MECHANISMS OF HEAT ACCLIMATION AND EXERCISE PERFORMANCE

by

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There has been a lot of research investigating the effects of heat stress and exercise on the physiological adaptations to heat acclimation. It is well documented that heat acclimation improves heat tolerance and performance in a hot environment; however, some of the mechanisms of adaptation are not clear. Furthermore, the role of heat acclimation on exercise performance in cool environments is currently unknown. Therefore, in Chapter IV we aimed to determine the effects of heat acclimation on lactate threshold and maximal oxygen uptake (VO_{2max}) in cool and hot conditions. We also sought to investigate the effects of heat acclimation on leg blood flow and oxygen delivery during a single-leg knee extensor exercise. We found that heat acclimation improved lactate threshold and

VO_{2max} in cool and hot environments but did not alter the leg blood flow and oxygen delivery during the leg kicking exercise. In Chapter V we investigated the heat acclimation effects on performance during a 1-hour time trial in hot and cool environmental conditions and the potential mechanisms by which this occurs. A secondary objective was to study whether the pacing strategy was modified during the time trial post-heat acclimation. The results demonstrated that heat acclimation improved time trial performance in both thermal environments by approximately 7% but pacing strategy was not altered. The purpose of the studies in Chapter VI were twofold. First, we sought to investigate how heat acclimation affects the skin blood flow and sweating responses to pharmacological treatment with specific dosages of the muscarinic receptor agonist acetylcholine. Second, we examined the maximal skin blood flow responses to a period of heat acclimation by locally heating the forearm with a water spray device for 45 minutes and measured brachial artery blood flow via ultrasound. We found that heat acclimation increased sweat rate and skin blood flow responses to given concentrations of acetylcholine, suggesting a role for peripheral mechanisms. On the other hand, maximal skin blood flow remained unchanged after heat acclimation.

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CHAPTER I

INTRODUCTION

Historical perspective and statement of the problem

Competition among humans is timeless. In fact, humans have been involved in sporting activities since at least ancient times, as exemplified by the Greek Olympic Games, which were first recorded in 776 BC in Olympia, Greece. Indeed, ethnographic and archaeological evidence such as cave paintings and the accounts of early European explorers indicate sports may well go back to the very beginning of humankind. The fact that regular exercise may contribute to improved performance is not confined to the 20th century. Since the dawn of athletic competition during the original Olympic Games in Ancient Greece, athletes, as well as their coaches and trainers, have been in constant search to find innovative ways to gain an edge on their competition. Wining was a measure of power and status. Now, success in sports is a business of invaluable potential. Therefore, the pursuit to enhance performance in sports has gained tremendous attention.

Performance during sporting competitions is influenced by many factors, including the environmental conditions in which they take place. It is clear that hot temperatures can potentially have a huge impact on the

human body during exercise. Year 490 BC: a Greek messenger, Pheidippides, ran 150 miles from Athens to Sparta to request help when the Persians invaded the city of Marathon. Two days later he ran the 22 miles from Marathon to Athens (the origin of the marathon race) to announce the Greek victory over Persia at the battle of Marathon. After saying the word "Nenikékamen" (which means "we have won"), he collapsed and died on the spot. Historians assumed that the Marathon battle date was 12 August 490 BC, which means that Pheidippides' epic run took place in the middle of the hot Greek summer. This story, whether accurate or not, is one classic example of how the environment (among other factors) can seriously affect the human body during exercise.

Organized research aimed to learn more about body functions during exercise dates back to the 18th century. In fact, one of the pioneer scientists in exercise and environmental physiology, David Bruce Dill, proposed that the first experiment in exercise physiology was conducted by the French scientist Laurent Lavoisiser in 1789. However, most of the interest in research related to the measurement of exercise at different environmental conditions was originally sparked by war. The First World War (1914-1918), without question, had a significant impact in the field of exercise physiology. Scientists became interested in physical fitness and how to train military personnel so they were ready for combat duty. As with World War I, World

War II (1939-1945) had a major impact on the research development of exercise physiology.

Based on the extensive scientific research on exercise and heat stress, we can now provide with reasonable ideas about the "physiological' reasons of why Pheidippides' story ended so tragically after he ran for days during the battle of Marathon in the middle of the Greek summer. The two main "candidates" responsible for this outcome are dehydration and hyperthermia. During prolonged exercise in the heat (as during Pheidippides epic run), excessive sweating and restrained fluid intake can reduce total body water and thus, blood volume. In addition, the increase in muscle metabolism induced by the run in combination with heat stress from the hot Greek summer can increase the risk for hyperthermia, resulting in cardiovascular complications, central nervous system and motor function impairment, and in the case of Pheidippides, death.

Within the last 20 or 30 years, there has been a lot of research focused on the specific physiological changes that take place during exercise in a hot environment and how this may affect performance. The combination of intense dynamic exercise and heat stress imposes a serious challenge to the human cardiovascular system. Demands for blood flow to the exercising muscles plus the requirements for blood flow to the skin for thermoregulatory purposes outstrip the ability of the cardiovascular system to provide adequate blood flow to both vascular beds. This results in a

competition for the available cardiac output. A decreased active muscle blood flow will limit the intensity and duration of exercise, while reduced skin blood flow will impair heat dissipation resulting in increased body temperatures. There is enough evidence to suggest that when active muscle and skin are competing for blood flow, muscle wins. During prolonged exercise at low intensities, the compromised skin blood flow will impair heat dissipation, resulting in higher core temperatures and consequently fatigue. Although research has shown that increases in blood flow to the skin microcirculation do not reduce muscle blood flow during submaximal exercise, during high intensity exercise of short duration fatigue is preceded by decreases in cardiac output, which leads to reductions in muscle blood flow and oxygen delivery. In summary, it appears that when exercising at a low intensity for long periods of time in the heat, fatigue develops at a critical elevated core temperature, but if the exercise is of short duration and high intensity, the decrease in active muscle oxygen delivery is the culprit for the onset of fatigue.

There have been many real competition examples in which high ambient temperatures caused detrimental effects on performance. One of the most recent and remembered competitions is the 2007 Chicago Marathon, where the ambient temperature by the middle of the race was almost 90°F and humidity was above 80%. The race was called off a few hours after it began, but it could not stop the race from claiming hundreds of

heat related medical emergencies, including one fatality. The average wining time for that race since 2000 is approximately 2 hours and 6 minutes and in 2007 was 2 hours and 11 minutes. This is a “real life” example of how heat stress can negatively affect performance.

