}$ performance in 15 well-trained ($\dot{V}O_{2max}$ = 68.6 ± 4.2 ml·kg^{-1}·min^{-1}) male cyclists. The researchers found no significant difference between mean power output at OBLA (288 ± 29 W) and mean TT$_{40}$ power output (282 ± 25 W). These variables were also moderately correlated ($r = 0.60$; $p < 0.05$). Interestingly, the average power output (509 W) maintained during the successful 1-hour track world record attempt of Miguel Indurain coincided with the power output (505 W) he reached at OBLA during GXT nineteen days before the event (Padilla et al., 2000). There is also evidence that

OBLA reflects MLSS. For example, an early study found that elite cross-country skiers could maintain running velocity corresponding to 4 mmol·L^{-1} BL concentration for at least 45 min (Kindermann et al., 1979). Subsequently, Heck at al., (1985) reported strong correlation (r = 0.97) between running velocity at OBLA and running velocity at MLSS. More recently, Van Schuylenbergh et al., (2004) found no difference between HR (175 ± 3 beats·min^{-1}) and power output (314 ± 12 W) at OBLA and those obtained at MLSS (175 ± 2 beats·min^{-1}, 311 ± 9 W, respectively) in 11 professional cyclists. OBLA also correlated with MLSS (r = 0.50; p < 0.05).
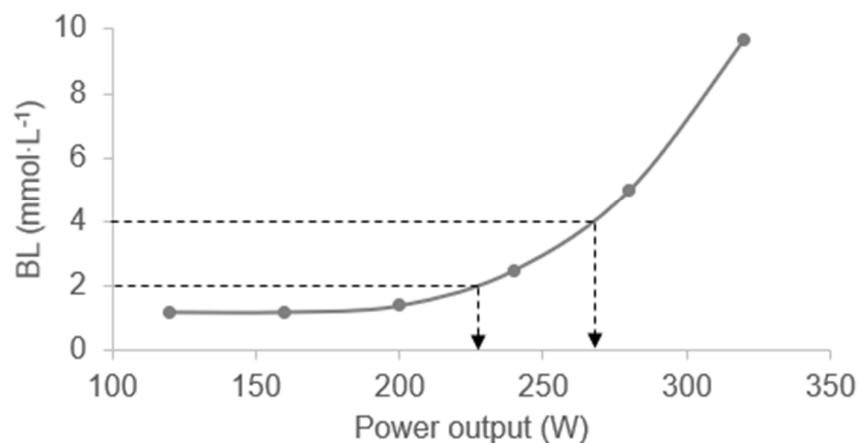
Figure 1.1. Determination of the first and second lactate threshold

In the 1990s, the maximal deviation method (Dmax) emerged as an alternative to OBLA (Cheng et al., 1992). Maximal deviation method is an objective and individual method that estimates the second threshold by identifying the point on the BL-workload curve that yields the maximum distance to the straight line formed by the first and the last point on the curve (Figure 1.2). Bishop et al., (1998) found that power output at Dmax to be significantly and highly correlated (r = 0.84; p < 0.001) with average power output achieved during 1-hour TT performance in 24 trained female cyclists. These results are consistent with those of Bentley et al., (2001) who observed significant correlation (r = 0.77; p < 0.05) between power output at Dmax and average power output during 90-min TT. Furthermore, power output at Dmax and power output at MLSS were not significantly different (p < 0.05) and were highly correlated (r = 0.97) in 20 (10 male and 10 female) well-trained cyclists (Czuba et al., 2009). Taken together, the results from these studies suggest that the Dmax method is effective for

endurance performance prediction in cycling. The disadvantage of Dmax method is the fact that the athlete needs to perform the GXT to exhaustion (Bentley et al., 2007), whereas the determination of OBLA generally requires submaximal efforts. Additional methods for identification of thresholds on the BL-workload curve also have been described in the literature, but it is beyond the scope of the current thesis to review all the existing methods. More information on this topic can be found elsewhere (Faude et al., 2009).
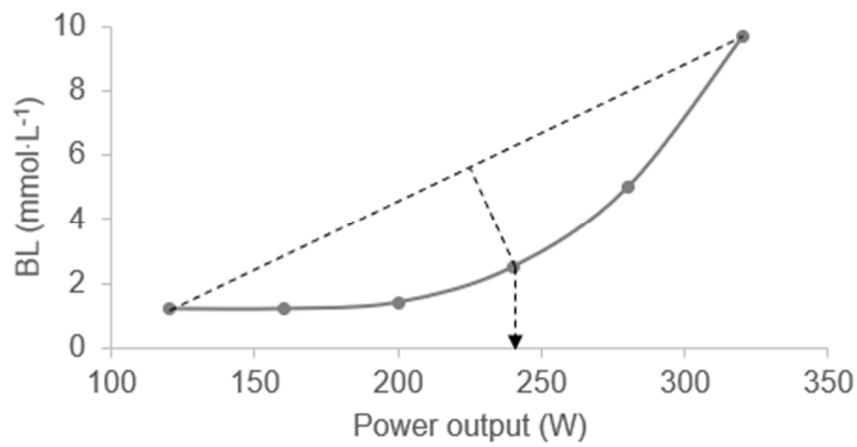
Figure 1.2. Determination of LTP in accordance with the Dmax method

An appropriate GXT for the assessment of LT and OBLA in a cyclist involves increasing power output by 30 W every 5 min and determining BL from fingertip blood samples taken within the last 30 seconds of every stage. Many exercise physiology laboratories around the world have adopted GXTs with stage duration of 3 min, but research indicates that this duration is not long enough to allow steady-state in BL concentrations (Bourdon, 2012). The consequence of this would be an overestimation of the workload at LT/OBLA. A number of reports have documented that exercise durations of at least 5 min are required to attain steady-state BL concentrations and thus allow an accurate determination of the threshold markers (Bourdon, 2012). There are, however, a number of other factors that may affect the test results. For example, BL response during a GXT is known to be influenced by glycogen stores and hydration status. With respect to the former, Hughes et al., (1982) reported that performing graded bicycle exercise in a glycogen depleted state resulted in significantly lower BL accumulation and shifted the LT towards higher workloads compared with the control

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condition. The effects of hydration status on the occurrence of LTP were studied by Moquin & Mazzeo, (2000). The researchers revealed that LTP occurred at 3.6% ($p <$ 0.05) lower power output after 2.6% BM loss by fluid restriction compared with a euhydrated state. Similar results were obtained by Kenefick et al., (2002) who found that 4% exercise-induced hypohydration caused an earlier occurrence of LT during incremental treadmill exercise. Additionally, the use of caffeine (Gaesser & Rich, 1985) and creatine (Chwalbiñska, 2003) influence LT. Finally, environmental conditions like temperature (Fink et al., 1975; Young et al., 1985) and altitude (Friedmann et al., 2005) can also alter BL response to exercise.

All these examples illustrate the fact that testing protocol, pre-test diet and environmental conditions on the day of the test should be strictly controlled during a longitudinal study if accurate comparisons of threshold measures over time are to be made. The BL response to incremental exercise and derived thresholds have been documented to be reproducible under standardised conditions (Bourdon, 2012). It is generally accepted that a rightward shift in BL thresholds to higher workload after a training intervention indicate an improved endurance capacity (Faria et al., 2005a; Faude et al., 2009; Jones, 2006; Jones & Carter, 2000). Such a change may occur form reduced rate of BL production or improved removal of BL owing to improved peripheral adaptations, i.e., increased mitochondrial and capillary density (Coyle, 1999; Jones & Carter, 2000; Williams, 1990). These adaptations are covered in section 1.4.

As an alternative to BL indices, the ventilatory threshold (VT) has been used by some researches to establish the highest sustainable exercise intensity for prolonged endurance events. VT is most commonly determined by the V-slope method proposed by Beaver et al., (1986) which is based on the observation of nonlinear increase in $\dot{V}CO_2$ in relation to $\dot{V}O_2$ during a GXT. Utilizing this method in their study, Hopkins & McKenzie, (1994) found that VT was significantly and highly correlated with TT_{40} ($r = -0.81$; $p < 0.05$) in 8 male competitive cyclists. Subsequently, Hoogeveen & Hoogsteen, (1999) also reported significantly strong relationship ($r = -0.82$; $p < 0.001$) between TT_{40} and VT, determined by the V-slope method. Another commonly used method for VT determination relies on the identification of workload at which there is a systemic increase in the ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) without concomitant

increase of the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$). According to the model based on two VT breakpoints (Davis, 1985; Meyer et al., 2005), this VT indicates the first VT (VT_1). VT_2 is defined as the workload corresponding to a systemic increase in both the ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) and ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$). VT_1 correlates well to the intensity of exercise during endurance events lasting >1 hour (Bentley et al., 2007; Laursen et al., 2005), whereas Perrey et al., (2003) reported that VT_2 reflects the intensity during a 30-minute cycling TT.

Since the aforementioned testing procedures are costly and they are therefore not readily available, many coaches and cyclists are left to rely on alternative field tests. Among these tests, perhaps the most popular is the one developed by Professor Francesco Conconi (Conconi et al., 1982; Conconi et al., 1996), which is simply based on analysis of HR response to increasing workload. In their earliest study (Conconi et al., 1982), the researchers found that the HR-running velocity (RV) relationship is linear from low to submaximal velocities and curvilinear from submaximal to maximal velocities. Furthermore, the point at which HR departs from linearity, coined as heart rate deflection point (HR_{DP}), coincided with BL concentration of 4 mmol·L^{-1}. From these observations, Conconi et al., (1982) proposed that the RV at the HR_{DP} can be used to estimate LTP in runners. The HR_{DP} concept was latter also applied to other endurance sports such as road cycling (Droghetti et al., 1985). However, the findings reported by Bourgois et al., (2004) call into question the validity of this threshold concept. In that study, the researchers found a BL steady state in only 4 out of 11 cyclists who performed a 30-minute prolonged exercise test on a bicycle ergometer at a power output corresponding to HR_{DP}. Two other cyclists also succeeded in completing the test, but failed to attain a BL steady state, whereas the remaining 5 riders were unable to complete the test due to exhaustion. Similar results have been reported for rowers (Bourgois & Vrijens, 1998) and distance runners (Jones & Doust, 1997).

1.3.3. Peak power output (PPO)

Several studies have documented that PPO, defined as the maximum wattage achieved at the end of a maximal incremental cycling test, is highly related to cycling performance (Faria et al., 2005a). Hawley & Noakes (1992) reported a highly significant correlation ($r = -0.91$; $p < 0.001$) between PPO obtained during an incremental test to exhaustion and 20 km time trial (TT_{20}) performance in 100 highly-

trained (54 male and 46 female) cyclists. Based on these findings, the authors concluded that PPO can be used as a predictor of cycling performance. This finding is further supported by Bishop et al., (1998) who found a correlation of 0.81 between the PPO and 1-hour cycling performance in 24 trained female cyclists. Furthermore, PPO was also highly correlated ($r = -0.87$; $p < 0.01$) to 40 km cycle time in a triathlon race (Bentley et al., 1998). Likewise, studies conducted on rowers (Bourdin et al., 2004) and swimmers (Hawley et al., 1992) have shown that measurement of maximal muscle power is a strong predictor of athletic potential.

The PPO is determined by measuring the highest power output for the last fully completed stage during an incremental test to exhaustion. The stages can last from 1 to 4 minutes. If the last exercise stage is not completed, an equation is used to determine PPO that takes into account the fraction of the non-completed stage (Kuipers et al., 1985). Nevertheless, PPO is a function of the test protocol. PPO values of $450 - 500$ W, $6.5 - 7.5$ W $\cdot$ kg^{-1} are reported during tests with one-minute increments in professional cyclists, whereas 400-450 W, $6.0 - 6.5$ W $\cdot$ kg^{-1} have been found when increments of four minutes were utilized (Atkinson et al., 2003). A power output/weight ratio of at least 5.5 W $\cdot$ kg^{-1} has been suggested as a prerequisite for elite-level cyclists (Faria et al., 2005a).

1.3.4. Gross efficiency (GE)

Another important characteristic of successful cyclists is high gross mechanical efficiency (Santalla et al., 2012). GE is a measure of effective work (the power output) and is expressed as a percentage of total energy expended that produces external work (Faria et al., 2005a). A mean GE of 24.5 ± 0.7% has been measured in professional road cyclists (Lucía et al., 2002), whereas mean GE values in the range between 18.1 ± 1.2% and 20.6 ± 1.1% have been reported for less accomplished riders (Hopker et al., 2009; Hopker et al., 2012; Moseley & Jeukendrup, 2001; Sidossis et al., 1992). The highest recorded GE in the study with professional riders was 28.1% (Lucía et al., 2002). Most recently, a case study on the four-time Tour de France winner (2013, 2015-2017), Chris Froome reported GE of 23.3% (Bell et al., 2017). The high GE observed in professional cyclists is believed to be a result of the large training volume typically performed by theses cyclists. In addition, several factors have been

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suggested to influence GE including: pedalling cadence, genetics, diet and overtraining (Faria et al., 2005b).

Although the exact mechanism for improvement of GE remains to be fully understood, better GE is undoubtedly beneficial for cycling performance. Indeed, Horowitz et al., (1994) compared two groups of trained cyclists with similar $\dot{V}O_{2max}$, but significantly different GE (20.2% vs 22.1%) and showed that average power output maintained during a 1-hour performance test was significantly higher (344 ± 8 W vs 314 ± 10 W) in the group with higher GE. Some authors have even proposed that GE is more important for cycling performance than $\dot{V}O_{2max}$ and LT (MacRae, 2006). This opinion was based on the report of Coyle who documented an increase in GE from 21.18% to 23.05% over a seven-year period in the seven-time champion of the Tour de France (1999 – 2005), Lance Armstrong, despite unchanged $\dot{V}O_{2max}$ and LT values (Coyle, 2005). Similar findings were subsequently observed in a longitudinal study over five racing seasons in a cohort of 12 male professional cyclists (Santalla et al., 2009). The researchers found an increase in GE from 23.61 ± 2.78% to 26.97 ± 3.7% ($p < 0.01$) without significant changes in $\dot{V}O_{2max}$.

Despite the importance of GE for cycling performance, this performance factor has received much less attention from exercise physiologists, coaches and cyclists than, for example, $\dot{V}O_{2max}$. GE is usually assessed in a laboratory setting with a cyclist pedalling on an ergometer while attached to a device that monitor $\dot{V}O_2$ and $\dot{V}CO_2$. The duration of the stages should be long enough (≥ 3 min) to allow a steady state in $\dot{V}O_2$ and $\dot{V}CO_2$. The following equitation is used to calculate the GE:

GE% = [work output (W) / energy expended] x 100

Energy expenditure is estimated from $\dot{V}O_2$ and respiratory exchange ratio (RER) using the formula of Lusk, (1924).

1.3.5. Muscle fibre type

Traditionally, textbooks on exercise physiology (Hale, 2003; Hoffman, 2014; Kraemer et al., 2011; Williams, 1990) identify two basic types of skeletal muscle fibre in humans, which according to their contractile and metabolic characteristics are classified as slow twitch (ST) and fast twitch (FT). FT fibres can be further divided into type IIA and type

IIB fibres. ST fibres have relatively slow contraction speed, high oxidative capacity and high fatigue resistance. Because of these characteristics, ST fibres are recruited most commonly during low-intensity endurance events. IIB fibres, on the other hand, have fast contractile speed, high glycolytic capacity and as such, they are better suited for anaerobic physical activities. Type IIA fibres are described as a hybrid of ST fibres and type IIB fibres because they have a capacity for ATP production via both glycolytic and oxidative system. The characteristics of these fibres are dependent on an individual's physical fitness level. In endurance-trained individuals, type IIA fibres have similar metabolic properties as ST fibres (Ingjer, 1979a).

Most skeletal muscles contain both ST and FT fibres. In highly-trained athletes, the prevalence of a particular fibre type seems to correspond to the metabolic demands of the given sport. Sprinters have a predominance of FT fibres, whereas endurance athletes possess a high percentage of ST fibres (Costill et a., 1976a; Gollnick et al., 1972). Indeed, studies reveal that skeletal muscles of elite endurance athletes may contain more than 90% ST fibres (Bergh et al., 1978; Costill et a., 1976b). Furthermore, a high percentage of ST fibres has historically been associated with superior performance in endurance events (Saltin et al., 1977). In this regard, the study of Coyle et al., (1991) is highly relevant for road cyclists. These investigators determined the fibre composition from the *vastus lateralis* muscle of 15 competitive male cyclists who were divided into two groups based on their TT_{40} performance. A significantly higher percentage of ST muscle fibres (66.5% vs. 52.9%) was reported for the group with better TT_{40} performance (53.9 min vs. 60 min). A study from the same laboratory also found that GE was significantly correlated ($r = 0.75$; $p < 0.001$) with the percentage of ST muscle fibres within the *vastus lateralis* of 19 well-trained male cyclists (Coyle et al., 1992). In addition, Ivy et al., (1980) reported a significant positive correlation ($r = 0.74$; $p < 0.01$) between the percentage of ST fibres and LT obtained during bicycling ergometry.

The importance of ST fibres for road cycling performance is clearly illustrated in the above studies. However, it remains unclear whether the amount of ST fibres in elite endurance athletes is a consequence of exercise-induced conversion form FT fibres, or whether these athletes have advanced to elite level because they have a very high proportion of ST fibres. Equivocal evidence exists whether endurance training can

alter the proportion of ST and FT fibres. Whereas some researchers have reported that endurance training can increase the percentage of ST fibres, others have failed to observe change in the percentage of ST fibres in response to endurance training (Ratamess & Izquierdo, 2008).

1.3.6. Body composition

A relationship between an athlete's body composition and competitive success has been established in a variety of sports (Slater et al., 2012). With respect to road cycling, maintenance of low body fat level is essential, especially for hill climbing when cyclists must overcome the force of gravity. In such a situation, carrying an extra non-functional mass places the rider at a disadvantage (Burke et al., 1990; Lucía et al., 2001; Swain, 1994). However, lean BM also has been found to relate to average power output maintained during a 1-hour TT (Coyle et al., 1991). Therefore, the measurement of body composition is of interest to coaches and cyclists. Currently, dual-energy X-ray absorptiometry (DXA) is considered the gold standard in body composition science (Menaspà & Impellizzeri, 2017). This method is based on measurement of the attenuation of an x-ray beam from a source with two energies passing through the body (Ellis, 2000). Although DXA allows precise measurement of lean mass, fat mass and bone mass, this method is expensive and not readily available. This has led to the use of less costly and easily accessible methods such as bioelectrical impedance analysis (BIA). This method estimates fat-free mass and fat mass by sending a low electrical current through the body tissues (Ellis, 2000). Rubiano and colleagues (2000) validated BIA against DXA and reported a correlation coefficient of 0.94. Alternatively, body composition can be determined by an equation based on measurements of skinfold thickness.

In 2 different publications, the average percentage of body fat in professional road cyclists has been reported to be 8.08 ± 1.4% (Fernandez et al., 2000) and 8.3 ± 0.2% (Lucía et al., 1999), respectively, using skinfold measures. The low body fat levels observed in these studies are not surprising given the fact that professional cyclists ride in excess of 30,000 km per year (Faria et al., 2005a; Lucía et al., 2001; Mujika & Padilla, 2001).

1.3.7. Other factors

Apart from the physiological characteristics described above, nutritional and pacing strategies can also have a strong influence on cycling performance (Atkinson et al., 2003).

1.3.7.1. Nutrition

1.3.7.1.1. Carbohydrate

As mentioned in section 1.2, the relative contribution of carbohydrate and fat to total energy expenditure during exercise depends mainly on exercise intensity. Generally, during prolonged exercise at intensities greater than 65% of $\dot{V}O_{2max}$, carbohydrate becomes a dominant energy source (Hawley, 2002). Therefore, increasing muscle glycogen stores through adequate carbohydrate intake is vital before prolonged, intensive training sessions and long-distance road races in order to maximise performance and prevent premature fatigue associated with glycogen depletion. Indeed, a strong relationship has been observed between pre-exercise glycogen levels and cycling time to exhaustion (Abbiss & Laursen, 2005; Jeukendrup, 2011; Williams, 1990). For example, a study conducted in the late 1960s by Bergström et al., (1967) demonstrated that time to exhaustion during cycling ergometry at 75% of $\dot{V}O_{2max}$ was significantly longer under the condition of increased muscle glycogen (166.5 ± 17.8 min) compared with a normal muscle glycogen trial (113.6 ± 5.3 min) and a reduced muscle glycogen trial (56.9 ± 1.7 min). Glycogen manipulation was performed by varying the amount of carbohydrate in the diets three days preceding the trials. In another study, the same workgroup showed that muscle glycogen content can be doubled when a glycogen-depleting exercise precedes a high-carbohydrate diet (Hultman & Bergström, 1967).

The findings from the Bergström laboratory prompted researchers to develop a nutritional strategy which has become widely known as 'glycogen loading'. Originally, this strategy consisted of a 3-day period of heavy exercise with minimal carbohydrate intake, followed by a 3-day period with light exercise and a high-carbohydrate diet (Karlsson & Saltin, 1971). This nutritional protocol resulted in a drastic increase in muscle glycogen content and improved endurance running performance compared with a normal mixed diet (Karlsson & Saltin, 1971). Subsequently, research has shown that the glycogen depletion phase of this protocol was not necessary and a

comparable increase in muscle glycogen level could be achieved by 3 days of a high-carbohydrate diet (70% of the total caloric intake) in association with a reduction of training load (Burke et al., 2011; Sherman et al., 1981). A study by Bussau et al., (2002) reported that trained athletes can maximise their muscle glycogen stores in as little as 24 h of inactivity and carbohydrate intake of 10 $g \cdot day^{-1} \cdot kg^{-1}$ BM. This protocol can be easily adapted by cyclists before races. However, it should be noted that elevated muscle glycogen may not improve performance when the exercise duration is about 1 hour (Abbiss & Laursen, 2005), such as a TT race. According to Hespel, (2014), muscle glycogen loading before TT races is redundant because normal muscle glycogen content is sufficient to support performance during these events. Nevertheless, adequate carbohydrate intake before a TT race is important, given the fact that 85% of the energy during such an event is derived from carbohydrate (Abbiss & Laursen, 2005).

Generally, cyclists are advised to consume 150-300 g of carbohydrates 3-4 hours before a race (Hespel, 2014; Jeukendrup, 2000). A pre-race meal satisfies hunger, refills liver glycogen and may contribute to muscle glycogen content, particularly after an overnight fast. Indeed, Wee et al., (2005) observed a 15% increase in muscle glycogen concentration 3 hours after the participants consumed a high carbohydrate breakfast containing 2.5 $g \cdot kg^{-1}$ BM of carbohydrate. The exercise performance during a constant-load cycling test after such a carbohydrate breakfast is significantly greater than the performance of participants who had no breakfast (Schabort et al., 1999). However, eating a meal 3 hours before the race may not be practical for a cyclist participating in a race that starts early in the morning. In such a case, consuming a small amount of carbohydrate (~1 $g \cdot kg^{-1}$ BM) within 1 hour before the race also can be beneficial to performance. The support for this advice comes from the study by Sherman et al., (Sherman et al., 1991) who assessed cycling performance in one trial that included 1-hour pre-exercise liquid breakfast containing 1.1 $g \cdot kg^{-1}$ BM of carbohydrate and in another trial in which participants ate no breakfast. The exercise protocol consisted of cycling for 90 min at 70% of $\dot{V}O_{2max}$ followed by a TT. The cyclists covered the TT significantly faster after the ingestion of the carbohydrate solution than following the ingestion of the placebo. (~41 min vs. ~47 min; $p < 0.05$). A liquid meal is perhaps the best choice if the meal is to be consumed closer to the start of the race.

During intensive training sessions and races that last longer than 90 min, fatigue is typically caused by muscle glycogen depletion and/or hypoglycaemia (Ivy, 1999). The consumption of carbohydrate during prolonged exercise is an effective strategy to enhance performance by providing an exogenous energy source to the active muscles. Consuming carbohydrates during exercise is especially important in a situation when a cyclist has not consumed a pre-ride meal. The amount of carbohydrate a cyclist needs to consume during a ride depends on the duration of the event (Hespel, 2014). For events lasting between 1 and 2.5 h, a carbohydrate intake of $30 - 60$ g $\cdot$ h^{-1} is sufficient to maintain performance (Burke et al., 2011; Hespel, 2014; Thomas et al., 2016). For longer events (>2.5 h), the recommended carbohydrate intake is 90 g $\cdot$ h^{-1} (Burke et al., 2011; Hespel, 2014; Thomas et al., 2016). The carbohydrate consumed during exercise can be in solid, liquid or gel form, as there are little differences in carbohydrate oxidation rates between these forms (Pfeiffer et al., 2010a; Pfeiffer et al., 2010b). In practice, professional cyclists consume sports drinks, bars, gels and conventional foods such as sandwiches and pastries during races (Hespel, 2014). Surprisingly, Garcia-Roves et al., (1998) noted a carbohydrate intake in a range between 10 and 43 g $\cdot$ h^{-1} in 10 professional cyclists during the stages of the Vuelta a España. The low carbohydrate intake was suggested to be caused by aggressive racing tactics, which prevent greater intake of food and drink.

Fast replenishment of muscle and liver glycogen stores after exercise is important, particularly when the time between training sessions/races is short. In the absence of significant muscle damage, muscle glycogen stores can be refilled within 24 by consuming sufficient amounts of carbohydrate (7-10 g$\cdot$kg^{-1} BM) (Burke et al., 2011). However, it is essential that the consumption of carbohydrate starts soon after the exercise, as the highest rates of muscle glycogen resynthesis occur during the first hour post-exercise due to the increased activity of the enzyme glycogen-synthase (Bergström & Hultman, 1966). Ivy et al., (1988) showed that muscle glycogen content at 6 h post-exercise was significantly increased when carbohydrates were consumed immediately after exercise than when the consumption was delayed by 2 h.

1.3.7.1.2. Hydration

Hydration status is another nutrition-related factor that can influence fatigue. The advice that athletes need to start exercise in a well-hydrated state (i.e. euhydration) is based on observations that hypohydration (i.e. pre-exercise body fluid deficit) results in a number of consequences during exercise including elevated HR and body core temperature, increased muscle glycogen utilization and impaired performance (Casa et al., 2000; Coyle, 2004; Sawka et al., 2007). The negative effects of hypohydration on endurance performance have been well-documented in the classic study by Armstrong et al., (1985) who had 8 male runners compete in track races at distances of 1500 m, 5000 m and 10 000 m when hypohydrated and euhydrated. Hypohydration was caused by oral ingestion of 40 mg of furosemide (a diuretic drug) 5 h before the races, which led to reductions in BM of ~2%. Compared to running in euhydrated condition, hypohydration increased time to complete the races by 3.1% (1500 m), 6.7% (3000 m) and 6.3% (10 000 m). Despite this, and the efforts of various sports organisations (Casa et al., 2000; Sawka et al., 2007) to educate athletes about the importance of adequate hydration, it is still not uncommon for athletes to begin practice/competition hypohydrated (Chapelle et al., 2017; Love et al., 2018; Magal et al., 2015; Magee et al., 2017).

To achieve euhydration before exercise, a cyclist is advised to start drinking ~ 5-7 mL/kg BM approximately 4 hours beforehand and then monitor urinary response (Jeukendrup, 2011; Sawka et al., 2007). In a case of highly concentrated or dark urine, which indicates hypohydration, he/she should drink another ~ 3-5 mL/kg about 2 hours before the event (Jeukendrup, 2011; Sawka et al., 2007). Finally, 200-300 mL of fluid should be consumed within 10 min before the start of exercise, especially on a hot day when considerable amounts of sweat could be lost. There is some evidence that achieving hyperhydration (via saline drinks) may improve thermoregulation and endurance performance, particularly in the heat (Sims et al., 2007a; Sims et al., 2007b). However, this strategy may increase the risk of having to void during exercise (Jeukendrup, 2011). More importantly, hyperhydration also may increase the risk of hyponatremia (see below), if fluids are replaced excessively during exercise (Hew-Butler et al., 2015; Jeukendrup, 2011).

During exercise, it is essential to drink sufficient amounts of fluids to adequately offset the body water losses caused by sweating and maintain normal hydration status. However, the decrements in exercise performance do not occur until there is at least 2-3% decrease in BM through sweating (Goulet, 2012; Jeukendrup, 2011). Conversely, persistent fluid intake beyond sweat loss can lead to dilution of plasma sodium concentration to such a critical level (<135 $mmol \cdot L^{-1}$) that it does not only reduce exercise performance but may also ultimately lead to fatality (Coyle, 2004; Hew-Butler et al., 2015; Sawka et al., 2007). The occurrence of this condition during or within 24 hours of prolonged exercise is defined as exertional-associated hyponatremia (Hew-Butler et al., 2015). Unfortunately, when it comes to determining how much fluid is needed to maintain euhydration during exercise, there is no single amount that will meet the needs of all individuals in all situations. Recently published hydration guidelines by the influential sports organisations (McDermott et al., 2017; Thomas et al., 2016) and investigators (Maughan & Shirreffs, 2010) acknowledge that each athlete has unique fluid needs and accordingly recommend individualized fluid replacement strategies based on an athlete's sweat rate, with the goal of preventing excessive dehydration (>2% BM loss) and changes in electrolyte balance. An alternative recommendation for fluid intake during exercise in endurance events is to drink only according to the dictates of thirst (Hew-Butler et al., 2015; Noakes, 2003). Although this drinking strategy has been associated with low risk of EAH (Scotney et al., 2015), using thirst to guide fluid intake during exercise may result in significant dehydration and consequently performance impairment (Bardis et al., 2017).

Complete replacement of sweat losses by fluid intake during exercise may not always be feasible because sweat rate may reach values higher than 2 $l \cdot h^{-1}$ (Jeukendrup, 2000), which exceeds the maximum intestinal absorptive capacity for water (Coyle, 2004; Sawka, 1992). When dehydration occurs, fluid losses need to be replaced after exercise. To achieve effective rehydration, it is recommended to drink an amount of fluid equal to 125-150% of that lost through sweating to allow for ongoing obligatory urine losses in the post-exercise period (Maughan & Shirreffs, 2010; Sawka et a., 2007). In addition, it is essential to include moderate to high amounts (>50 $mmol \cdot l^{-1}$) of sodium in the drink to help retain most of the ingested fluids (Maughan & Leiper, 1995). The sodium may also come from solid food consumed with the drink (Maughan et al., 1996).

Given the negative effects of body water deficit on endurance exercise performance, the assessment of hydration status has received much attention in the scientific literature. The available hydration markers are typically divided into three main categories including markers that measure total body water, haematological markers and urinary markers (Armstrong, 2005). Determination of total body water can be achieved by isotopic dilution with tritium or deuterium, or by using BIA. Changes in BM can also be used as a noninvasive marker to assess changes in total body water in response to exercise or heat exposure. Plasma osmolarity is the most commonly used haematological hydration marker, and increases linearly by 5 mOsmol·L^{-1} for every 2% loss of BM during exercise in the heat (Popowski et al., 2001). Although isotope dilution techniques and plasma osmolarity are considered the most accurate and reliable measures of hydration status, these methods can be costly and require technical expertise. Since urinary markers are simple and inexpensive, they are the most commonly used hydration markers in hydration studies. These markers include urine osmolality (UO$_{SM}$), urine specific gravity (USG) and urine colour. Urine colour is usually determined according to the 8-colour scale developed by Armstrong et al., (1994). However, ingestion of medications, vitamins and certain foods may affect urine colour and thus the accuracy of this method. Urine osmolality measures the concentration of urine and USG measures the relative density of urine. According to the American College of Sports Medicine (Sawka et a., 2007), UO$_{SM}$ ≥700 mOsmol·L^{-1} and USG ≥1.020 g·mL^{-1} represent hypohydration. Based on the findings of strong association ($r = 0.97$; $p < 0.0001$) between UO$_{SM}$ and USG, Armstrong at el., (1994) suggested that these two hydration markers can be used interchangeably in the assessment of the hydration status.

1.3.7.1.3. Caffeine

Caffeine, chemically 1,3,7-trimethylxanthine, is found in a variety of foods, drinks and sports nutrition supplements. Since the late 1970s, caffeine has been recognized as an ergogenic aid for endurance athletes (Costill et al., 1978). These researchers reported that cycling time to exhaustion at 80% of V̇O$_{2max}$ was 15 min longer after ingestion of 330 mg of caffeine compared with a placebo trial. These findings were confirmed by numerous additional studies (Astorino et al., 2011; Bridge & Jones, 2006; Bruce et al., 2000; Desbrow et al., 2012; Graham & Spriet, 1991; Graham & Spriet, 1995; Ivy et al., 1979; Jenkins et al., 2008; McNaughton et al., 2008; Pasman &

Jeukendrup, 1995) demonstrating the ergogenic effects of caffeine in endurance sports. These studies showed that caffeine in dosages ranging from 2 to 9 mg·kg^{-1} BM used approximately 1 hour before exercise provide performance benefits. In addition, one study found that taking 1.5 mg·kg^{-1} BM of caffeine during the latter stages of prolonged cycling can also enhance performance (Cox et al., 2002).

Importantly, the ergogenic effects of caffeine are similar in both habitual and non-habitual consumers (Jeukendrup, 2011). Furthermore, recent research has found that caffeine intake of up to ~7.0 mg·kg^{-1} BM does not affect the fluid status and the ability to thermoregulate during exercise (Spriet, 2014). However, in novice caffeine users, caffeine can cause several adverse effects including anxiety, jitteriness, diarrhoea, gastrointestinal stress, insomnia, high blood pressure and tachycardia (Paluska, 2003). Therefore, a cyclist who is unaccustomed to caffeine should experiment with caffeine during a training ride before using it in a competitive environment.