So how can we improve athletes’ performance at high environmental temperatures? Adequate physical training, good hydration and proper nutrition are strongly advised in order to maximize performance in the heat. Moreover, exposing the athlete to chronic heat or “heat acclimation” prior to the competition will further enhance their performance. Reports of heat acclimation effects on work performance go as far back as the 1940s, with studies done on humans working in mines and on soldiers. Heat acclimation protocols vary considerably but generally consist on chronic heat exposures at ambient temperatures high enough to elevate core temperature and induce profuse whole body sweating. It is well documented that heat acclimation improves heat tolerance and performance in a hot environment. Some of the physiological adaptations include plasma volume expansion, increased sweat rates, and skin blood flow, and reduced core temperature, heart rate, and perceived exertion at a given level of intensity, leading to an overall improved cardiovascular stability. Although much research on heat acclimation has been done, some specific questions in regards to the effects of heat acclimation on central cardiac function and the dynamics of muscle blood flow and oxygen

delivery remain to be elucidated. We previously discussed that during short duration, high intensity exercise in the heat, the primary source of fatigue is a decrease in muscle oxygen delivery due to the inability of the cardiovascular system to further increase cardiac output and thus, muscle blood flow. Exercises that activate a small muscle mass and thus, are not limited by cardiac output (i.e. single-leg kicking) can be used to isolate possible peripheral adaptations that occur in skeletal muscle and its blood/oxygen supply after a period of heat acclimation. Furthermore, another issue that has not been explored is whether heat acclimation can alter performance in a cool environment. Specifically, we aimed to determine whether heat acclimation could be used to improve cool weather performance and the potential mechanisms by which this occurs.

Finally, studies that focus on the local skin adaptations to heat acclimation are warranted. One of the classic thermoregulatory adaptations to heat acclimation is an increase in sweat rate and skin blood flow at a given core temperature. What we do not fully understand is whether these responses are purely centrally mediated or if there is an augmented cutaneous vascular function independent of core temperature. Furthermore, another issue that remains to be explored is whether heat acclimation affects maximal skin blood flow. We will investigate this by stimulating the cutaneous circulation by locally heating the forearm with a water spray

device and by infusions of acetylcholine and sodium nitroprusside before and after a period of heat acclimation.

Significance

The research objectives outlined in the dissertation will advance the basic scientific and mechanistic literature of the effect of heat acclimation on endurance-trained cyclists. Moreover, these set of studies can further the practical knowledge of the use of heat acclimation as a natural way to improve performance in elite cyclists. The competitiveness in sports have become so fierce that any small improvement in performance could make a big difference in the outcome, so athletes and their coaches have been forced to find innovative ways to gain an edge over the competition. For example, a 1% decrease in performance in the 2007 Chicago Marathon (i.e. 1.5 minutes) was the difference between winning the race or not making it to the top-three podium. Therefore, heat acclimation could be used as a training tool to improve performance in cool environmental conditions and that could potentially have a big impact in the world of sports competitions. In addition, advancing the knowledge on this topic can be very beneficial not only for athletes and their coaches, but also other populations that might be at risk when exposed to hot environments such as the elderly, hypertensive, diabetic and multiple sclerosis patients. The overarching goal of this research is to further understand the mechanisms of heat acclimation

on cardiovascular regulation and thermoregulatory responses during exercise in the heat and cool environments, and its effects on performance.

Specific aims

The studies discussed in this dissertation were designed to address the following specific aims:

1. In Chapter IV we aimed to study the effect of heat acclimation on lactate threshold and VO_{2max} of highly trained cyclists in a hot and cool environment. In addition we sought to investigate the effect of heat acclimation on the dynamics of muscle blood flow and oxygen delivery during a single-leg knee extensor exercise.

2. In Chapter V we tackled the heat acclimation effects on a 1-hour time trial performance of highly trained cyclists in a cool and hot environment.

3. The purpose of the studies in Chapter VI were two-fold. First, we sought to investigate how heat acclimation affects the skin blood flow and sweating responses to pharmacological treatment with specific dosages of the endothelium dependent muscarinic receptor agonist acetylcholine. Second, we examined the maximal skin blood flow responses to a period of heat acclimation.

Hypotheses

The following hypotheses were tested:

1. In Chapter IV we hypothesized that following heat acclimation, VO_{2max} and lactate threshold will be increased in hot and cool environments. Furthermore, femoral blood flow at peak kicking workload will not change but oxygen delivery will decrease due to the increased plasma volume and will match the decreased muscle's oxygen needs.

2. In Chapter V we hypothesized that heat acclimation will improve the 1-hour time-trial cycling performance in both cool and hot environments.

3. In Chapter VI we hypothesized that, to a specific dose of acetylcholine infused via microdialysis technique, the skin blood flow and sweating response will be greater after a period of heat acclimation. In addition, maximal skin blood flow will not change after a period of heat acclimation.

CHAPTER II

REVIEW OF THE LITERATURE

The review of the literature will first address the basic physiology of exercise performance and the limiting factors. The following reviews tackle the systemic and active muscle hemodynamics during exercise heat stress. The effect of heat acclimation on the cardiovascular and thermoregulatory systems will be the focus on the remaining part of the review of the literature. The review on the mechanisms of heat acclimation and its effect on performance will shed some light on the lacking knowledge in the literature and aid the development of the specific hypotheses for each of the studies presented in this dissertation.

Physiology of performance in the heat

There has been extensive research in the field of exercise and heat physiology. It is well documented that heat stress can impair performance during prolonged exercise of approximately one hour and longer (intensities varying from 40 to 80% of VO_{2max}) (Gonzalez-Alonso *et al.*, 1999, Kay *et al.*, 2001, Nybo *et al.*, 2001, Tucker *et al.*, 2004, Tucker *et al.*, 2006). In addition, heat stress can also decrease performance during maximal exercise lasting approximately 3 to 10 minutes (Arngrimsson *et al.*, 2003,

Gonzalez-Alonso & Calbet, 2003, Nybo & Nielsen, 2001b, Pirnay *et al.*, 1970). This earlier onset of fatigue during prolonged exercise in the heat is attributed to high core and brain temperatures (Nybo & Nielsen, 2001b). An impairment in oxygen delivery to the exercising muscles becomes relevant during high-intensity exercise of short duration, where cardiac output declines significantly and muscle blood flow decreases, such that increased oxygen extraction cannot compensate for the reduced oxygen delivery (Gonzalez-Alonso & Calbet, 2003, Gonzalez-Alonso *et al.*, 1998). In the following sections, I will be reviewing each potential factor that limits exercise performance.

Cardiac output and active muscle blood flow

The capacity of a skeletal muscle to increase its blood supply is huge. Blood flows as high as 250-400 ml (100 g^{-1} of tissue) min^{-1} or more can occur when exercise is limited to a small muscle mass (Andersen & Saltin, 1985, Armstrong & Laughlin, 1983, Rowell *et al.*, 1986). These levels of muscle blood flow, however, could not be achieved during whole-body dynamic exercise that are associated with VO_{2max}. Instead, other circulatory control elements prevent muscle vascular conductance from reaching such high levels in order to prevent blood pressure from being threatened. In fact, this inability to regulate blood pressure during whole body dynamic exercise is seen in conditions where sympathetic vasoconstrictor function is

compromised (Krediet *et al.*, 2004, Puvi-Rajasingham *et al.*, 1997).

In one classic study, Rowell and colleagues (Rowell *et al.*, 1966) investigated the cardiovascular responses in unacclimated and sedentary men to short duration exercise in the heat. They found that a high ambient temperature caused significant decreases in cardiac output. This failure to adequately increase cardiac output constitutes an important contributory factor limiting sedentary, unacclimated men's capacity to exercise in the heat. Gonzalez-Alonso and colleagues (Gonzalez-Alonso & Calbet, 2003) extended this knowledge and looked at the primary factors that limit VO_{2max} in trained men in thermoneutral and hot conditions. They found a decrease in VO_{2max} and time to fatigue in the hot condition compared to a thermoneutral environment. In addition, cardiac output decreased before fatigue, while heart rate was still rising. The authors measured a decrease in the oxygen delivery and leg blood flow in the hot condition and attributed it to the decrease in cardiac output. The authors concluded that the decreased VO_{2max} in the heat is directly related to the inability of the heart to maintain cardiac output and oxygen delivery to the active muscle. Recently, Mortensen and colleagues (2005) examined systemic and muscle hemodynamics during maximal exercise involving large active muscle mass (cycling) and small active muscle mass (one-legged knee extensor exercise) in trained male subjects under thermoneutral conditions. Only during the cycling trial there was an attenuation in leg blood flow, leg

oxygen delivery and VO_2 observed immediately preceding fatigue. The authors suggested that this is largely related to the inability of the cardiovascular system to continue to increase cardiac output to match the metabolic demands of the exercising muscles. On the other hand, when exercising with a small muscle mass (and thus, no cardiac limitation), leg blood flow and leg oxygen delivery continued to increase until onset of fatigue.

Although not fully confirmed, both baroreflex and active muscle metaboreflexes have been implicated in this attenuation of muscle vasodilation during intense whole body dynamic exercise (Rowell *et al.*, 1996). Both reflexes act in competition with the local control mechanisms of functional hyperemia to limit the increase in muscle blood flow. As mentioned previously, Mortensen *et al.* (2005) showed an attenuation in the rate of rise in leg muscle blood flow with increases in exercise intensity above 50% of VO_{2max} during cycling, and a plateau in the cardiac output above 90% of VO_{2max}, accompanying a leveling off in the limb muscle and systemic vascular conductance. Furthermore, they also showed in the same subject that cardiac output and muscle blood flow increased linearly during incremental one-legged knee extensor exercise (i.e. small muscle mass) to exhaustion (Mortensen *et al.*, 2005). Together, these observations support the possibility that the restrictions in active muscle blood flow might be at least in part related to the insufficient cardiac output as the limits of

cardiac pumping capacity are reached.

The idea that thermoregulatory reflexes (i.e increased skin blood flow) may also limit the functional muscle hyperemia during exercise is not well supported. Evidence from different studies show that even during extreme body heating, active muscle blood flow remained unchanged (Gonzalez-Alonso *et al.*, 1998, Nielsen *et al.*, 1990, Nielsen *et al.*, 1993, Savard *et al.*, 1988). Moreover, studies have shown that during submaximal exercise in the heat, the cardiovascular system was able to maintain active muscle blood flow by adequately increasing cardiac output by 3 L min^{-1} to supply the additional demand for an elevated skin blood flow and further decreasing visceral blood flow (Nielsen *et al.*, 1990, Nielsen *et al.*, 1993, Savard *et al.*, 1988). Therefore, these observations do not support the thought that skin blood flow may cause the restriction of active muscle blood flow during submaximal exercise. However, this might not be the case when performing dynamic exercises that require maximal efforts.

Muscle metabolism

As discussed earlier, during maximal exercise, severe heat stress (high core temperature and skin temperature) results in a greater decline in cardiac output, mean arterial pressure, and muscle blood flow, leading to a reduction in oxygen delivery, oxygen uptake, and earlier onset of fatigue compared to control conditions (Gonzalez-Alonso & Calbet, 2003). In

addition, decreased tolerance in the heat compared to thermoneutral conditions has been associated with increased rate of glycogenolysis (Fink *et al.*, 1975), and earlier increases in blood lactate accumulation (Young *et al.*, 1985). This blood lactate concentration reflects the balance between muscular production of lactic acid, efflux into the blood, and removal of lactate from the blood.

During submaximal exercise in the heat, anaerobic metabolism is increased (Dimri *et al.*, 1980, Young *et al.*, 1985), and several studies have shown that plasma lactate levels are higher than in a thermoneutral environment (Papadopoulos *et al.*, 2008, Powers *et al.*, 1985, Tyka *et al.*, 2000, Tyka *et al.*, 2009, Young *et al.*, 1985). The physiological events that mark this event are characterized by the inability of the rate of fat oxidation to meet the ATP demands of muscles contracting. Consequently, intracellular signaling events stimulate glycogenolysis and glycolysis. Ultimately, the rate of pyruvate delivery to the mitochondria progressively exceeds the ability of the mitochondria to oxidize pyruvate and this leads to accelerated generation of lactic acid (Hermansen & Stensvold, 1972, Hermansen *et al.*, 1967, Holloszy & Coyle, 1984, Holloszy *et al.*, 1977, Nagle *et al.*, 1970, Poortmans *et al.*, 1978, Robergs *et al.*, 2004). This exponential rate of blood lactate accumulation has been shown to be caused by an exponential increase in muscle lactate production (Ivy *et al.*, 1987). These changes in plasma lactate may also be attributed to the rise in

core temperature and subsequent increased Q_{10} effect, which enhances overall substrate metabolism (including lactate) (Nadel, 1985). In addition, plasma lactate levels can be affected by the heat-related redistribution of the cardiac output. It has been shown that the vasoconstriction in the splanchnic circulation accompanying heat exposure and exercise decreases lactate removal (Rowell *et al.*, 1968). Nevertheless, the elevated plasma lactate concentration during exercise in the heat suggests that either lactate production is increased (Ivy *et al.*, 1987) and/or lactate removal is hampered in the heat (Rowell *et al.*, 1968).