1.3.7.2. Pacing

Pacing is a term used to describe how an athlete distributes work rate or energy expenditure throughout an exercise task. Adopting an appropriate pacing approach or strategy will maximise performance by allowing the cyclist to maintain the highest sustainable work rate and prevent premature fatigue before the end of the event (Abbiss & Laursen, 2008; Thompson, 2014). In contrast, choosing a too fast pace will cause depletion of the limited anaerobic capacity with a concomitant build-up of fatigue-related metabolites early in the event and ultimately lead to poor performance. A number of different pacing profiles have been described in the literature including all-out pacing, positive pacing, negative pacing, even pacing, parabolic-shaped pacing and variable pacing (Abbiss & Laursen, 2008; Thompson, 2014).

The all-out pacing strategy is usually observed in short duration events (≤ 30 - 60 seconds) in which athletes accelerate as quickly as possible to their peak speed and then attempt to maintain it until the end of the event (Thompson, 2014). However, as fatigue is rapidly developed after the acceleration phase, this pacing strategy should only be used in short disciplines, such as 200 m and 1000 m track cycling.

A positive pacing strategy involves completing the first half of an event faster than the second half. Typically, the athlete starts the event with a pace faster than he/she can

maintain for the entire event, leading to a slower finish of the event. It has been reported that the adoption of this pacing strategy leads to an increased $\dot{V}O_2$, greater accumulation of fatigue-related metabolites and increased ratings of perceived exertion during the early stages of an exercise session (Abbiss & Laursen, 2008). Positive pacing profile has been observed during swimming, running, rowing and cycling events that last up to a few minutes as well as during ultra-endurance events (Abbiss & Laursen, 2008).

In contrast to positive pacing strategy, negative pacing strategy involves completing the second half of an event faster than the first half. This pacing strategy has been associated with reduced rate of glycogen depletion, decreased $\dot{V}O_2$, lowered accumulation of fatigue-related metabolites early in the event and improved endurance performance (Abbiss & Laursen, 2008). Indeed, Mattern et al., (2001) showed that adoption of a negative pacing strategy during TT_{20} resulted in significantly better performance than self-paced trials.

Even pacing, which is characterized by minimal accelerations and decelerations from the selected pace, has been suggested to be an 'optimal' strategy for prolonged events under stable external conditions (Abbiss & Laursen, 2008). A good example in which adoption of an even pacing strategy led to successful competitive outcome is provided by Padilla et al., (2000) who analysed the speed of a cyclist who set a world record in 1-hour track cycling. Researchers reported that the cyclist's speed per lap deviated very little from the target speed of 53 km/h or the actual average speed of 53.040 km/h. Similarly, Wilberg and Prat (1988) analysed the race profiles of Canadian national and international 4000 m individual pursuit cyclists and found that cyclists that adopted even pacing strategy were more successful than those who do not.

A parabolic-shaped pacing strategy involves negative and positive pacing in different segments of the event, which may result in U, J and reverse J-shaped pacing profile. However, these pacing profiles have not been well described in the scientific literature until recently. A J-shaped pacing strategy was observed by Garland, (2005) who examined the speed of elite rowers during 2000-m rowing races and time trials. In these events, the initial 500 m were covered in the fastest time, followed by a decrease in the speed throughout the middle 1000 m, and then an increase in the speed again during the last 500 m.

Variable pacing strategy is characterized by fluctuations in pace and is typically adopted in response to inconsistent conditions associated with outdoor events. In cycling, Swain, (1997) demonstrated that the best performance during a TT_{40} on a hilly course with equal sections of tailwind and headwind is achieved by increasing power output on the uphill and headwind sections and decreasing power output on the downhill and tailwind sections. The findings that variations in power output is the best pacing strategy for cycling races under variable external conditions were subsequently confirmed by other researchers (Atkinson & Brunskill, 2000; Atkinson et al., 2007). However, it should be noted that variable power output distribution during a flat TT race with no wind can be detrimental to performance (Thompson, 2014).

1.4. Key adaptations to endurance training

Long-term endurance training, such as cycling, leads to a number of physiological adaptations that serve to improve endurance exercise performance. The most prominent adaptations reported to result from endurance training are increased mitochondrial density and increased capillarity (Hawley & Nadermujika, 2008; Holloszy & Booth, 1976; Taylor & Bachman, 1999).

As described in section 1.2, mitochondria are essential for energy production during prolonged sporting events. It is therefore not surprising that endurance training acts as a potent stimulus for mitochondrial biogenesis. Exercise-induced mitochondrial biogenesis is a complex process that leads to an increase in mitochondrial content and function (Bishop et al., 2014). This process is initiated by the activation of signal kinases such as calmodulin kinase (CaMK), 5' AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK), as a consequence of the exercise-induced metabolic perturbations in skeletal muscle (Coffey & Hawley, 2007). Their activation leads to an increased expression of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α), which has been described as a master regulator of mitochondrial biogenesis (Coffey & Hawley, 2007; Puigserver & Spiegelman, 2003). Activated PGC-1α translocates into the nucleus and activates nuclear respiratory factor 1 (NRF 1) and 2 (NRF 2), both of which activate mitochondrial transcription factor A (TFAM). TFAM then translocates into mitochondria and initiates the transcription and replication of mitochondrial DNA (mtDNA) (Hood, 2009). Recent evidence indicates that tumour suppressor protein p53 also plays a role

in mitochondrial adaptation through interactions with TFAM (Saleem & Hood, 2013). Furthermore, there is evidence to suggest that heat shock protein 72 (HSP72) is involved in the process of mitochondrial biogenesis, though its exact role is yet unknown (Henstridge, 2014).

The most commonly used biomarker for mitochondrial content in skeletal muscle is the activity of citrate synthase (CS) (Bishop et al., 2019; Bishop et al., 2014), which is one of the key enzymes of the Krebs cycle. Recall from section 1.2 that the Krebs cycle facilitates the production of ATP in mitochondria. Mitochondrial function refers to the ability of mitochondria to maintain cellular energetic homeostasis and is often expressed as oxidative capacity. Although it is reasonable to expect that both mitochondrial content and function increase in parallel in response to a training stimulus, available evidence indicates that this is not always the case. Indeed, several studies have reported a dissociation between training-induced adaptations in mitochondrial content and function (Bishop et al., 2019). For example, Granata et al., (2016) reported that 4 weeks of sprint-interval training significantly enhanced mitochondrial respiration without changes in mitochondrial content. In contrast, Montero et al., (2015) found that 6 weeks of endurance training led to an increase in mitochondrial content without significant changes in mitochondrial respiration. This points out the necessity to measure both mitochondrial content and function when conducting training studies. It should be mentioned, however, that adaptations of both mitochondrial content and function to exercise training occur very rapidly. Changes in mitochondrial content have been seen as early as 24 h post single training session (Little et al., 2011), whereas changes in mitochondrial function have been reported after only five training sessions (Starritt et al., 1999).

The increased mitochondrial content has been reported to result in decreased carbohydrate utilization and enhanced reliance on fat as a fuel source during submaximal exercise (Holloszy & Coyle, 1984; Kiens et al., 1993). This shift in substrate use by working muscles attenuates the rate of glycogen breakdown and reduces the formation of lactic acid, which can lead to improved endurance performance (Hawley & Stepto, 2001). In fact, a strong relationship has been reported between exercise performance and muscle mitochondrial content (Ihsan et al., 2014). In addition, a recent study reported that skeletal muscle oxidative capacity was the

main predictor of 26-km TT performance in a group of highly-trained cyclists (Jacobs et al., 2011).

In addition to regulating mitochondrial biogenesis, PGC-1α also drives angiogenic adaptation through induction of vascular endothelial growth factor (VEGF), a factor deemed critical to this process. Angiogenesis refers to the formation of new capillaries from existing capillaries through either sprouting or intussusception (Prior et al., 2004). This results in an increase in the number of capillaries surrounding each muscle fibre. Importantly, the formation of new capillaries can occur in all fibre types. Research indicates that highly-trained endurance athletes can have up to 50% greater capillary density than sedentary individuals (Ingjer, 1979b). Comparison between elite national riders and good regional cyclists revealed that the former group of cyclists had a 23% higher capillary density (Coyle et al., 1991). An improved capillary to fibre ratio allows for greater exchange of gases, waste, heat and nutrients between the circulation and exercising muscles, which can have a direct positive effect on endurance performance (Jones & Carter, 2000; Prior et al., 2004). The importance of muscle capillarization for cycling performance has been emphasized by Coyle et al., (1988). In that study, the authors documented a strong correlation ($r = 0.74$; $p < 0.003$) between capillary density and time to fatigue at 88% of $\dot{V}O_{2max}$ in 14 well-trained male cyclists.

Another major adaptation to endurance training is an expansion in blood volume. On average, endurance-trained individuals have 20-25% greater blood volume than sedentary individuals (Convertino, 1991). The increase in blood volume, however, results primarily from the increase of plasma volume (PV), and this is especially true during the short-term training programs (Convertino, 1991). In a review of 18 studies, Sawka et al., (2000) found that PV can increase by ~10% after 4 consecutive days of endurance exercise training. The paper also reveals that the increase in PV plateaus after approximately two weeks of exercise training. Exercise-induced PV expansion is thought to be mediated by the increase in plasma albumin and increased release of antidiuretic hormone and aldosterone. The former mechanism serves to retain more fluid in the plasma as a result of increased blood osmotic pressure, whereas the latter mechanism increases the PV by stimulating the kidneys to retain water. Furthermore, increased PV leads to increased maximal cardiac stroke volume and increased maximal cardiac output, which in turn result in a reduced HR at rest and during

exercise and increased $\dot{V}O_{2max}$ (Convertino, 1991; Guy et al., 2015). In addition, PV expansion also provides some thermoregulatory benefits such as increased cutaneous blood flow and improved sweat response (Convertino, 1991; Guy et al., 2015).

The beneficial effects of PV expansion on endurance exercise performance have been documented in several studies. In a study conducted on 10 trained male cyclists, Luetkemeier & Thomas (1994) found that hypervolemia induced by 3 consecutive days of exercising (~90 min/day at 67-70% $\dot{V}O_{2max}$) led to significant improvement in TT performance compared with euvolemic state (81.41 ± 5.52 min vs. 90.87 ± 5.27 min; $p < 0.05$). In the same study, similar improvement in TT performance (81.36 ± 5.06 min) was observed following acute PV expansion with dextran infusion (400 ± 121 ml). In another study, Coyle et al., (1990) showed that acute PV expansion by 282 ± 16 ml of 6% dextran solution significantly increased $\dot{V}O_{2max}$ and exercise time to fatigue in 9 untrained male individuals. These results were subsequently confirmed by Berger et al., (2006) who reported increases in $\dot{V}O_{2max}$ and time to exhaustion following intravenous infusion of PV expander (7 ml·kg^{-1}) in 8 recreationally active men. However, it must be noted that the association between PV expansion and performance enhancement is not a universal finding (Karlsen et al., 2015; Keiser et al., 2015; Warburton et al., 2000). Furthermore, the fitness level seems to be a modulator of the functional effects of PV expansion (Warburton et al., 2000).

The magnitude of physiological adaptations in response to endurance training depends largely on the intensity and volume of training as well as the training state of the individual (Bosquet et al., 2002). Untrained individuals generally respond well to any type of training and it appears that the intensity and duration of exercise sessions are less important for this cohort, as long as they train regularly. In contrast, well-trained athletes are highly adapted and their response to exercise stimulus is therefore considerably reduced (Wilmore & Costill, 1994). In this population, the intensity of the training session is a crucial factor in influencing the adaptive responses of physiological systems to exercise (Bosquet et al., 2002; Evertsen et al., 1999; Midgley et al., 2006). Indeed, it has been demonstrated that when a trained person reaches a $\dot{V}O_{2max}$ of over 60 ml·kg^{-1}·min^{-1}, further improvements in endurance performance or related physiological determinants can only be attained through high-intensity interval

training (Laursen & Jenkins, 2002; Londeree, 1997). Other factors that can also influence the magnitude of improvements in physiological adaptations include genetics, nutritional status, age, and lifestyle habits such as alcohol consumption, tobacco use and sleeping patterns.

1.5. High-intensity interval training (HIT) for cycling

The main goal of any training program is to maximize performance during competition. One method of training that has been traditionally used by endurance athletes to improve performance in events of various durations is high-intensity interval training (HIT) (Billat, 2001). This type of training consists of repeated short to moderate duration (30 seconds to 5 minutes) bouts of high-intensity exercise separated by periods of passive or active recovery (Tschakert & Hofmann, 2013). The advantage of this training concept is its ability to train both aerobic and anaerobic energy systems simultaneously (Hazell et al., 2010; Meckel et al., 2012). This is important given the fact that both energy systems contribute to ATP resynthesis during road cycling. Moreover, it appears that further improvements in endurance performance in already highly-trained endurance athletes can only be achieved through HIT (Laursen & Jenkins, 2002). HIT is usually introduced in the weeks leading into the cyclists' competition phase, and is also performed throughout the whole racing season. HIT programs usually manipulate the intensity, duration and number of intervals to achieve the desired performance effects. However, it is currently unclear which HIT approach is most effective.

HIT has a long history and was part of the training programs of many successful athletes before there was any scientific information on this topic. As reviewed by Billat, (2001) HIT has been applied in competitive sports since the early 1900s and was made widely popular in the 1950s by the Olympic champion, Emil Zatopek of Czechoslovakia. HIT helped him to win three long-distance running gold medals at the Helsinki Olympic Games in 1952. About a decade after this historical success, the first scientific studies on HIT were published (Astrand et al., 1960; Christensen et al., 1960). Since then numerous researchers have investigated the physiological and performance responses to HIT. However, very few of these studies have been conducted on highly-trained competitive athletes. This owes to the fact that it is difficult

to convince elite athletes to take part in training intervention studies (Laursen & Jenkins, 2002). The studies pertaining to HIT in road cyclists are outlined below.

Back in the 1990s, sport scientists in South Africa carried out a series of studies that investigated the effects of HIT in well-trained competitive cyclists. The participants in these studies were all men with a training history of at least 3 years and had $\dot{V}O_{2max}$ values of ≥ 65 ml·kg^{-1}·min^{-1}. The cyclists in the studies of Lindsay et al., (1996) and Weston et al., (1996) and Westgarth-Taylor et al., (1997) replaced 15 ± 2% of their normal base training with HIT, which took place once or twice a week for up to 6 weeks. Each HIT consisted of six to nine 5-min repetitions at 80% of PPO (~86% $\dot{V}O_{2max}$) separated by 1 minute of recovery. Before and after the training interventions, all cyclists performed several exercise performance tests on separate occasions, including GXT and TT_{40}.

In the studies of Lindsay et al., (1996) and Weston et al., (1996) 6 HIT sessions performed over a 4-week period led to significant improvements in the ride to fatigue at 150% of PPO (+22%, for both studies), PPO (+3.5 – 4.3%) and TT_{40} performance (+2.2 – 3.5%). Lindsay et al., (1996) also reported that cyclists were able to sustain significantly higher absolute (301 vs 326 W) and relative power output (72% vs 75% of PPO) during TT_{40} after HIT. In addition, Weston et al., (1996) observed a high correlation ($r = – 0.91$; $p < 0.01$) between baseline PPO and TT_{40} performance, which reinforces the idea that PPO can serve as a strong predictor of cycling performance (see section 1.3.3). Furthermore, biopsies taken from the *vastus lateralis* of the cyclists in the study of Weston et al., (1996) showed significant increases in muscle buffering capacity after the training intervention, which was related ($r = – 0.74$) to the improved TT_{40} performance. However, maximal activity of mitochondrial enzymes did not change with HIT. In contrast, rapid increases in maximal activity of CS are observed after HIT in recreationally active individuals (Little et al., 2010; Perry et al., 2010). One possible explanation as to why the HIT did not induce further adaptation in the mitochondria in the study of Weston et al., (1996) is that the intervention period was not long enough to detect measurable changes in that system in already well-trained athletes. Alternatively, there may be a ceiling for adaptation in mitochondrial oxidative capacity in skeletal muscles of well-trained athletes.

In the study of Westgarth-Taylor et al., (1997) 12 HIT sessions undertaken over 6 weeks increased PPO values by 4.9% (from 404 to 424 W) and decreased 40-km cycling time by 3.5% (from 57.2 to 55.8 min). These researchers observed that HIT increased the absolute (291 vs 327 W) and relative (72% vs 78% of PPO) power output during TT_{40}, which is in line with the findings of Lindsay et al., (1996). The study also investigated the metabolic adaptations to the HIT protocol and found that changes in metabolism occurred at absolute intensities, but not at relative intensities. Indeed, HIT decreased the rate of carbohydrate oxidation and plasma lactate accumulation during 10-min rides at power output of 60, 70 and 80% of pre-HIT PPO. However, the rates of carbohydrate oxidation and plasma lactate accumulation were similar before and after HIT when the cyclists exercised at 60, 70 and 80% of their post-HIT PPO. Thus, reduction in the carbohydrate oxidation and plasma lactate accumulation at the same absolute sub-maximal power output after HIT were probably a result of the cyclists exercising at lower relative intensities.