The effects of exercise on plasma lactate under cool conditions have been studied but more research on this area is necessary (Bergh *et al.*, 1979, Claremont *et al.*, 1975, Fink *et al.*, 1975, Flore *et al.*, 1992, Kruk *et al.*, 2000, Minaire *et al.*, 1982, Therminarias *et al.*, 1989). The literature on the dynamics of plasma lactate and cold exposure is conflicting. One study found no difference in the plasma lactate concentration between exercise in 0°C versus 20°C (Quirion *et al.*, 1988), while another study suggested that severe cold temperatures (−2°C) may delay the onset of blood lactate accumulation (OBLA) (Therminarias *et al.*, 1989). In addition, moderate cold exposure (10°C) has also been shown to decrease the plasma lactate levels (Flore *et al.*, 1992), although these changes were less marked than those observed during severe cold stress. These discrepancies may be attributed to differences in methodologies, degree of cold stress, fitness of

subjects and type of exercise.

The delay in blood lactate accumulation in a cool environment when compared to thermoneutral conditions could be explained by different theories (Flore *et al.*, 1992). First, lactate production in the muscle could be reduced and this could be explained by a decreased anaerobic glycogenolysis rate during cold exposure (Fink *et al.*, 1975), secondary to an increase in oxygen delivery to the active muscles. In addition, the vasoconstriction in the skin and consequently the reduction of cutaneous blood flow during cold exposure, may enhance blood flow to active muscles. Another possibility is that the delay in the onset of blood lactate accumulation (OBLA) observed during cold exposure is due to an increased lactate utilization by the heart, liver and skeletal muscle. Claremont et. al. (1975) suggested that cold-induced peripheral vasoconstriction may increase the blood flow and thus lactate removal by the liver (Claremont *et al.*, 1975). Furthermore, part of the blood flow diverted from the skin may increase blood flow, and thus lactate uptake, in the inactive muscles.

Heat acclimation and muscle metabolism

At a given exercise intensity, heat acclimation reduces the blood lactate concentration (Febbraio *et al.*, 1994, Young *et al.*, 1985) but the mechanism(s) remain unclear. Some studies suggested that heat acclimation induces metabolic adaptations during exercise by reducing the

aerobic metabolic rate (Sawka *et al.*, 1983, Young *et al.*, 1985), or decreasing the rate of glycogenolysis (Febbraio *et al.*, 1994, Febbraio *et al.*, 1996, Kirwan *et al.*, 1987). Alternatively, the increased plasma volume (and thus, total blood volume) (Bass *et al.*, 1955, Harrison *et al.*, 1981, Senay *et al.*, 1976, Wyndham *et al.*, 1968) may allow for an increased blood flow through the splanchnic circulation, enhancing lactate removal (Rowell *et al.*, 1968) and thus delaying blood lactate accumulation. The effects of heat acclimation on plasma lactate levels in cool environments have not been explored.

Regulation of skin blood flow

There are two types of human skin. The majority of the body is covered by non-acral skin, which is "hairy". The skin of the lips, nose, ears, palms of the hands and fingers, and plantar aspects of the feet are acral skin, also called glabrous skin. For the purpose of this dissertation, we will focus on "non-acral" skin when referring to the cutaneous microvasculature. Human skin is comprised of two layers: the epidermis (superficial) and dermis (deep layer). The epidermis contains mostly kerastinized squamous epithelial cells, and the dermis has a more complex structure containing blood vessels, nerves, sebaceous glands, sweat glands and hair follicles. The proximity of the blood vessels to the surface of the skin allows for a

great temperature gradient and heat exchange between the blood and the external environment.

There are two branches of the sympathetic nervous system that control blood flow to the skin: a vasoconstrictor system and an active vasodilator system. The vasoconstrictor system is adrenergic, and releases norepinephrine that binds to α_1- and α_2 adrenergic receptors. The active vasodilator system remains moderately understood. These nerves are believed to be cholinergic, releasing acetylcholine and an “unknown” cotransmitter to mediate vasodilation (Kellogg *et al.*, 1995). The strongest evidence supporting this theory comes from a study where blockade of muscarinic receptor in the skin with atropine suppressed sweating but not skin blood flow during passive heat stress. Furthermore, pre-synaptic blockade of cholinergic nerves by injections to areas of the skin with botulinum toxin inhibited both sweating and skin active vasodilation. The chemical properties of botulinum toxin prevent cholinergic nerves from releasing acetylcholine and any other colocalized neurotransmitter. Thus, this data suggests that cholinergic nerve activation mediates skin active vasodilation through the release of an unknown cotransmitter and not through acetylcholine (Kellogg *et al.*, 1995).

Skin blood flow during exercise

During exercise, the release of energy as heat and the concomitant rise in core temperature result in the temperature gradient between the body core and the skin to narrow, and for thermoregulatory purposes skin blood flow must therefore increase (Johnson, 1992, Wendt *et al.*, 2007). When thermoregulatory and non-thermoregulatory responses occur simultaneously, as they do during exercise, the cutaneous circulation is subjected to conflicting demands (Kellogg *et al.*, 1991). This competition for blood flow between active muscles and skin results in a compromised skin blood flow causing higher core temperatures. The attenuation of the skin blood flow response during exercise has two main causes. First, the threshold core temperature at which skin vasodilation begins is shifted to a higher level during exercise compared to rest (Bevegard & Shepherd, 1966, Bevegard & Shepherd, 1967, Johnson & Park, 1981, Johnson *et al.*, 1974, Johnson, 1992, Kellogg *et al.*, 1991, Kenney & Johnson, 1992, Smolander *et al.*, 1991, Zelis *et al.*, 1969). Second, as exercise in the heat progresses, core temperature continues to rise steadily, but skin blood flow reaches an upper limit when core temperature reaches approximately 38°C (Brengelmann *et al.*, 1977, Gonzalez-Alonso *et al.*, 1999). This occurs at a skin blood flow to be estimated around 50% of maximal, whereas, during resting conditions, skin blood flow would continue to rise with core temperature until a true maximal level is achieved.

In summary, the demands of dynamic exercise of high intensities distill down to demands for blood flow. Active muscles require blood flow and oxygen for ATP synthesis to meet the energetic demands for muscular activity. On the other hand, blood flow to the skin is required to meet the demands for thermoregulation. The combination of demands for blood flow results in a competition for the insufficient available cardiac output (Rowell, 1974) between active muscle and skin.

Heat acclimation effects on skin blood flow

Another issue that has not been thoroughly explored is the specific mechanism by which skin blood flow is increased after heat acclimation. It is well documented that heat acclimation increases skin blood flow at a given core temperature (Nadel *et al.*, 1974, Roberts *et al.*, 1977, Takeno *et al.*, 2001, Yamazaki & Hamasaki, 2003). For example, Roberts et.al. (1977) reported that a 10-day period of heat acclimation lowered the internal temperature threshold for cutaneous vasodilation, without significant change in the slope of the relations. The authors postulated that these changes were caused by a central mechanism. Conversely, other studies showed that heat acclimation increases the slope of the relation of forearm vascular conductance to internal temperature during exercise in the heat, implying some peripheral vascular changes (Sawka *et al.*, 1989, Takeno *et al.*, 2001). Differences in the heat acclimation protocols (ambient

temperature, intensity and duration of exercise) and the type of heat test (i.e. rest or during exercise) can account for such inconsistencies. However, in all these studies changes in cutaneous blood flow were induced by raising internal temperature. Therefore, it remains uncertain if these changes are centrally mediated or if there are local structural changes occurring within the cutaneous vasculature. More specifically, skin blood flow may be augmented by an increased ability of the skin vessels to vasodilate (i.e. increased maximal skin blood flow), or there may be an improved vasodilatory response (i.e. increased sensitivity) for a specific stimulus.

Regulation of sweating

There are two main types of human sweat glands: the apocrine and eccrine gland. The eccrine sweat gland is the primary gland responsible for thermoregulatory sweating and thus, will the focus of this dissertation. Eccrine sweat glands are located nearly over the entire body surface and its structure consists of a bulbous secretory coil, which is located in the lower dermis, and a duct, which extends through the entire dermis and opens directly into the skin surface.

Acetylcholine is the primary neurotransmitter released from cholinergic sudomotor nerve terminals and binds to muscarinic receptor in on the eccrine sweat gland (Randall & Kimura, 1955, Thaysen & Schwartz,

1955), although exogenous administration of α- or β- adrenergic agonists can also stimulate sweating (Quinton, 1987, Randall & Kimura, 1955, Robertshaw, 1975, Sato, 1977). Nevertheless, most of the experimental evidence suggests that the thermoregulatory sweating occurs primarily through stimulation of muscarinic receptors. Supporting this theory, evidence showed that local and systemic administration of atropine (i.e. muscarinic receptor antagonist) greatly attenuates or abolishes sweating during thermal challenge or during exogenous administration of acetylcholine or its analogs (Foster & Weiner, 1970, Kellogg *et al.*, 1995, Kolka & Stephenson, 1987, Longmore *et al.*, 1986, Low, 2004).

When acetylcholine binds to muscarinic receptors on the sweat gland, intracellular Ca^{2+} concentration increase, leading to increases in permeability of Cl^- and K^+ ion channels and the release of a fluid precursor form the secretory cells (Sato *et al.*, 1989). This fluid solution that travels up the secretory coil into the sweat duct has been found to be hypotonic in the dermal level relative to the epidermis (Sato, 1973). This evidence lead to the conclusion that, as the fluid travels up the secretory coil and duct towards the skin surface, sodium and chloride are reabsorbed, resulting in the sweat fluid at the skin being hypotonic relative to the plasma. However, as sweat rate increases due to exercise or heat stress (or both), fewer ions are reabsorbed due to the increased sweat secretion into the ducts, which leads to higher ion losses. Therefore, sweat sodium content is greatly

influenced by sweat rate (Bulmer & Forwell, 1956, Quinton, 1987, Sato & Dobson, 1970, Sato, 1973, Schwartz & Thaysen, 1956, Schwartz *et al.*, 1953).

Although the main stimulus for sweating is core temperature, mean skin temperature can also modify sweating responses. Nielsen and Nielsen (1965) were one of the first to show that a rapid decrease in mean skin temperature reduced sweat rate when internal temperature remained stable (Nielsen & Nielsen, 1965). Later, Nadel and colleagues were among the first to directly investigate the relationship between the sweat rate responses relative to dynamic increases in internal temperature in humans (Nadel *et al.*, 1971b, Nadel *et al.*, 1974). Animal models confirmed that sweating is primarily controlled by central brain temperature and secondarily affected by mean skin temperature (Smiles *et al.*, 1976). Given the observations that internal and mean skin temperature can control sweating (Hardy & Stolwijk, 1966, McCook *et al.*, 1965, Nadel *et al.*, 1971a, Saltin & Gagge, 1971, Saltin *et al.*, 1970, Wurster & McCook, 1969), the concept of "mean body temperature" was introduced, which represent the fraction of internal and skin temperature (i.e. 0.9*internal temperature + 0.1*mean skin temperature) (Gagge & Nishi, 1977, Gisolfi & Wenger, 1984), and it is now being frequently used when expressing sweating responses (Ogawa *et al.*, 1979, Yamazaki *et al.*, 1994, Yoshida *et al.*, 1995).

Heat acclimation effects on sweating

Due to the improvements in cardiovascular function that follow a period of heat acclimation, we know that skin blood flow and sweat rate are higher at a given exercise intensity or core temperature (Fox *et al.*, 1963b, Nielsen *et al.*, 1993, Senay *et al.*, 1976, Wyndham *et al.*, 1976). The current thinking is that this is a predominantly centrally mediated response (Colin & Houdas, 1965, Kuno, 1956, Nadel *et al.*, 1974, Roberts *et al.*, 1977, Shvartz *et al.*, 1979, Wyndham *et al.*, 1976). Roberts *et. al.* (1977) showed that heat acclimation increases sweat rate by lowering the internal temperature threshold for sweating, and also by increases in the slope of the sweat rate: internal temperature relationship. Therefore, what we do not know is whether or not there are functional changes in the peripheral thermoregulatory apparatus for a specific stimulus (Chen & Elizondo, 1974, Collins *et al.*, 1965, Fox *et al.*, 1964, Inoue *et al.*, 1999, Ito & Adachi, 1934). A very well designed study by Chen & Elizondo (1974) showed evidence that the increased sweat output following heat acclimation is due primarily to an increased sweating capacity of the sweat gland apparatus. In other words, there might be some underlying adaptations that can modify sweating independent of a central drive. Some studies observed increased sweating during exogenous administration of sudorific agents (i.e. methacholine or acetylcholine) after heat acclimation in humans (Collins *et al.*, 1966, Inoue *et al.*, 1999). In addition, other studies showed that if local

skin temperature is maintained at a cool temperature throughout heat acclimation, sweat responses at that location were not modified by heat acclimation (Chen & Elizondo, 1974, Fox *et al.*, 1964), suggesting physiological changes at the sweat gland.