In the last of the series of studies from South Africa, Stepto et al., (1999) investigated the effects of different HIT protocols on TT_{40} performance. Nineteen cyclists were randomly assigned to one of five HIT groups: 4 x 8 min at 80% PPO, 8 x 4 min at 85% PPO, 12 x 2 min at 90% PPO, 12 x 60 seconds at 100 % PPO or 12 x 30 seconds at 175% PPO. After 6 sessions over a 3-week period, the greatest improvement (+ 2.8%) in TT_{40} performance was observed with a HIT protocol consisting of 8 x 4 min at 85% PPO. The authors expected this outcome, given that this intensity was closely matched to the average time-trial intensity. This finding lends support to the idea that cyclists should train for racing at exercise intensities specific to their event. Furthermore, 30 seconds supramaximal work bouts were nearly as effective for improving TT_{40} performance (+ 2.4%) as the 4-minute interval. The other HIT protocols failed to significantly improve TT_{40} performance. Again, PPO was highly correlated with TT_{40} performance ($r = - 0.82$; $p < 0.0002$). However, the limitation of this study was the small sample size in each group ($n = 4$).

In a similar study, Laursen et al., (2002) examined the effects of three different HIT protocols on cycling performance in 38 highly-trained ($\dot{V}O_{2peak}$ = 64 ± 5.2 ml·kg^{-1}·min^{-1}) male cyclists and triathletes. Before and after 4 weeks of training, all participants performed a GXT, a time to exhaustion test at their PPO and a TT_{40}. The participants

were assigned to one of the following groups. Group 1 performed 8 intervals at PPO for a duration equal to 60% of the time to exhaustion, with a 1:2 recovery ratio. Group 2 performed 8 intervals at the same intensity as group 1, but the recovery time was based on HR returning to 65% of maximal HR. Group 3 completed twelve 30-seconds intervals at 175% of PPO, with 4.5 min of recovery between each interval. The participants in the control group were instructed to maintain their regular low-intensity training program. All HIT groups trained twice per week.

All HIT protocols led to substantial improvements in PPO (+ 3.0 – 6.2%) and TT_{40} performance (+ 4.4 – 5.8%), which was not the case with the control group. Furthermore, participants in group 1 (+ 5.4%) and group 2 (+ 8.1%) significantly improved their peak oxygen consumption ($\dot{V}O_{2peak}$). The findings of this study are in agreement with the findings of Stepto et al. who showed that supramaximal HIT can significantly improve TT_{40} performance. However, the improvements in TT_{40} and PPO in this study were slightly higher than those reported in the above studies. Therefore, Laursen et al., (2002) suggested that their HIT protocols provide a higher training stimulus for adaptations and performance improvement than the intervals used in the previous studies.

In a separate paper, Laursen et al., (2005) described the physiological adaptations associated with their HIT protocols. The variables of interest were V_1, V_2, anaerobic capacity (estimated via accumulated oxygen deficit method) and resting PV. After 4 weeks of training, V_1, V_2 and anaerobic capacity were significantly increased in all HIT groups, but not in the control group. However, resting PV remained unchanged after the training period. Furthermore, improvements in TT_{40} performance were modestly related to significant increases in $\dot{V}O_{2peak}$, V_1, V_2 and anaerobic capacity ($r = 0.41, 0.34, 0.43, 0.40$, respectively; all $p < 0.05$). Since PV was unchanged, the authors suggested that improvements in exercise performance seen after HIT in well-trained endurance athletes are achieved through peripheral adaptation, rather than central adaptation.

The effects of different HIT protocols on cycling performance were also investigated in the most recent study by Seiler et al., (2013). Thirty-five (29 male and 6 female) recreational cyclists ($\dot{V}O_{2peak} = 52 \pm 6$ ml·kg^{-1}·min^{-1}) were randomized to four training groups. The cyclists in the HIT groups completed 2 interval training sessions per week of either 4 x 4 min, 4 x 8 min or 4 x 16 min. All three groups completed an additional 2

to 3 weekly exercise sessions at low intensity. The control group performed 4 to 6 unsupervised exercise sessions per week at a low intensity. A GXT and time to exhaustion test at 80% of $\dot{V}O_{2peak}$ were performed before and after 7 weeks of training. The cyclists were instructed to perform the interval training sessions at their maximal sustainable intensity, which led to training being performed at 88% of HR_{max} in the 4 x 16 min group, 90% of HR_{max} in the 4 x 8 min group and 94% of HR_{max} in the 4 x4 min group. Only 4 x 8 min group improved significantly in all performance measures ($\dot{V}O_{2peak}$, PPO, power at 4 mmol/L and ride to fatigue at 80% of $\dot{V}O_{2peak}$) after the intervention period. Furthermore, ride to fatigue at 80% of $\dot{V}O_{2peak}$ was significantly improved in all HIT groups, but not in the control group. The authors concluded that accumulating 32 min of work at 90% of HR_{max} induced greater adaptive gains than accumulating 64 min of work at 88% of HR_{max} or 16 min of work at 94% of $HR_{max.}$ Whether similar performance improvements can be achieved in well-trained cyclists is unknown.

Swart et al., (2009) compared the effects of power-based intervals versus HR-based intervals on cycling performance. In this study, 17 trained male cyclists ($\dot{V}O_{2max}$ = 58.6 ml·kg^{-1}·min^{-1}) were randomly assigned to two interval training groups and a control group. Each cyclist performed 2 supervised training sessions per week over 4 weeks. HIT groups performed 8 intervals of 4 minutes at either 80% of PPO or at the HR corresponding to 80% of PPO. Control group completed a self-paced 40-km ride at an intensity below 70% of PPO. Testing took place at baseline and postintervention. The results have showed significant improvements in TT_{40} and PPO in both HIT groups compared to the control group. However, there were no significant differences between the HIT groups. These findings were subsequently confirmed by Robinson et al., (2011) in recreational cyclists. The authors found that HIT (twice a week over 5 weeks) prescribed using either power or HR resulted in similar improvements in TT_{20} performance and power at LT. In addition to documenting the beneficial performance effects of HIT, these studies clearly indicate that both methods can be equal effective for regulating intensity during interval training sessions.

Collectively, the findings from these studies indicate that short-term HIT is an effective strategy to improve exercise performance in already well-trained cyclists, though the

mechanisms behind these improvements are not clear. As little as six HIT sessions are sufficient to enhance TT performance.

1.6. Cycling in the heat

It is well-established that endurance capacity is diminished when cycling in the heat (Périard & Racinais, 2019). For example, Galloway & Maughan (1997) showed that cycling time to exhaustion at 70% of $\dot{V}O_{2max}$ was significantly shorter in hot (30.5°C) condition when compared with the air temperature of 10.5°C (51.6 ± 3.7 min vs. 93.5 ± 6,2 min; $p < 0.05$). Racinais et al., (2015b) also reported that time to complete a 43-km cycling TT was significantly longer in hot (37°C) than in cool (8°C) conditions (77.1 ± 6.2 min vs. 66.1 ± 3.2 min; $p < 0.01$). Hyperthermia-mediated alteration in the function of the cardiovascular and central nervous system has been pointed out as the main factor responsible for the degradation of performance during prolonged, submaximal exercise in the heat (Nybo, 2010). In extreme situations, exercising in the heat can cause a rise in hyperthermia to such a critical level that it does not only impair performance, but also may ultimately lead to exertional heat stroke (EHS) (Armstrong et al., 2007, Kjertakov & Epstein, 2013). Although EHS incidents have been more frequently reported in American-type football players and distance runners than in road cyclists, any athlete who exercises under high heat stress is at risk of this potentially fatal heat illness (Armstrong et al., 2007).

Various strategies have been introduced in order to minimise the risk of heat illness and decline in performance during training and competition in the heat. This section will focus on heat acclimation. Other heat-mitigation strategies are covered elsewhere (Armstrong et al., 2007; Racinais et al., 2015a).

1.6.1. Heat acclimation

Repeated exposure to exercise in a hot environment has been recommended as a strategy to induce physiological adaptations that will improve thermoregulation, attenuate physiological strain and enhance exercise performance during subsequent heat exposure (Maughan & Shirreffs, 2004; Périard et a., 2015). The physiological adaptations include, but are not limited to, improved cardiovascular stability (Périard et a., 2016), reduced resting and exercising body core temperature (Taylor, 2014), increased skin blood flow and sweating (Périard et a., 2015) and improved skeletal muscle metabolism (Febbraio et al., 1994; Kirwan et al., 1987). The process of

adaptation to heat can be induced in naturally hot environments (i.e. acclimatisation) and in artificial hot environments (i.e. acclimation). Both strategies will be referred to as heat acclimation (HA) throughout the text.

1.6.1.1. Heat acclimation methods

Heat acclimation can be achieved by using one or a combination of the following protocols: self-regulated exercise, fixed intensity and controlled hyperthermia or also known as isothermal strain (Périard e a., 2015). Although a combination of exercise in the heat is the most effective approach for attaining HA, passive heat exposure can also induce some adaptations.

Self-regulated HA method allows athletes to choose their own exercise intensity based on their fitness level and perceived stress. This approach also minimises the potential for premature termination of HA session due to hyperthermia. However, self-regulated protocols are of limited usefulness from a research aspect because the intensity of exercise and thus metabolic heat production is not standardised. As such, the use of this HA protocol is limited to practical settings only.

Fixed intensity method requires athletes to exercise at fixed intensity during HA session. Historically, this is the most commonly used HA method and as a result, ample evidence exists regarding the physiological responses to such method (Pryor et al., 2018; Racinais et al., 2019). From this evidence, it could be concluded that the best protocol consists of exercise for 60-100 min per day, at intensities > 40% $\dot{V}O_{2max}$ for 10-14 days in conditions that simulate the anticipated environment. Although fixed intensity protocols are easy to administer, they also have disadvantages. Given that this method requires athletes to exercise at the same absolute intensity, the thermal strain they experience will be variable because of difference in metabolic heat production at individual's relative percentage of peak workload. As such, this method may not always induce the same adaptive response across participants. Furthermore, since both the exercise intensity and heat stress are constant throughout the programme, the relative thermal strain diminishes as adaptation takes place and as a consequence, the stimulus for adaptation is reduced. Thus, fixed intensity protocols appear inefficient and call for gradual progression in either heat stress or exercise intensity to maintain the thermal load throughout the duration of the HA programme and maximise the stimulus for adaptation. Consequently, progressive HA protocol has

been developed by Costa et al., (2014) and successfully implemented in preparation for ultramarathon running in the heat. The researchers had 6 non-heat acclimatised male ultra-endurance runners complete three, 2-h treadmill running sessions at 60% $\dot{V}O_{2max}$ in 30°C, followed by three more sessions in 35°C and observed a significantly lower body core temperature, HR and thermal comfort rating during the sixth session compared with the fourth session. Furthermore, the third session resulted in a significantly lower increase in these variables than in the first session. However, the lack of a control group who underwent traditional fixed intensity HA protocol makes it difficult to compare the improvements in this study to existing literature.

Controlled hyperthermia method involves elevation and maintenance of constant body core temperature during a HA session. This approach provides equal thermal strain across participants and it is thought to offer more complete adaptation to fixed intensity HA method. In comparison with the latter method, the isothermal method ensures that the strength of the adaptation stimulus is kept constant throughout the duration of the HA protocol. Recently, Garret et al., (2009) demonstrated the effectiveness of the isothermal method in a short-term (5-day) HA programme during which 10 non-acclimatised moderately trained males exercised daily for 90 min in 40°C at a fixed body core temperature of 38.5°C. Prior to and post HA period, a heat stress test was conducted by having the participants cycle for 90 min at 40% of PPO in 35°C. The researchers reported significantly lower values for body core temperature and HR at the end of the second heat stress test compared with the values obtained at the end of the first test. More recently, Gibson et al., (2015) directly compared isothermal and fixed intensity methods of HA and did not find differences in physiological adaptation between these two methods after 5 and 10 days of HA. The findings of this study do not support the belief that isothermal HA method is superior to the fixed intensity HA method.

Passive HA involves use of exogenous heat stress to induce hyperthermia, with a minimal metabolic contribution. Passive heating can be achieved through hot-water immersion and by exposure to heated environmental chamber, steam room or sauna (Heathcote et al., 2018; Taylor, 2014). Although passive HA may provide some cardiovascular and thermoregulatory benefits, this method has been reported to be less effective than active HA (Taylor, 2014). This is not surprising knowing that the

elevation of skin and body core temperature, a physiological response that drives the HA adaptations, is heightened when exercising in the heat, resulting in sustained adaptation stimulus (Périard et a., 2015).

1.6.1.2. Induction and decay of HA

In general, HA is a rapid process that begins on the first day of heat exposure. During the initial exposure to heat stress, the physiological strain is high, as demonstrated by an elevated HR and body temperature. However, the physiological strain caused by heat stress progressively decreases each day of HA period (Périard et a., 2015). The timeline of physiological adaptations to heat can be classified into short term (< 7 days), medium term (8-14 days) and long term (> 14 days) (Périard et a., 2015; Pryor et al., 2018). Importantly, 75-80% of adaptations that influence exercise performance occur in the first 4-7 days of HA process (Pandolf, 1998). Cardiovascular changes are the earliest adaptive response to chronic heat exposure and they are characterised by a reduction in resting and exercising HR and hypervolemia (Armstrong & Maresh, 1991; Périard et a., 2015). Expansion of PV leads to increased stroke volume, which is thought to be the primary mechanism for reduced HR. These improvements reduce the cardiovascular strain experienced by an athlete for any given exercise intensity. PV has been reported to increase in a range between 3% to 27% within the first 5 days of onset of a HA protocol (Périard et a., 2016; Racinais et al., 2019). The magnitude of increase in PV depends on the HA protocol, the hydration status when measured and the participant's fitness status (Périard et a., 2015). There is also some evidence that dehydration can lead to a greater PV expansion during a HA intervention compared with euhydration (Garrett et al., 2014).

The reduction of body core temperature at rest and during exercise occurs following 5 to 8 days of onset of HA process (Armstrong & Maresh, 1991). The reported reduction of body temperature in response to HA is between 0.2 – 1.0 °C, depending upon the protocol and length of HA (Garrett et al., 2009; Garrett et al., 2014; Gibson et al., 2015; Gill & Sleivert, 2001; Lorenzo et al., 2010; Weller et al., 2007). This adaptation allows exercising athletes to experience a greater magnitude of body temperature increase before the threshold associated with the performance decrement is surpassed (Nielsen et al., 1993). However, the exact mechanisms behind the HA-induced decrease in body temperature remain to be elucidated (Taylor, 2014).

An enhanced sweating response is observed after 9 days of exercising in the heat (Armstrong & Maresh, 1991; Périard et a., 2015). This adaptation is characterised by reduced body temperature threshold for the onset of sweating, increased skin blood flow and increased sweat rate. All these changes allow an athlete to more effectively thermoregulate during exercise in the heat. However, increased capacity for evaporative heat loss also results in greater body water loss and thus increased risk of dehydration and associated ill effects. A well-trained, heat acclimated endurance athlete may experience sweat losses in excess of $3\,l \cdot h^{-1}$ during intensive exercise in the heat (Armstrong et al., 1986). In addition to increased sweating with HA, sweat becomes more dilute, resulting in less loss of electrolytes (Allan & Wilson, 1971; Chinevere et al., 2008). Another adaptation reported to occur after 9 days of HA is reduced blood and muscle lactate concentrations during submaximal exercise (Young et al., 1985). Although complete cardiovascular and thermoregulatory adaptations to heat are usually achieved by 10 to 14 days of heat exposure (Armstrong & Maresh, 1991; Périard et a., 2015; Racinais et al., 2019), it is thought that longer HA protocols may provide further performance-enhancing adaptations (Pryor et al., 2018). However, this has not been tested in athletes. Ergogenic effects of short-term (≤ 7 days) and medium-term (7 - 14 days) HA programs on exercise performance in the heat have been demonstrated in numerous papers (Chalmers et al., 2014; Tyler et al., 2016).

Unfortunately, the benefits obtained during HA will gradually disappear after heat exposure is ceased. The adaptations that are first induced during HA have a faster rate of decay than the adaptations that take longer to develop. For example, Flouris et al., (2014) showed that following a 14-day fixed intensity HA regimen, 26% and 60% of the HA-induced adaptations in body core temperature and HR, respectively, were lost after 26 days without heat exposure. In contrast, the beneficial effects of the 5 days of HA reported in the study from Garret et al., (2009) were completely lost after 2 weeks of cessation of exercising in the heat. The findings of these studies also indicate that the rate of decay of HA depends on the length of the acclimation protocol. Importantly, 2-4 days of re-acclimation have been shown to be enough to regain complete HA after 26 days without heat exposure (Weller et al., 2007).