In summary, there are still a number of important questions left to pursue with respect to basic thermoregulatory adaptations caused by heat acclimation. Also, effect of heat acclimation on performance in cool environments was one of the main goals of this dissertation. The performance variables used on this dissertation were maximal oxygen consumption (VO_{2max}), lactate threshold and time trial performance. These performance parameters will be discussed in the following section.

Measured performance parameters

Maximal oxygen uptake

The upper limit of aerobic metabolism is the called 'maximal' oxygen uptake (VO_{2max}). By examining the Fick principle ($VO_2 = Q_c$ x a-vO_2 difference), VO_{2max} represents the integrative ability of the cardiovascular system to generate a high cardiac output, high muscle blood flow and muscle oxygen extraction, and in some cases the ability of the lungs to adequately oxygenate the blood (Bassett & Howley, 2000, Dempsey, 1986, Kanstrup & Ekblom, 1984, Mitchell *et al.*, 1958, Saltin & Strange, 1992). As early as the 1930s (Robinson *et al.*, 1937) very high values for VO_{2max} in

athletes were observed and identified as a marker of elite performance. Values between 70 and 85 ml kg^{-1} min^{-1} are commonly seen in very highly trained male athletes (Saltin & Astrand, 1967). Female values are, on average, 10% lower due to the lower hemoglobin concentration and higher levels of body fat (Pate *et al.*, 1987, Pollock, 1977, Saltin & Astrand, 1967). On average, elite endurance athletes have VO_{2max} values 50-100% greater than those seen in normally active healthy young subjects. These striking differences that allow for such high VO_{2max} values with training include increased blood volume and cardiac stroke volume, and increased capillary density and mitochondrial density in the trained skeletal muscle (Costill *et al.*, 1976a, Costill *et al.*, 1976b). Maximal cardiac output in athletes has been measured since the 1950s (Ekblom & Hermansen, 1968, Grimby *et al.*, 1966a, Mitchell *et al.*, 1958) and is well established that the ability of athletes to further increase cardiac output plays a major role in the VO_{2max} difference compared to sedentary subjects. In fact, a good correlation between aerobic work capacity, and such circulatory dimensions as heart volume, stroke volume, and blood volume has been demonstrated (Astrand *et al.*, 1964).

The reports on the effects of high ambient temperatures on VO_{2max} are very conflicting, to say the least. For instance, human subjects that have been acutely exposed to hot conditions have been reported to have lowered (Arngrimsson *et al.*, 2004, Klausen *et al.*, 1967, Mortensen *et al.*,

2005, Nybo *et al.*, 2001, Rowell *et al.*, 1969, Sakate, 1978, Saltin *et al.*, 1972, Sawka *et al.*, 1985, Taylor *et al.*, 1955) or unchanged VO_{2max} values (Arngrimsson *et al.*, 2004, Rowell *et al.*, 1965, Williams *et al.*, 1962). These discrepancies can be attributed to the different methodologies used, including different ambient temperature/humidity, subjects' fitness level, and degree and/or duration of heat stress prior to exercise. For example, in some of the studies that found little or no change in the VO_{2max} value, exposure to the heat was for relatively short durations, and/or rectal temperatures often were not elevated to high levels. More recently, studies suggested that the largest decreases in VO_{2max} were achieved with very high core and skin temperatures; high skin temperatures alone was insufficient to cause a large reduction in $VO2_{max}$ (Arngrimsson *et al.*, 2004, Mortensen *et al.*, 2005, Nybo *et al.*, 2001).

Effects of heat acclimation on VO_{2max}

Maximal aerobic power is a good predictor of endurance performance. As mentioned earlier, as early as the 1930s (Robinson *et al.*, 1937) very high values for VO_{2max} in athletes were observed and identified as a marker of elite performance. However, the direct effects of heat acclimation on heat stress and VO_{2max} was not studied until Sawka et al. in 1985. Their data demonstrated that heat stress reduced VO_{2max} relative to the level achieved in a moderate environment. More importantly, the heat

acclimation protocol did not modify the approximate 0.25 L*min^{-1} decrement in VO_{2max} between environments. In addition, heat acclimation resulted in a significant increase in the VO_{2max} in both environmental conditions, which the authors attributed to a "training effect" (Sawka *et al.*, 1985). However, a control group would be necessary to confirm this theory. Subjects who participated in the study had VO_{2max} values ranging from 39-53 ml * kg^{-1} * min^{-1}, which corresponds with fair-to-high fitness levels (Astrand & Rodahl, 1977). Furthermore, the methodology is unclear if the subjects had their core temperature increased prior to the VO_{2max} test in the heat. As previously discussed, high core (and skin) temperatures are necessary to have significant impact on VO_{2max} (Arngrimsson *et al.*, 2004, Mortensen *et al.*, 2005, Nybo *et al.*, 2001). To our knowledge, research which attempts to investigate the influence of acute and chronic heat exposure (heat acclimation) on highly trained (VO_{2max} > 65 ml * kg^{-1} * min^{-1}) cyclists' maximal aerobic power in the hot and cool environment has not been published.

Anaerobic threshold

As already mentioned, VO_{2max} is a good predictor of aerobic performance. However, another parameter that has gained much interest in the last 25-30 years in the so-called anaerobic threshold or 'lactate threshold'. A battery of tests have been developed to determine the

intensity of exercise associated with the anaerobic threshold. They covered the testing of maximal lactate steady state, lactate minimum, lactate threshold, onset of blood lactate accumulation, individual anaerobic threshold, ventilatory threshold and electromyographic threshold (Glass *et al.*, 1998, Nagata *et al.*, 1981, Svedahl & MacIntosh, 2003, Tyka *et al.*, 2000). Measurements of blood lactate have been used as indicators of muscular stress during exercise for almost 90 years. In fact, it had been recognized since 1933 that the production of lactic acid by the muscle during exercise is indicative of metabolic stress (Margaria *et al.*, 1933). However, regardless of the name used, this physiological event is caused by the inability of the rate of fat oxidation to meet the ATP demands of muscles contracting. Consequently, intracellular signaling events stimulate glycogenolysis and glycolysis, which causes the rate of pyruvate delivery to the mitochondria to progressively exceed the ability of the mitochondria to oxidize pyruvate, leading to increased production of lactic acid (Hermansen & Stensvold, 1972, Hermansen *et al.*, 1967, Holloszy & Coyle, 1984, Holloszy *et al.*, 1977, Nagle *et al.*, 1970, Poortmans *et al.*, 1978, Robergs *et al.*, 2004). This exponential rate of blood lactate accumulation has been shown to be caused by an exponential increase in muscle lactate production (Ivy *et al.*, 1987). Although the physiological determinants of lactate threshold are exceptionally complex in nature, they are determined mainly by the oxidative capacity of the skeletal muscle (Davies *et al.*, 1982,