1.6.1.3. Effects of HA on endurance exercise performance in temperate/cool environments

Although the ergogenic effects of HA for exercise performance in hot conditions have been well-known for years, the potential of HA to improve performance in nonthermally challenging environments has been recognised recently (Corbett et al., 2014). Lorenzo et al., (2010) were the first researchers to report that HA enhances exercise performance in both hot (38°C, 30% humidity) and cool (13°C, 30% humidity) environmental conditions. In that study, highly trained cyclists were assigned to either HA group (10 male and 2 female) or control group (7 male and 1 female). The HA group cycled for 90 min at 50% of $\dot{V}O_{2max}$ for ten consecutive days in 40°C, and the control group completed 10 days of identical exercise in 13°C. Prior to and following the intervention, the participants performed $\dot{V}O_{2max}$ test, LT test and 1-hour TT test in both hot and cool conditions. Compared with training in cool conditions, HA resulted in significant improvement in power output at LT (5% in both cool and hot conditions) $\dot{V}O_{2max}$ (5% in cool and 8% in hot conditions) and TT performance (6% in cool and 8% in hot conditions). These improvements coincided with an increase of PV by 6.5% and maximal cardiac output by 9.1% in cool and by 4.6% in hot conditions. These findings are further supported by Rendell et al., (2017) who recruited 8 trained ($\dot{V}O_{2max}$ = 58.6 ± 8.9 ml·kg^{-1}·min^{-1}) male cyclists to examine the effects of 11 days of controlled hyperthermia HA protocol on cycling performance in temperate conditions (22°C, 55% humidity). The researchers found an increase in PV of 5.9% and significant improvement in PPO, power output at LT and 30 min TT performance. Interestingly, supplementing the HA with an overnight hypoxic stimulus did not increase exercise performance significantly more than HA alone. In another study, Neal et al., (2016) reported an enhanced power output at LT and PPO in temperate conditions (22°C, 60% humidity) after only five days of HA in 10 trained ($\dot{V}O_{2max}$ = 63.3 ± 4 ml·kg^{-1}·min^{-1}) male cyclists and triathletes. However, despite these improvements, TT_{20} performance was not changed. Another two HA studies also failed to demonstrate an improvement in TT performance in cool conditions in trained cyclists (Karlsen et al., 2015; Keiser et al., 2015). Similarly, McCleave et al., (2016) did not observe improvements in 3 km running performance under the environmental conditions ranging between 13 and 19°C after 9 exercise sessions in the heat performed over a period of 3 weeks.

Nevertheless, the findings of Lorenzo et al., (2010) and Rendell et al., (2017) indicate that HA has a potential to be used as a preparatory strategy for performance in cooler environments. It should be noted, however, that heat training is not without limitations. For example, given that the capacity to exercise is significantly reduced in hot environments (Galloway & Maughan, 1997; Racinais et al., 2015b), the quality of training may be compromised. Furthermore, this training strategy also can be impractical for athletes living in cooler climates.

To overcome these barriers, some researchers have advanced the idea that the desired physiological and thermoregulation adaptations can be elicited by applying heat stress (e.g. sauna bathing or hot water immersion) immediately following the exercise session, a scenario that would not interfere with a normal training program (Casadio et al., 2017). The rationale behind this idea comes from the observation that thermal strain imposed by the sauna bathing can be maximised if the sauna session is implemented right after the exercise when the core temperature is still elevated (Ridge & Pyke, 1986). Given this, it is not unreasonable to expect that post-exercise heat exposure may provide a strong-enough stimulus for performance-enhancing adaptations. The support for this idea comes from a study by Scoon et al., (2007) who documented that post-exercise sauna bathing improves endurance performance in temperate environment (Personal communication with the authors). In this study, the authors had male trained distance runners take 30 min sauna baths at ~90°C immediately following running on 12 occasions over 3 weeks and observed a 7.1% greater PV and 32% improvement in run time to exhaustion at 5 km run speed in a temperate environment compared with a 3-week period of normal training. Based on the observed high correlation ($r = 0.96$) between the change in PV and the change in running performance, the authors have suggested that the expansion of PV was responsible for the performance enhancement. The mechanisms underlying exercise/heat-induced PV expansion and associated performance-beneficial effects have been described in section 1.4.

Furthermore, Scoon et al., (2007) have speculated that adaptations other than PV expansion that lead to increased oxygen delivery to the muscles also could have contributed to the enhancement of running performance. With respect to this notion, there is evidence to suggest that heat stress may increase muscle capillarization, an

adaptation that may confer performance improvements by improved delivery of oxygen to mitochondria (Prior et al., 2004). To date, several studies have documented that heat therapy increases angiogenesis in animal models (Akasaki et al., 2006; Gong et al., 2006; Huang et al., 2012), and a most recent study by Kuhlenhoelter et al., (2016) yielded promising findings regarding the ability of heat stress to induce angiogenesis in humans. This study evaluated the acute effects of lower limb heating (via liquid-circulating garment) on the expression of angiogenic factors, including VEGF, and found that in comparison to control intervention (33°C), heat treatment (48°C) significantly increased expression of these factors. Over the long term, such responses may augment angiogenesis. At present, however, no studies have examined the effects of repeated heat application on chronic angiogenic adaptation in humans.

Another adaptation that might have been elicited by the sauna bathing is increased mitochondrial biogenesis. Indeed, utilising a study design similar to that in the Scoon study, Tamura et al., (2014) have shown that post-exercise heat exposure can additively enhance training-induced mitochondrial adaptation. In that study, mice that were placed in a heat chamber at 41°C for half an hour immediately after treadmill running (5 days a week) demonstrated greater enhancement of mitochondrial biogenesis (evidenced by an increase in mitochondrial enzyme activities and respiratory chain protein content) compared with an exercise-only group after three weeks of training. Theoretically, increased mitochondrial capacity should lead to improved endurance performance by reducing muscle glycogen depletion during exercise as a result of decreased carbohydrate utilization and enhanced reliance on fat as a fuel source, but the implications of the findings of the aforementioned study for exercise performance are unknown as this was not assessed. This study also attempted to elucidate the mechanisms underlying heat stress-induced mitochondrial adaptation by examining the activation status of upstream regulatory factors of PGC-1α, and it was observed that post-exercise heat exposure significantly increased p38 MAPK activation, but paradoxically, AMPK was downregulated, whereas the activity of CaMK was not affected. From these findings, the authors suggested that heat stress-induced mitochondrial adaptation was mediated primarily by p38 MAPK signalling. These data, however, were collected following acute post-exercise heat exposure, and the signalling pathways were not examined following the chronic phase

of the study. Furthermore, PGC-1α was not measured, which is unexpected given the known role it has in regulating mitochondrial biogenesis. Thus, the exact mechanism by which heat stress-induced mitochondrial adaptation has not been defined.

Elsewhere, Liu and Brooks (2011) reported that exposing C2C12 myotubes to 40°C for 1 hour upregulated AMPK activity and increased PGC-1α expression. Most recently, however, Petrie et al., (2016) failed to validate the latter finding in human model. In that study, 30 min of sauna bathing resulted in downregulation of PGC-1α. This implies that the heat stress may attenuate mitochondrial biogenesis in humans, by supressing PGC-1α expression. Nevertheless, the potential for using heat stress as a stimulus to induce adaptation at cellular level in athletes should not be dismissed because of the following. First, it's possible that the authors of the aforementioned study failed to detect any heat-induced increase in PGC-1α levels since the measurement of this variable took place only at one-time point after the treatment (i.e. at 3 hours post-treatment). For instance, the observed increase of PGC-1α in the study of Liu & Brooks (2011) took place at 24 hours post-treatment, although its levels were unchanged at 2 hours post-treatment. Second, absence of PGC-1α expression does not necessary translate into blunted mitochondrial adaptation. This notion is supported by the work of Leick et al., (2008) in which PGC-1α knockout mice were still able to increase mitochondrial content in response to exercise. Third, as Petrie's study has addressed only the effects of acute heat exposure, it is impossible to predict what influence chronic heat stress would have on mitochondrial biogenesis in humans. In this respect, the research of Tamura et al., (2014) provides a strong base to study the effects of post-exercise heat exposure on mitochondrial adaptation in athletes and its potential implications on endurance performance. Furthermore, as the mechanisms responsible for heat-induced mitochondrial adaptation have not been fully elucidated, it also will be of great interest to address this issue.

The study of Scoon et al., (2007) clearly indicates that post-exercise sauna bathing is an efficacious approach in potentiating physiological and performance adaptations. Sauna bathing, however, may not be feasible in many situations. A recent study by Zurawlew et al., (2016) lends support to the idea that hot water immersion could be a viable alternative to sauna bathing. The researchers recruited seventeen physically active, non-heat acclimatised males who completed a 6-day intervention consisting of

daily running for 40 min at 65% of $\dot{V}O_{2max}$ in temperate conditions (18°C, 40% humidity) followed immediately by either hot (40°C; $n = 10$) or thermoneutral (34°C; $n = 7$) water immersion up to the neck for 40 min. Before and after the intervention, the participants completed a 5-km run TT in temperate (18°C, 40% humidity) and hot (33°C, 40% humidity) conditions. Compared with the thermoneutral-water condition, the hot-water group demonstrated an increase in PV of 3% and improvement in 5-km run TT performance in hot conditions of 4.9%, though the running performance was unchanged in temperate conditions. A possible explanation as to why the intervention did not confer ergogenic benefits under cooler conditions, is that the study period was not long enough to allow some important physiological adaptations to develop. This warrants further investigation.

Future studies may need to modify the water immersion protocol developed by Zurawlew et al., (2016) because this protocol caused premature cessation of hot water immersion sessions due to either extreme hyperthermia (>39.5°C) or intolerable discomfort (Zurawlew et al., 2018a; Zurawlew et a., 2018b; Zurawlew et al., 2016). In a study that compared the physiological responses to 30 min of lower body hot (42°C) water immersion versus an equal duration of exercise at 65-75% of age-predicted maximal HR, Thomas et al., (2016) reported that the heating protocol was well-tolerated in their participants. Importantly, hot water immersion treatment led to increased core and muscle temperatures by 1.3 ± 0.4°C and 4.7 ± 0.9°C, respectively, values which were significantly greater than those measured in the exercise group. If applied immediately after exercise, this hot water immersion protocol could provide strong-enough heating stimulus to maintain already elevated core and muscle temperatures and thus potentially prolonging the training stimulus.

AIM OF THE STUDY

The aim of the study is to investigate the effects of post-exercise hot water immersion over a period of 3 weeks on subsequent performance and selected physiological adaptations in trained road cyclists.

Objectives

- To determine whether 3 weeks of post-exercise hot water immersion can improve TT_{20} performance.
- To determine whether 3 weeks of post-exercise hot water immersion can improve LTP, $\dot{V}O_{2max,}$ and PPO.
- To determine the effects of post-exercise hot water immersion on mitochondrial function.
- To determine the effects of post-exercise hot water immersion on PV.
- To determine the effects of HIT on TT_{20} performance, LTP, $\dot{V}O_{2max}$, PPO, mitochondrial function and PV.

HYPOTHESES

- Regular post-exercise hot water immersion will improve TT_{20}, LTP, $\dot{V}O_{2max}$ and PPO in trained road cyclists.
- Regular post-exercise hot water immersion will have beneficial effects on mitochondrial function and PV.
- HIT will have beneficial effects on TT_{20} performance, LTP, $\dot{V}O_{2max}$, PPO, mitochondrial function and PV.

CHAPTER TWO - METHODOLOGY

2.1. Participants

G*power 3.1.9.2 software was used to calculate the sample size required to achieve statistical significance in TT_{20} performance post-intervention. The calculation was based on the paper by Scoon et al., (2007) who found that, relative to control, 3 weeks of post-exercise sauna bathing increased run time to exhaustion at 5 km run speed by 32% (14.1 ± 2.1 min vs. 18.2 ± 2.0 min). From these findings, it was calculated that a minimum of 12 participants (6 participants per group) would be required to achieve a statistical power of 0.80 and a significance level of 0.05. Initially, there were seventeen trained male cyclists who volunteered for this study. However, since three participants failed to finish the study, the final sample size for analysis was fourteen. The participants were recruited from local cycling clubs and from advertisements posted on social media. To qualify for this study, participants had to be: 1) male, 2) aged 18 – 45, 3) cycling at least three times a week, 4) free from known cardiovascular, haematological or metabolic disorders. After being fully informed of the study requirements and screening for possible exclusion criteria (Appendices A and B), participants signed informed consent (Appendix C). The study was approved by the Victoria University Research Ethics Committee (HRE-18-033) and conducted in accordance with the Declaration of Helsinki.

2.2. Experimental design

Prior to the baseline testing, all participants performed a familiarisation session of both a GXT and a TT_{20} in order to increase the reliability of the subsequent testing (Zavorsky et al., 2007). At baseline a GXT, a TT_{20} and a muscle biopsy were performed on three different days separated by one day of rest. Following this, the participants were matched by their TT_{20} performance and $\dot{V}O_{2max}$ and assigned to either a hot water immersion (HWI) group or a control group (CON). This approach results in homogeneity of the training groups. The rationale behind this idea comes from the observation that randomising participants to training groups has produced non-homogeneity in the groups that affected the study outcome (Laursen et al., 2002). The intervention period lasted for 3 weeks during which the participants performed three high intensity interval training session per week and each training session was followed by immersion in either hot water (HWI) or thermoneutral water (CON). The

acute effect of water immersion on body core temperature and thigh muscle (*vastus lateralis*) temperature were measured during the first water immersion sessions and 30 min post-immersion. Venous blood was sampled at rest before this training session. Within one week of the last training session the testing and the blood and muscle sampling were repeated. A schematic of the experimental design for the entire study is shown in Figure 2.1.
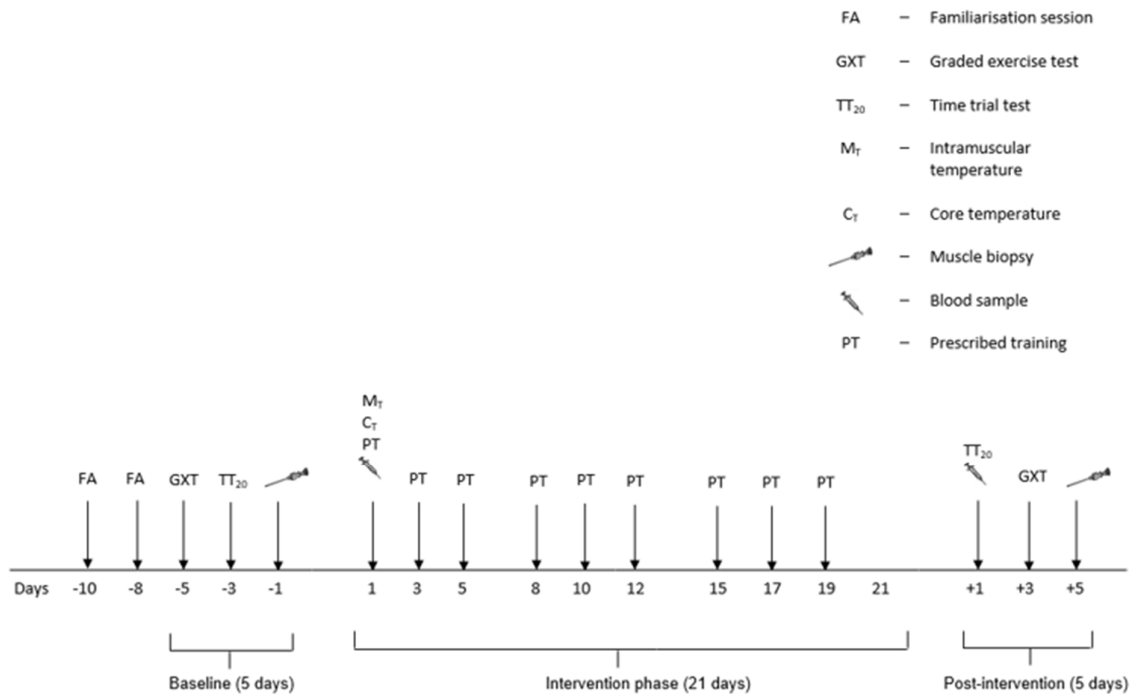
Figure 2.1. Study overview

2.3. General procedures

All testing and training were conducted in the exercise physiology laboratory at the Victoria University. The air temperature in the laboratory was maintained between 18°C and 21°C and the participants were fan-cooled during exercise. The participants were asked to maintain their usual diet and to abstain from alcohol and any antioxidant supplements throughout the study. Recently, these supplements were shown to impair training-induced mitochondrial adaptations (Gomez-Cabrera et al., 2008; Paulsen et al., 2014; Strobel et al., 2011). Dietary supplement use was assessed prior to the study by using a previously developed questionnaire (Kjertakov et al., 2013), which helped to identify a possible use of antioxidant supplements among the participants. Participants were allowed to drink water *ad libitum* during all testing and training

sessions, except for the first training session (see section 2.11). Heart rate was recorded during all training sessions and performance tests using a Polar RS 800 heart rate monitor (Polar Electro OY, Kempele, Finland). To confirm stable training status prior to the study, the participants were asked to maintain their normal training and keep a training log during the last 4 weeks prior to the commencement of the study. Examination of the training logs revealed no significant difference between the CON group and the HWI group in self-reported training hours (38.6 ± 10.1 vs. 33.7 ± 14.9 hours, respectively, p = 0.61) during the 4-week period prior to baseline testing.

2.4. Testing procedures

For each participant, all tests were performed at the same time of the day in order to avoid possible diurnal effects (Reilly et al., 1984). The participants were instructed to refrain from intensive or prolonged exercise the day before each test. They were asked to report for testing in well-hydrated state, at least 2 hours postprandial and not to have consumed caffeine for a minimum of 24 h. Upon arrival at the laboratory, hydration status was measured via USG using an optical refractometer (URC-NE, Atago, Japan) and hypohydration threshold was set at USG ≥ 1.020 (Sawka et a., 2007). If a participant arrived at the laboratory hypohydrated, he was provided with 600 ml of water and hydration status was reassessed after 30-40 min, as recommended by Logan-Sprenger & Spriet, (2013). This time frame was sufficient for all participants to become euhydrated. Participants were also asked to record their dietary intake for the 24 hours preceding the first test and asked to repeat the same nutritional intake prior to each subsequent test.

2.5. Graded exercise test

A continuous graded exercise test was conducted on an electronically-braked cycle ergometer (Lode Excalibur Sport, v2.0, Groningen, The Netherlands) to determine each participant's LTP, $\dot{V}O_{2max}$ and PPO, as described previously (Bell et a., 2017; Kristoffersen et al., 2014; Rønnestad et al., 2015). Briefly, for LTP determination, the initial workload was set at 100 W, with increments of 30 W every 5 min until BL concentration reached ≥4 mmol·L^{-1}. Blood samples were obtained from a fingertip at rest, and during the last 30 s of each stage and immediately analysed using a hand-held lactate analyser (Lactate Pro 2, Arkray Inc., Japan). The validity of this device has been previously confirmed (Bonaventura et al., 2015). The fingertip was cleaned

with an alcohol swab, dried and then punctured with a lancet (Accu-Check Safe-T-Pro Plus, Roche Diagnostics, Mannheim, Germany). The data obtained from the test were entered in Lactate-E computer software (Newell et al., 2007) through which LTP was identified as the power output corresponding to BL concentration of 4 mmol·L^{-1}. After completing the LTP test, the participants rested for 15 min before they performed the $\dot{V}O_{2max}$ and PPO test. Following a light warm up, this test started at 30 W below the last completed stage for the LTP test, with subsequent increments of 30 W every minute until exhaustion. Oxygen consumption and $\dot{V}CO2$ were continuously measured with a gas analyser (Quark, Cosmed, Rome, Italy), which was calibrated immediately before each test. Breath-by-breath data for each participant were edited to remove occasional breaths caused by, for example, coughs, swallows, or sighs, and which were not related to the physiological response of interest, as described previously (Rossiter et al., 2006). Editing was performed on $\dot{V}O_2$ data applying the criterion of breaths lying outside three SDs of the local mean. Finally, $\dot{V}O_{2max}$ was defined as the highest recorded $\dot{V}O_2$ reading over a 30-s period. Peak power output was defined as the power output of the last completed stage of the $\dot{V}O_{2max}$ test. If the last exercise stage was not completed, PPO was calculated according to the equation described by Kuipers et al., (1985):

PPO = W_{com} + (t/60 x 30)

Where W_{com} is the power output (W) of the last completed stage; t is the time (in seconds) that the final, uncompleted exercise stage was sustained.

2.6. 20 km time trial

The TT_{20} test was conducted on an electronically-braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA, USA) and preceded by a structured warm up protocol. The warm up consisted of cycling for 10 min at intensity corresponding to ~65% of each participant's real maximal heart rate, which was determined during the GXT, and 4 x 20 s accelerations at LTP power output at the beginning of the 4th, 5th, 6th and 7th minute (Ihsan et al., 2010). The participants were instructed to ride the TT_{20} as fast as possible. They were also allowed to adjust the power output throughout the trial. The time to complete the test, average power and average heart rate were recorded.

2.7. Training intervention

The training sessions were conducted on the same electronically-braked cycle ergometer as the TT_{20}. All participants followed the same structured training programme, which included 3 interval training sessions per week, with at least 48 h between the sessions. All training sessions began with a 10-min light warm up after which a series of 5-min intervals were performed. To ensure a progressive training stimulus during the training period, 4 intervals were performed during the first three training sessions, 5 intervals during the fourth, fifth and sixth sessions and 6 intervals on the last three sessions. The intervals were performed at a power output corresponding to LTP, with 1 min active rest at 50 W between each interval. The training sessions were concluded with a 5-min cool down. Both the warm up and cool down were performed at 80% of LTP power output. A similar training protocol to this improved performance of well-trained cyclists (Lindsay et al., 1996; Stepto et al., 1999; Swart et al., 2009; Weston et al., 1996). Exercise intensities were set as a percentage of LTP, rather than $\dot{V}O_{2max}$, as cardiovascular and metabolic strains are similar when individuals with different training status exercise at a percent of LTP, but can differ significantly when training at a percent of $\dot{V}O_{2max}$ (Baldwin et al., 2000). After each training session, the participants were given 3 min to change out of the cycling gear. Thereafter, they were immersed up to their sternum (arms were not immersed) in a semirecumbent posture for 30 min in an inflatable bath (iCool, Brisbane, Australia) with continuous water recirculation and temperature control. The HWI participants were immersed in 42°C water, whereas a thermoneutral water temperature of 34°C was used for CON. To aid glycogen resynthesis, the participants were provided with 1.0 g per kg BM carbohydrate in the form of a sports drink (Powerade®, The Coca-Cola Company, USA), which they were instructed to consume within 30 minutes post-exercise (Pritchett et al., 2011). The participants were also asked to record all the training they were doing outside the laboratory. They were instructed to record the time (in hours) and intensity (on a scale from 1 to 10, with 10 being maximal exertion) of each training session. There were no significant differences between the CON group and the HWI group in self-reported training hours (16.3 ± 3.7 vs. 20.4 ± 8.5 hours, respectively, $p = 0.39$) or training intensity (5.3 ± 1.3 vs. 4.2 ± 0.3, respectively, $p = 0.2$) during the intervention phase.

2.8. Estimation of plasma volume (PV)

Venous blood (~1 mL) was sampled at rest before the first training session and before the test on the day of the second TT_{20}. Each of these days was preceded by a rest day in order to avoid exercise-induced PV shifts. Since hydration status may influence PV (Sawka et a., 2000), all blood samples were taken in euhydrated state, as verified by USG <1.020. Blood samples were taken from an antecubital vein using a sterile needle (BD PrecisionGlide™ Needle, Singapore) and syringe (BD 5ml Syringe, Luer-Lok™ Tip, Singapore) by a trained researcher. Ten minutes prior to venepuncture, the participants were placed into a supine position, an appropriate arm was chosen and the area was cleaned using alcohol swab (Briemarpak skin cleansing swab, Briemar Nominees Pty Ltd, VIC, Australia). A tourniquet was applied to aid vein selection and needle insertion before the drawing of blood into the syringe. Following the blood collection, the tourniquet was released and the needle removed from the vein, the punctured site was then covered with a sterile dressing and secured using tape. Changes in plasma volume (ΔPV) were estimated from the changes in concentration of haemoglobin (Hb) and haematocrit (Hct), as described by Dill & Costill, (1974):

$$\Delta PV (\%) = 100 \times [(Hb_{post}/Hb_{pre}) \times ((1 - Hct_{post})/(1 - Hct_{pre}))] - 100\%$$

Where Hb is in g/dL and Hct is in percentage. All samples were assayed in duplicate using Sysmex KX-21N Automated Hematology Analyzer (Block Scientific, NY, USA).

2.9. Muscle biopsy

Twelve participants had a muscle biopsy taken. All muscle biopsies were performed in the morning between 07:30 and 09:00 by an experienced medical doctor. The participants were instructed to arrive at the laboratory in a fasted state and not to perform intense exercise 24 hours prior to each biopsy. Upon arrival, the participants were placed into a supine position and the middle portion of the *vastus lateralis* muscle was shaved and cleaned using an antiseptic (Povidone-Iodine Prep Pad, Lights Medical Manufacture CO., LTD, China) before local anaesthetic (1% Xylocaine) was injected in the skin. After ~10 min, a small incision (0.6 cm long) was made in the skin and muscle fascia using a sterile surgical blade (Sterile R, Swann-Morton®, UK). A Bergström needle with the aid of suction (Evans et al., 1982), was inserted into muscle to a depth of 2-3 cm and a small piece of tissue was removed. The muscle sample was placed on filter paper to remove excess blood before being immersed in a 2 mL tube containing

biopsy preserving solution (BIOPS). Following the biopsy, the incision was closed using sterile strips (3M Health Care, MN, USA) and covered by a transparent waterproof dressing (6 cm x 7 cm, Tegaderm Film, 3M Health Care, MN, USA). Then a pressure bandage was applied which was maintained for 24-48 hours. The post-intervention biopsy was obtained from the same leg using a separate incision at ~2 cm from the first biopsy.

2.9.1. Mitochondrial respiration

Within 30 min of muscle sampling, a small portion of muscle fibres were gently separated under a microscope using fine-tipped forceps in ice-cold BIOPS (containing 2.77 mM CaK_2EGTA, 7.23 mM K_2EGTA, 5.77 mM Na_2ATP, 6.56 mM $MgCl_2$, 20 mM taurine, 50 mM K^+-4-morpholinoethanesulfonic acid, 15 mM Na_2phosphocreatine, 20 mM imidazole and 0.5 mM dithiothreitol adjusted to pH 7.1). Subsequently, fibres were permeabilised by gentle agitation for 30 min at 4°C in BIOPS supplemented with saponin (50 µg/mL). Fibres were then washed three times for 7 min at 4°C in MiR05 (mitochondrial respiration medium containing 0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM potassium-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 mg/mL bovine serum albumin at pH 7.1), weighed and transferred into the chamber of an Oxygraph-2k respirometer (Oroboros, Innsbruck, Austria), which contained 2 ml of MiR05. Temperature in the chamber was maintained at 37°C throughout all experiments. Chamber O_2 concentration was maintained between 200 and 500 $nmol·mL^{-1}$ to prevent potential limitations in oxygen diffusion. Oxygen concentration (in $nmol·mL^{-1}$) and oxygen flux (in $pmol·s^{-1}·mg^{-1}$) were recorded using DataLab 5.2 software (Oroboros, Innsbruck, Austria) All respiration measurements were done in duplicate.

To assess mitochondrial respiration, a standard substrate-uncoupler-inhibitor titration (SUIT) protocol was applied, as described previously (Pesta et al., 2012). First, malate (5 mM) and pyruvate (5mM) were added in absence of adenylates to measure leak respiration through Complex I (CI). ADP (2.5mM) was then added to measure maximal oxidative phosphorylation (OXPHOS) capacity through CI (CI_P), followed by addition of 10 mM of succinate for measurement of OXPHOS capacity with parallel electron supply form CI and Complex II ($CI+CII_P$). Subsequently, cytochrome c (5 µM) was added to test the integrity of outer mitochondrial membrane. Following confirmation of intact membrane, uncoupler FCCP (carbonyl cyanide *p*-

trifloromethoxyphenylhydrazone; a total of 1.5 μM in steps of 0.05 μM) was added to measure maximal noncoupled electron transport system (ETS) capacity through CI and CII (CI+CII$_E$). Finally, addition of rotenone (1 μM), an inhibitor of CI, allowed to determine ETS capacity through CII (CII$_E$), while addition of antimycin A (5 μM), an inhibitor of Complex III, allowed measurement of residual $\dot{V}O_2$ (ROX). All respiratory values were corrected by subtracting the corresponding ROX value for the sample.

2.10. Monitoring intramuscular temperature (M$_T$)

Prior to the water immersion after the first training session, the participants were placed into a supine position and the middle portion of the *vastus lateralis* muscle was shaved and cleaned using an antiseptic before an 18-gauge needle (Optiva IV Catheter 18G X 1.75", Smiths Medical, USA) was inserted to a depth of 3 cm below the skin. The needle was removed, leaving a flexible catheter. A thermistor probe (Model T – 204A, Physitemp Instruments, USA) was then inserted through the catheter. Once the probe was in place, the catheter was removed and the probe was secured to the leg with a plastic adhesive cover (10 cm x 12 cm, Tegaderm Film, 3M Health Care, MN, USA). On average, time taken to complete this procedure was 8 ± 0.8 min. The other end of the probe was connected with a computer and M$_T$ was continuously recorded during the water immersion and 30 min post-immersion (the participants rested in a supine position at room temperature) using DasyLab 12.0 software (Measurement Computing Corporation, MA, USA). This is a well-established, accepted and well-practiced technique used to document M$_T$ (Broatch et al., 2014; Hafen et al., 2018; Racinais & Girard, 2012; Shute et al., 2017; Thomas et al., 2016; Zak et al., 2018).

2.11. Monitoring body core temperature (C$_T$)

Core temperature was recorded simultaneously with M$_T$, using CorTemp^{TM} telemetric system (CorTemp, HQInc, Palmetto, FL, USA) consisting of ingestible temperature sensor pill that transmits a radio wave signal to a small data recorder. Numerous studies have shown that this is a valid method for assessing C$_T$ (Casa et al., 2007; Easton et al., 2007; Ganio et al., 2009; Gant et al., 2006; Hosokawa et al., 2016; Kolka et al., 1997; Kolka et al., 1993; O'Brien et a., 1998). One day prior to the first training session, the participants were provided with a packaged temperature pill and were instructed to swallow the pill at least 4 hours before reporting to the laboratory the

following day. No fluid was allowed during this training session because ingestion of fluid may influence the temperature reading (Wilkinson et al., 2008).

2.12. Statistical analyses

Data are presented as means ± SD. Paired Student's *t*-tests were used to test the differences between the groups at baseline and data from the training logs. A paired Student's *t*-test was also used to compare ΔPV between the groups. All other outcome variables were analysed using a two-way repeated measures ANOVA. When significant main effects were observed, a Bonferroni post-hoc test was performed to locate the differences. *t*-tests were performed in Excel 2016 (Microsoft Corporation, Redmond, WA, USA), whereas ANOVAs were performed using IBM SPSS Statistics V21 (IBM Corporation, Armonk, New York, USA). Statistical significance was set at *p* < 0.05 for all tests.

CHAPTER THREE - RESULTS

There were no significant differences between the HWI group and the CON group in relation to age, height, weight, $\dot{V}O_{2max}$ and TT_{20} at baseline testing (Table 3.1).

Table 3.1. Participants' characteristics at baseline (mean ± SD)

	HWI (n = 7)	CON (n = 7)
Age (years)	30.7 ± 10.8	31.0 ± 10.1
Height (cm)	176.7 ± 4.1	176.4 ± 3.7
Body mass (kg)	75.1 ± 10.8	79.5 ± 11.4
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	60.5 ± 12.0	60.2 ± 11.1
TT_{20} (sec)	2009.8 ± 147.3	2010.4 ± 182.3

3.2. Temperature responses

3.2.1. Muscle temperature

Muscle temperature was significantly higher in the HWI group compared to the CON group form 15-30 min of immersion, and at all time points throughout the 30-min post-immersion period (Figure 3.1a).