Gregg *et al.*, 1989a, Gregg *et al.*, 1989b, Holloszy & Coyle, 1984, Holloszy *et al.*, 1977). In contrast to the "small window" for improving VO_{2max}, the oxidative capacity of skeletal muscle is highly plastic and studies have shown that this ability of the skeletal muscle to oxidize pyruvate can increase more than twofold in trained skeletal muscle of humans or animals who engage in a training protocol (Dudley *et al.*, 1982, Holloszy & Coyle, 1984, Holloszy *et al.*, 1977). This is one of the factors that allow elite athletes to achieve very high 'lactate threshold' values.

Determination of anaerobic threshold

The term anaerobic threshold (AT) has been generally used to define the peak work rate or oxygen uptake at which aerobic metabolic processes can no longer meet the skeletal muscle requirements for ATP (Wasserman *et al.*, 1967). As work rate increases above the AT, anaerobic glycolysis must increase to sustain adequate levels of ATP, which leads to an elevated muscle lactic acid concentration and a consequent metabolic acidosis (Sahlin, 1978).

Although the existence of AT in muscle energy metabolism and its good correlation with endurance performance is well accepted (Bishop *et al.*, 1998, Farrell *et al.*, 1979, Yoshida *et al.*, 1987), the procedure for detecting the point of AT is less well confirmed. As previously mentioned, a variety of terms have been used to describe this critical threshold, including

anaerobic threshold (AT) (Wasserman *et al.*, 1967), lactate threshold (LT) (McGehee *et al.*, 2005), onset of blood lactate accumulation (OBLA) (Sjodin & Jacobs, 1981), maximal lactate steady state (MLSS) (Billat *et al.*, 2003), ventilatory threshold (VT) (Plato *et al.*, 2008), and electromyographic threshold (Tyka *et al.*, 2009). Despite disagreements over the definition and causal mechanisms of the AT, it's practical importance has been adequately documented. There are two major ways to estimate the anaerobic threshold. One involves direct blood lactate measurements, while that other uses pulmonary gas exchange data. Next, I will discuss the most common indices to determine the AT.

Lactate indices

There are numerous approaches currently used to determine anaerobic threshold using blood lactate levels during incremental exercise. Some involve simple subjective observations (i.e. OBLA), while other methods use complex mathematical algorithms (i.e. semi-log model). For the purpose of dissertation, the most commonly used methods will be discussed in the following paragraphs.

The 4 mM Lactate Threshold (LT_4) method determines the power output or oxygen uptake at which blood lactate reaches a concentration of 4 mM. If the value of 4 mM is between two measured values, it is interpolated from the two closest samples (Kindermann *et al.*, 1979). Even though this

method is determined during incremental work, it has been shown to correlate to sustained aerobic performance. (Kindermann *et al.*, 1979, Sjodin & Jacobs, 1981). Although the value of 4 mM is usually found to be close to the maximal lactate steady state (Billat *et al.*, 2003), other studies have shown that this value could change with different sporting activities and could be as high as 5.4 mM in cycling (Beneke & von Duvillard, 1996). In addition, some individuals were able to sustain efforts that result in blood lactate concentration of > 6 mM for 30 minutes or longer (Harnish *et al.*, 2001, Myburgh *et al.*, 2001). Therefore, the ability of this method to predict anaerobic threshold may be in question.

The 1 mM Lactate Threshold (LT_1) method determines the power output or oxygen uptake at which blood lactate increases 1 mM above resting values (Coyle *et al.*, 1983). This method, and some of its variations (i.e. blood lactate increases of 0.5 mM or 0.75 mM above baseline values), has been commonly used to estimate anaerobic threshold (Dumke *et al.*, 2006, McGehee *et al.*, 2005, Thomas *et al.*, 2008), due to the relative ease and nonsubjective nature in discerning the anaerobic threshold. Furthermore, this approach takes into account individual variations in the subjects' resting steady state lactate levels.

The Maximal deviation method (Dmax) method uses computer algorithms to make objective determinations of the anaerobic threshold. The blood lactate concentration curve versus power output is fitted by a

third order polynomial regression. A straight line is formed with the two end data points of the curve. The power output on the regression curve that yields the maximal perpendicular distance to the straight line is considered Dmax (Cheng *et al.*, 1992). Like the LT_1 approach, the Dmax method also provides an objective means for determining anaerobic threshold. This method has been shown to correlate well with other approaches (McGehee *et al.*, 2005, Thomas *et al.*, 2008), but others showed little correlation with most conventional lactate indices (Dumke *et al.*, 2006).

The Inflection point between resting and rising phases in blood lactate (LT_{INT}) method also uses regression equations to determine the anaerobic threshold objectively. The blood lactate concentration curve versus power output or oxygen consumption is fitted with two regression lines. One line corresponds to the steady state values (slope of zero), while the second regression line uses the "rising" lactate stages. The power output or oxygen consumption at the intersection between both lines is considered the LT_{INT}. This approach has been shown to correlate highly with other methods (Coyle *et al.*, 1983, Farrell *et al.*, 1979, Senay & Kok, 1977).

Indirect methods using pulmonary gas exchange

As previously described, inadequate oxygen supply to the exercising muscles causes concomitant increases in anaerobic metabolism to

maintain energy requirements for ATP. The first outcome of inadequate oxygen supply is the formation of lactic acid. Due to the low p*K* of this molecule, 99% of lactic acid is dissociated and buffered mainly by the bicarbonate system. This bicarbonate system is highly effective in buffering lactic acid because its by-product (CO_2) can be readily exhaled into the atmosphere, which results in an increase in CO_2 production (VCO_2) and respiratory exchange ratio (RER). The increase in VCO_2 provides an additional ventilatory stimulus.

The Ventilatory Threshold (VT) method is determined at the power output or oxygen consumption where there is a disproportionate increase in ventilation (V_E) for an increment in VO_2 (Wasserman *et al.*, 1973). This method has been show to be strongly correlated with numerous lactate indices (Bosquet *et al.*, 2002), although these thresholds could occur at different power outputs. Furthermore, the relationship between lactate and ventilatory thresholds could be dissociated (Hughes *et al.*, 1982). This theory will be discussed later in this section.