3.2.2. Core temperature

Compared with the CON condition, C_T was significantly higher during the last 5 min of HWI and at 5 min and 10 min post-immersion (Figure 3.1b).
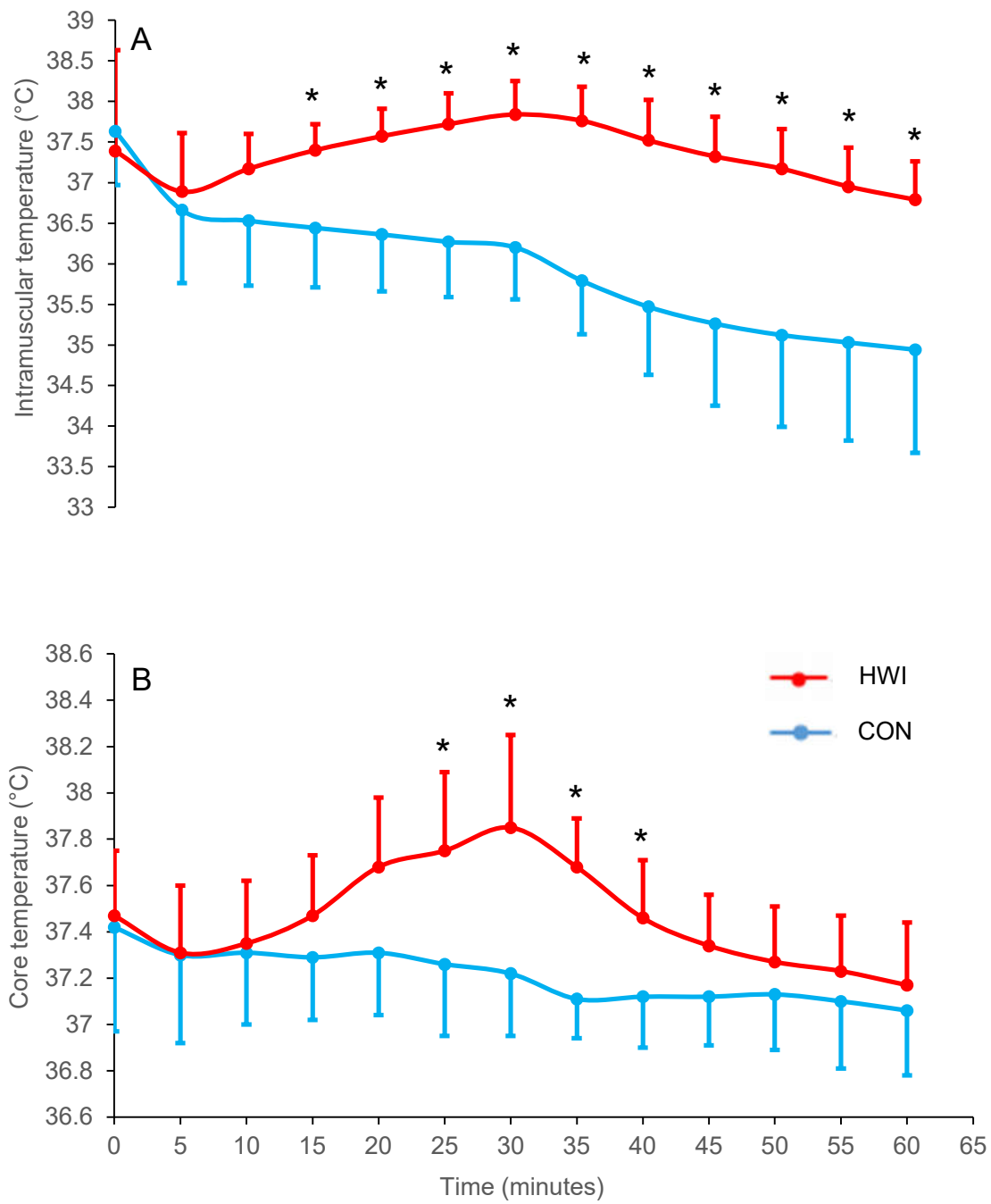
Figure 3.1. Muscle temperature (A) and core temperature (B) from start to end of water immersion and 30 min post-immersion measured at 5-min intervals. Data are means ± SD. *Significant difference between the groups, $p < 0.05$

3.3. Exercise performance

The TT_{20} performance improved by 1.7% but not to different extend between the groups (interaction: p = 0.99) (Figure 3.2), neither was the 4.3% increase in average power output different between groups (interaction: p = 0.91) (Figure 3.3).
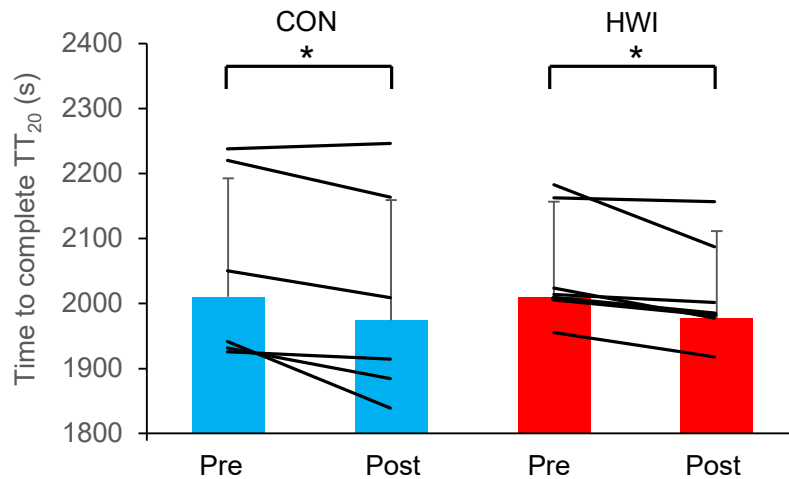
Figure 3.2. Time to complete the TT_{20} before and after the intervention in CON and HWI groups. Data are means ± SD (columns) and individual responses (lines). *p < 0.05
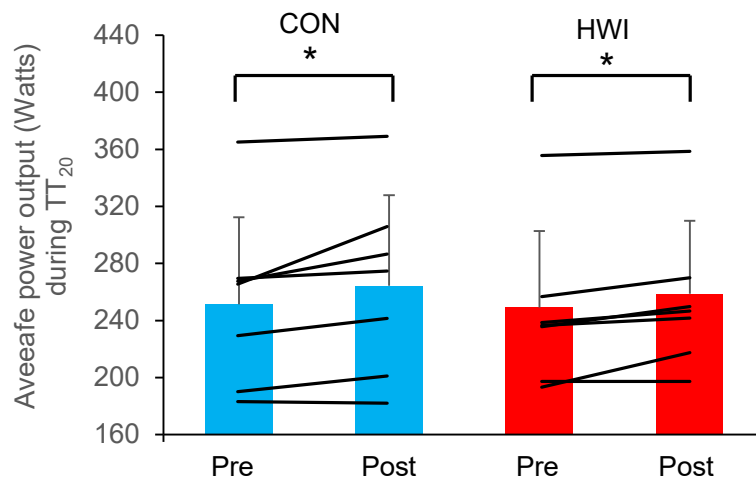
Figure 3.3. Average power output during the TT_{20} before and after the intervention in CON and HWI groups. Data are means ± SD (columns) and individual responses (lines). *p < 0.05

LTP, $\dot{V}O2_{max}$ and PPO did not change significantly after 3 weeks of intervention, either between the groups (p = 0.99, p = 0.96, p = 0.63, respectively) or over time (p = 0.57, p = 0.91, p = 0.27, respectively) (Figure 3.4).
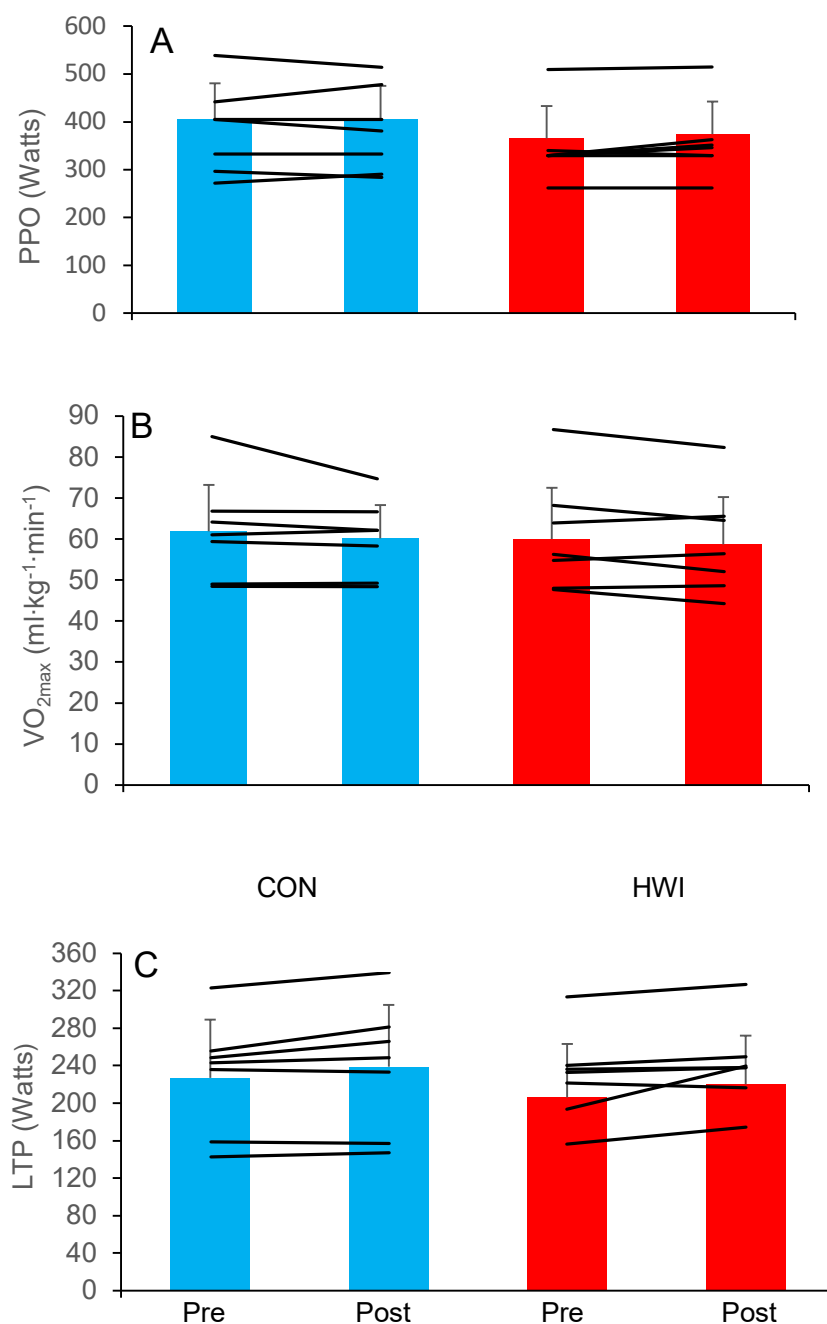
Figure 3.4. PPO (A), $\dot{V}O2_{max}$ (B) and LTP (C) before and after the intervention period for the CON and HWI groups. Data are means ± SD (columns) and individual responses (lines).

3.4. Mitochondrial respiration

No significant difference was found in any of the respiratory states measured (CI, CI_P, $CI+CII_P$, Cyt-c, $CI+CII_E$ and CII_E), either between the groups ($p = 0.27$, $p = 0.74$, $p = 0.37$, $p = 0.51$, $p = 0.64$, $p = 0.53$, respectively) or over time ($p = 0.67$, $p = 0.41$, $p = 0.72$, $p = 0.43$, $p = 0.40$, $p = 0.06$, respectively) (Figure 3.5).
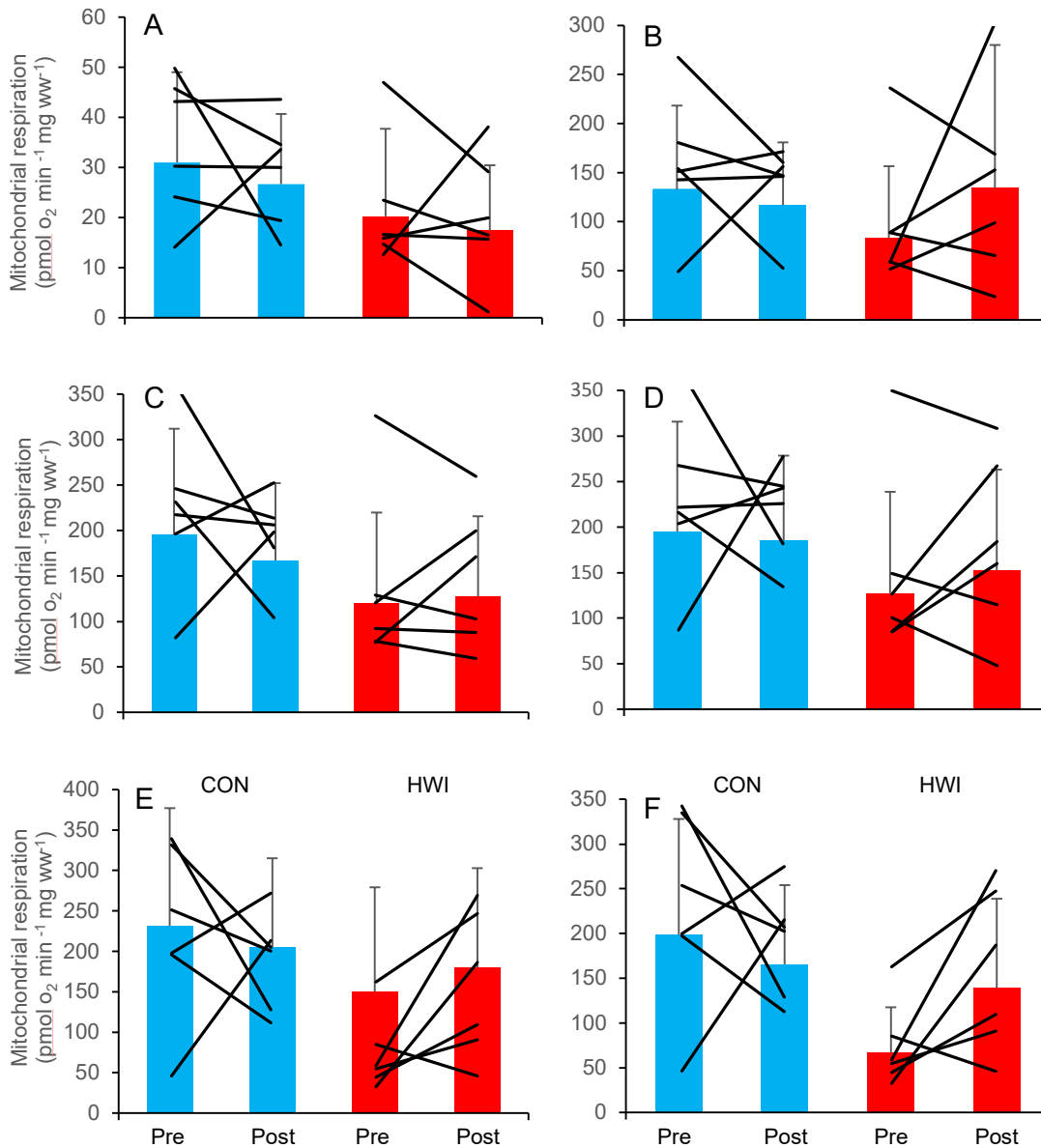
Figure 3.5. Mitochondrial respiration [CI (A), CI_P (B), $CI+CII_P$ (C), Cyt-c (D), $CI+CII_E$ (E) and CII_E (F)] before and after the intervention for the CON and HWI groups. Data are means ± SD (columns) and individual responses (lines).

3.5. Plasma volume

No significant difference (p = 0.56) was observed in the change of PV between the groups post intervention (Figure 3.6).
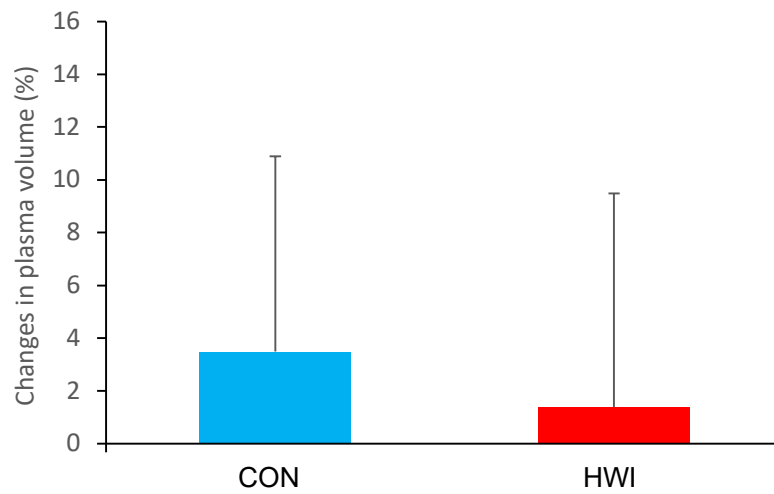
Figure 3.6. Percentage change in PV from pre-to post-intervention for the CON and HWI groups. Data are means ± SD.

CHAPTER FOUR – DISCUSSION

The aim of the current study was to investigate the effects of regular post-exercise hot water immersion on selected physiological adaptations and on exercise performance in a temperate environment in trained road cyclists. After 3 weeks of intervention, no significant differences were found in the TT_{20}, LTP, $\dot{V}O_{2max}$ and PPO between the HWI and CON groups. Thus, the first hypothesis was rejected. Since there were no significant differences in the changes in the mitochondrial function and PV between the groups, the second hypothesis was also rejected. However, the third hypothesis was partially confirmed since HIT only improved TT_{20} performance.

4.1. Effects of HWI on exercise performance and physiological adaptations

The idea that regular post-exercise heat stress application via HWI can be beneficial to endurance cycling performance in temperate environment was based on the study of Scoon et al., (2007) who observed a 32% improvement in endurance running performance in ~19°C after 12 sauna bathing sessions for 30 min immediately following training sessions. In contrast to that study, we failed to demonstrate the ergogenic effect in exercise performance after 9 x 30 min post-training HWI sessions. Since both studies used male participants with similar training status the inconsistency in the findings between the studies cannot be attributed to these factors. A possible explanation as to why we did not see a significant difference in exercise performance post-HWI intervention compared with CON is that the degree of hyperthermia during our heating treatment was not of sufficient magnitude to induce the desired performance adaptations.

In the current study, we observed mean values of C_T and M_T during the first 30-min HWI session (mean of 6 values taken at 5-min intervals) of 37.5°C and 37.4°C, respectively. Although Scoon et al., (2007) did not attempt to assess the body temperature response to sauna bathing in their participants, Ridge & Pyke, (1986) studied the effects of taking a 15-min sauna (~83°C) immediately after a 15-min bout of exercise in healthy males and measured a C_T of 38°C at the end of the sauna session. Given the fact that the duration of the sauna bathing sessions in the Scoon study was 30 minutes, it is possible that their participants have experienced an even greater increase in C_T than the value reported in the former study. According to

existing literature, an effective HA program requires maintenance of C_T at ≥38.5°C during heat exposure sessions (Heathcote et al., 2018; Pryor et al., 2019; Taylor, 2014). Whether such a degree of hyperthermia was achieved during the sauna bathing sessions in the Scoon study is unknown.

Nevertheless, 12 x 30 min sauna exposures were sufficient to elicit an increase in PV (a key marker of HA) of 7.1%, which was suggested by the authors to be the main mechanism responsible for improved endurance performance. The efficacy of post-exercise sauna bathing to induce expansion of PV was subsequently confirmed by Stanley et al., (2015) in seven well-trained male cyclists ($\dot{V}O_{2max}$ = 60.4 ± 4.7 ml·kg^{-1}·min^{-1}). The researchers found an increase in PV of 17.8% after a 10-day training block consisting of daily 30 min sauna exposures at 87°C following training. Unfortunately, this study did not focus on determining the effects of post-exercise sauna bathing on endurance cycling performance. In contrast to these two studies, our HWI group did not experience PV expansion of such magnitude. This is not surprising given the fact that the elevation of C_T during the heating treatment did not reach a degree that is considered crucial for HA. The rationale behind the decision to employ the current heating method was based on the expectation that HIT will lead to an increase in C_T to around 38.5°C, and that the chosen heating treatment will provide strong-enough thermal stimulus to successfully maintain the exercise-induced hyperthermia throughout the duration of the treatment. Previous research showed an increase in C_T to ~38.1°C in response to cycling for 30 min at 70% of $\dot{V}O_{2max}$ at 20°C (Aldemir et al., 2000). Knowing that the degree of hyperthermia during exercise is directly related to the intensity of exercise (Gleeson, 1998), it was reasonable to expect that HIT will lead to a greater increase in C_T than that reported in the above study. However, C_T measured immediately after cessation of the first HIT session in our participants was 37.95 ± 0.3°C and 38.01 ± 0.3°C for the HWI group and the CON group, respectively (data not shown). The C_T values dropped to 37.47 ± 0.2°C and 37.37 ± 0.4°C for the HWI group and the CON group, respectively (Figure 3.1) at the start of the water immersion due to the time gap between the end of the HIT session and the immersion session. Furthermore, the mean increase in C_T during our HWI protocol was 0.47 ± 0.3°C, which is about twofold lower than the value (1.3 ± 0.4°C) reported by Thomas et al., (2016) in response to 30 min of lower body hot (42°C) water immersion. Interestingly, the HWI protocol adopted by Zurawlew et al., (2016) induced

an increase in C_T above 39°C and lead to an increase in PV of 4.9% after 6 post-exercise HWI immersion sessions, but HWI group failed to improve running performance in temperate conditions. The inconsistency in the findings between that study and the study of Scoon et al., (2007) could possibly be explained by the difference in the number of heat exposure sessions. The greater number of post-exercise heat exposure sessions in the Scoon study may have allowed their participants to experience some peripheral (muscular) adaptations, such as, increased skeletal muscle oxidative capacity, which has been identified as an important determinant of endurance performance (Jacobs et al., 2011).

Indeed, the evidence is accumulating that passive heat exposure can increase mitochondrial biogenesis. Liu & Brooks (2011) were the first researchers to report that heat stress induces mitochondrial biogenesis in C2C12 myotubes. Subsequently, Tamura et al., (2014) have shown that post-exercise heat exposure can additively enhance training-induced mitochondrial adaptation in mice. However, since the findings of that study have not been confirmed in a human model, addition aim of our study was to assess changes in mitochondrial respiration in response to HWI. Unfortunately, the increase in M_T observed in the HWI group in the present study was likely not of sufficient magnitude to enhance the oxidative potential in skeletal muscles. Most recently, Hafen et al., (2018) reported a significant increase in CIP, CI+CII$_P$ and CI+CII$_E$ following repeated heating via pulsed shortwave diathermy. Although the intervention in that study lasted only 6 days, the duration of each heat treatment was 2 hours and M_T was maintained at 39 ± 0.31°C throughout the treatments.

4.2. Effects of HIT on exercise performance and physiological adaptations

A positive finding in this study was that 9 HIT sessions led to significant improvement in TT$_{20}$ performance. On average, both groups improved their TT times by 1.7%. This magnitude of TT improvement is comparable to that observed by Swart et al., (2009) and Weston et al., (1996), but smaller than the values (3.5-7.8%) reported by Laursen et al., (2002), Lindsay et al., (1996), Robinson et al., (2011) and Westgarth-Taylor et al., (1997) after short-term HIT programs. Although the ergogenic effect of HIT for exercise performance in already trained cyclists is well-documented, the mechanisms responsible for performance improvement brought about by this training program are not fully understood. The improvement of TT performance in our study, despite no

significant changes in any of the other variables measured, raises a further question as to the precise mechanism by which HIT augmented exercise performance. Although not statistically significant, it is possible that the increase in power output at LTP by 5.3% and 6.8% in the CON group and in the HWI group, respectively, may have been of sufficient magnitude to allow the participants to maintain higher average power output during the second TT_{20} test. However, it is also possible that our study lacked the statistical power to detect a significant difference in LTP between pre and post intervention period.

Alternatively, improvement of TT performance in response to HIT in our participants could be attributed to non-physiological factors. For example, a recent study compared HIT with continuous training of moderate intensity and found that HIT can increase muscle pain tolerance (O'Leary et al., 2017), which in turn may allow an athlete to perform closer to the limits of his/her maximal physical potential. Indeed, the willingness to endure pain during the exercise has been identified as an important determinant of cycling TT performance (Astokorki & Mauger, 2017). Additionally, psychological factors such as arousal, motivation and self-confidence have been associated with successful athletic performance (Tuffey, 2000). Some of these factors may also have contributed to TT_{20} performance. Although it cannot be ruled out that improved pacing may have contributed to TT_{20} performance improvement, all participants performed one TT familiarisation session before the baseline testing, which has been shown to be sufficient to offset potential learning effects during subsequent TTs (Laursen et al., 2003; Zavorsky et al., 2007).

The lack of significant changes in mitochondrial respiration after HIT is in contrast to the findings reported by Jacobs et al., (2013) and Vincent et al., (2015). In the former study, the researchers found a significantly greater increase in all respiratory states measured (except for CII_E) after only 6 HIT sessions in sixteen untrained ($\dot{V}O_{2peak}$ = 43 ± 6 ml·kg^{-1}·min^{-1}) male participants. Similarly, Vincent et al., (2015) found a significant increase in Cyt-c and $CI+CII_P$ after 8 HIT sessions in eight recreationally active ($\dot{V}O_{2peak}$ = 45.7 ± 2.1 ml·kg^{-1}·min^{-1}) males. Taken together, the findings of ours and these two studies suggest that baseline training status has an important impact on adaptive responses. This is in line with current literature (Midgley et al., 2006; Wilmore & Costill, 1994), indicating that, compared with untrained individuals, already

trained athletes have less room for further physiological adaptations in response to a given exercise stimulus.

Conclusion and future directions

Nine HIT sessions led to significantly improved TT_{20} performance, but post-exercise HWI protocol used in this study did not provide additional performance benefits. Available evidence indicates that post-exercise heat stress exposure results in favourable physiological adaptations and performance improvements in temperate environment, but the minimal 'dose' of heat stress necessary to elicit these benefits remains to be determined in future studies.

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RISK FACTOR ASSESSMENT QUESTIONNAIRE

Telephone: (03) 9919 9452

College of Sport and Exercise Science

Mobile:

Victoria University of Technology

Fax: (03) 9919 4891

P.O. Box 14428

Email:

MELBOURNE MC8001

Website:

NAME: _____   **DATE** _____

ADDRESS: _____   **SEX**   **M / F**

_____ **Postcode:** _____   **AGE** _____ **YRS**

TELEPHONE: Work: _____   **WEIGHT** _____ **KG**

TELEPHONE: Home: _____   **HEIGHT** _____ **CM**

EMAIL: _____

MEDICAL HISTORY:

In the past have you ever had (tick No or Yes)

Medical Condition	NO	YES	Medical Condition	NO	YES
Heart Attack	❏	❏	Congenital Heart Disease	❏	❏
Chest Pain (angina)	❏	❏	Disease of Arteries/Veins	❏	❏
Heart Murmur	❏	❏	Asthma	❏	❏
Heart Rhythm Disturbance	❏	❏	Lung Disease (eg. emphysema)	❏	❏
Heart Valve Disease	❏	❏	Epilepsy	❏	❏
Stroke	❏	❏	Injuries to back, knees, ankles	❏	❏

List any prescribed medications being taken

_____

_____

List any surgical procedures that you have had (write the year in brackets):

 example: appendix (1979)

_____

List any injuries in your past medical history

_____

_____

ALLERGIES: Do you have any allergies   **NO** ❏   **YES** ❏

If yes, give details: _____

_____

SYMPTOMS DURING OR AFTER EXERCISE

As a result of exercise, have you ever experienced any of the following:

Symptom during exercise	NO	YES	Symptom during exercise	NO	YES
Pain or discomfort in the chest, back, arm, or jaw	❏	❏	Palpitations (heart rhythm disturbance)	❏	❏
Severe shortness of breath or problems with breathing during mild exertion	❏	❏	Pain in the legs during mild exertion	❏	❏
Dizziness, nausea or fainting	❏	❏	Severe heat exhaustion	❏	❏

CARDIOVASCULAR RISK FACTORS:

Do you have (tick NO, YES or circle ? for DON'T KNOW)

Cardiovascular Risk Factors	<u>NO</u>	<u>YES</u>	DON'T KNOW
High Blood Pressure	❑	❑	?
High Blood Cholesterol/Triglycerides	❑	❑	?
Diabetes	❑	❑	?
Current Smoker	❑	❑	Average/day =
Ex-smoker	❑	❑	Average/day =
Do you drink alcohol regularly?	❑	❑	Average/day = drinks

FAMILY MEDICAL HISTORY:

Have members of your immediate family ever had any of the following conditions: (tick NO, YES or circle ? for DON'T KNOW).   If you answer Yes or ?, write beside this the member of the family affected (F=father, M=mother, B=brother, S=sister, GM=grandmother, GF=grandfather).

FAMILY MEDICAL HISTORY	NO	YES	?	FAMILY MEMBER	AGE (Years)	ALIVE NOW?)
Heart Attack	❑	❑	?	____	____	____
Chest Pain (Angina)	❑	❑	?	____	____	____
Stroke	❑	❑	?	____	____	____
High Blood Pressure	❑	❑	?	____	____	____
High Blood Cholesterol/Triglycerides	❑	❑	?	____	____	____
Diabetes	❑	❑	?	____	____	____

PERSONAL LIFESTYLE:

A.   Exercise

List the sports, exercise or physically active hobbies (eg. gardening or playing with the kids) that you are **currently** engaged in:

Sport/Activity	Day(s) of week **Sa-Su-Mo-Tu-We-Th-Fr**	Time of the day eg. 6 p.m.	Approximate duration eg. 30 minutes
		TOTAL	

B. Nutrition

List a typical day's eating pattern.

Breakfast	Lunch	Dinner	Snacks	Drinks

C. Rest/Recreation

How many hours sleep do you usually have? _____ hours/night

On average how much time do you spend each day on passive hobbies or just relaxing?

_____ minutes/hours per day.

Do you feel that you usually get enough restful sleep and time to relax?   **Yes/No**

Client Declaration I declare that the above information is To my knowledge true and correct, and that I have not omitted any information that is requested on this form. ***Signed:*** _____ ***Parent Signature: (for those under 18)*** _____ ***Date:*** _____/_____/_____	**OFFICE USE ONLY CLEARANCE TO UNDERGO AN** **EXERCISE TEST** This person has been cleared to undergo a Fitness test: ❑ Without medical supervision ❑ With medical supervision ❑ A fitness test is not advisable at this time **Signed: Dr/Mr/Mrs/Ms _____** **(Circle appropriate title:** **Physician/exercise physiologist)**

Please turn over and provide the information requested overleaf.

APPENDIX B

VENOUS CANNULATION & MUSCLE THERMISTOR QUESTIONNAIRE :

In order to be eligible to participate in the experiment investigating:

"The role of heat as a conditioning stimulus in endurance athletes"

you are required to complete the following questionnaire which is designed to assess the risk of you having an adverse event during venous blood sampling or muscle thermistor insertion.

NAME: _____

DATE: _____   **AGE:** _____ **years**

1. Have you or your family suffered from any tendency to bleed excessively? (e.g. Haemophilia) or bruise

very easily?   Yes   No   Don't Know

    If yes, please elaborate

_____

2.   Are you allergic to local anaesthetic?   Yes   No   Don't Know

    If yes, please elaborate

_____

3.   Do you have any skin allergies?   Yes   No   Don't Know

    If yes, please elaborate

_____

4.   Have you any other allergies?   Yes   No   Don't Know

    If yes, please elaborate

_____

5.   Are you currently on any medication?   Yes   No

    If yes, what is the medication?

_____

6.   Do you have any other medical problems?   Yes   No

        If yes, please elaborate

_____

7.   Have you ever fainted when you had an injection or blood sample taken?   Yes   No

                                                                                        Don't know

        If yes, please elaborate

_____

8.   Do you or other members of your family have Raynauds disease, or suffer from very poor

circulation in the fingers, leading to painful fingers that turn white/blue?   Yes   No

                                                                                      Don't know

        If yes, please elaborate

_____

9.   Do you suffer from thromboembolic disorders e.g. DVT, PE, AMI?

                                                                              Yes   No
                                                                              Don't know

        If yes, please elaborate
_____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____

Date: _____

APPENDIX C

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

INFORMATION TO PARTICIPANTS:

We would like to invite you to be a part of a study entitled:

"The role of heat as a conditioning stimulus in endurance athletes"

AIMS OF THE STUDY:

The aim of this project is to investigate the effects of 30 minutes of post-exercise hot water immersion over a period of 3 weeks on subsequent 20 km cycling time trial performance in a temperate environment and selected physiological and cellular adaptations in well-trained cyclists.

PROCEDURES INVOLVED AND NATURE OF THE PROJECT:

You will be asked to complete a medical questionnaire so we can be sure it is safe for you to participate in the study. You will then be asked to attend the Exercise Physiology Laboratory at Victoria University Footscray Park on 17 occasions; two familiarization sessions, four exercise testing trials (two before and two after the training period) a 3-week period of training (3 sessions per week) and two muscle biopsies (one before and one after the training period). The familiarization session and exercise testing trials will take less than 1 hour. Each training session will last 1 hour and will be followed by 30 min of water immersion. You will be allocated to either intervention (hot water immersion) or control (thermoneutral water immersion) group. You will be also asked for your permission for a medical doctor to draw venous blood samples on two occasions. During one of the water immersion sessions, muscle temperature will be monitored via insertion of an intramuscular temperature probe into your quadriceps muscle.

CERTIFICATION BY SUBJECT

I, _____(Phone No:)_____

of _____

certify that I am at least 18 years old* and that I am voluntarily giving my consent to participate in the study:

"The role of heat as a conditioning stimulus in endurance athletes"

being conducted at Victoria University by: **Dr. Aaron Petersen, Prof Robert Aughey, and Mr Metodija Kjertakov.**

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Dr Aaron Petersen

and that I freely consent to participation involving the below mentioned procedures:

- Pre-test screening
- Diet monitoring via a food diary
- Graded exercise test to exhaustion
- Performance test (20 km cycling time trial)
- Water immersion (hot or thermoneutral)
- Monitoring body core temperature
- Thigh muscle temperature probe insertion
- Muscle Biopsies
- Blood samples (venous and capillary samples)

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the information I provide will be kept confidential.

Signed: _____

Date: _____

Any queries about your participation in this project may be directed to the principal researcher

Dr. Aaron Petersen

Telephone number: 03 9919 9452

Fax number: 03 9919 4891

Email: aaron.petersen@vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact the Research Ethics and Biosafety Manager, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 or phone (03) 9919 4148