The Ventilation/Oxygen Consumption Threshold (V_E/VO_2) method has been widely used to estimate anaerobic threshold (Caiozzo *et al.*, 1982, Reinhard *et al.*, 1979, Thomas *et al.*, 2008). The plots V_E/VO_2 and V_E/VCO_2 curve versus power output are visually inspected and the threshold is determined when there is a systematic increase in the V_E/VO_2 without any concomitant increase in the V_E/VCO_2 (Caiozzo *et al.*, 1982). Studies have

shown that the V_E/VO_2 method was the best single index to detect AT from gas exchange data (Thomas *et al.*, 2008). Some advantages that make this method a better choice include a high correlation with plasma lactate thresholds indices, as well as high test-retest correlations with plasma lactate threshold (Caiozzo *et al.*, 1982).

The power output or oxygen consumption when the Respiratory Exchange Ratio equal to 1 is an attractive method because is an objective and easy-to-use indirect method giving a good index for the lactate threshold. Although this method is not commonly used, one study reported that the power output associated with a RER equal to 1 during incremental exercise could be used to estimate maximal lactate steady state (Laplaud *et al.*, 2006).

Although widely accepted, the use of respiratory events (i.e. ventilation) to characterize a metabolic event (metabolic acidosis) is based on several assumptions that may not always hold true. For instance, the change in muscle and blood lactate concentrations must occur almost simultaneously and the point of elevation in blood lactate can be used to characterize metabolic acidosis and the threshold of an altered V_E/VO_2 relationship. Several studies suggest that these relationships are not always held true. The time delay associated with lactate diffusion from the muscle (Harris *et al.*, 1977), the retention of a substantial part of the lactate within the muscle (Hermansen & Vaage, 1977), the diffusion hindrance for lactate

above certain concentration (Jorfeldt *et al.*, 1978), and the potential dissociation between the removal of lactate and hydrogen ions (H^+) from the muscle (Jones, 1980) are factors that might disrupt these relationships.

Currently, there is no “universal” method used to detect the AT. Researchers have used lactate indices as well as indirect methods using gas exchange. One of the major challenges of the indices used is the subjective evaluation of the data, although presently accepted as techniques for determining AT. For example, visual estimation of the VT may be modified after examination of other gas-exchange measures such as end tidal CO_2 partial pressure (PCO_2), mixed expired PCO_2, ventilatory equivalents for VO_2, and respiratory exchange ratio. To provide a more objective means of determining AT, some researchers have developed a computer algorithm to detect the departure from linearity in the V_E vs. VO_2 relationship (Orr *et al.*, 1982). Briefly, the computer accepts as input two arrays containing V_E and VO_2 data. A brute-force method is then used to fit two lines to the data. Regression lines are calculated for all possible divisions of the data into two contiguous groups, and the pair of lines yielding the least-pooled residual sum of squares is chosen as representing the best fit. This idea of using a computer model to determine the AT in a more objective has been adopted by many researchers (Beaver *et al.*, 1985, Beaver *et al.*, 1986, Cheng *et al.*, 1992, Hughson *et al.*, 1987, Morton *et al.*, 1994, Myers *et al.*, 1994, Peronnet & Morton, 1994, Sherrill &

Swanson, 1989, Sherrill *et al.*, 1990, Thomas *et al.*, 2008).

There are, however, some disadvantages of using the objective methods. One of the major limitations is that in about 30% of the cases, no deflection point can be detected with some of the methods due to irregular behavior of the physiological variable (Cheng *et al.*, 1992). In addition, if different variables form the same subject are treated (i.e. V_E, VCO_2, V_E/VO_2) with the same method or the same variable is treated with different methods, a discrepancy may occur because of the different criteria used in the different methods. It is difficult to determine if these discrepancies in the objective methods are compared to the obvious visual subjective threshold determination.

Effects of ambient temperature on the anaerobic threshold

As previously discussed, during submaximal exercise in the heat, anaerobic metabolism is increased (Dimri *et al.*, 1980, Young *et al.*, 1985), and several studies have shown that plasma lactate levels are higher than in a thermoneutral environment (Papadopoulos *et al.*, 2008, Powers *et al.*, 1985, Tyka *et al.*, 2000, Tyka *et al.*, 2009, Young *et al.*, 1985). Therefore, it is well established that high ambient temperatures decrease the work rate and the VO_{2max} at the AT compared to a less thermally challenging environment (Papadopoulos *et al.*, 2008, Smolander *et al.*, 1986, Tyka *et al.*, 2000). The basis for this was discussed in an earlier section (see

Muscle Metabolism).

The effects of cooler temperatures on AT has also been studied, although not as thoroughly (Flore *et al.*, 1992, Therminarias *et al.*, 1989). The literature on anaerobic threshold and cold exposure is conflicting. One study found no difference in the anaerobic threshold between exercise in 0°C versus 20°C (Quirion *et al.*, 1988), while another study suggested that severe cold temperatures (−2°C) may delay the onset of blood lactate accumulation (OBLA) (Therminarias *et al.*, 1989). In addition, moderate cold exposure (10°C) has also been shown to decrease the plasma lactate levels (Flore *et al.*, 1992), although these changes were less marked than those observed during severe cold stress. These discrepancies may be attributed to differences in methodologies, degree of cold stress, fitness of subjects and type of exercise.

One-hour time trial performance

Methods to evaluate aerobic and anaerobic performance in athletes have been studied extensively, although there is only scarce literature in regards to the reproducibility of endurance performance tests. The literature shows that no uniform endurance performance test exists, but a wide variety of different exercise protocols are used (Anantaraman *et al.*, 1995, Below *et al.*, 1995, Clark *et al.*, 2000, Coyle *et al.*, 1991, el-Sayed *et al.*, 1997, Hickey *et al.*, 1992, Jeukendrup *et al.*, 1996, Jeukendrup *et al.*, 1997,

Krebs & Powers, 1989). Traditionally, these methodologies were based on submaximal performance rides at a fixed percentage of VO_{2max} or maximal power output. One test, however, has reported to be highly reproducible (Jeukendrup *et al.*, 1996) and involved having the subjects perform a certain amount of work as fast as possible with the ergometer set in the linear mode (pedaling rate dependent). The total amount of work (in kilojoules) was calculated according to the formula:

$$\text{Total work} = .75 * W_{max} * 3600$$

where W_{max} is the maximum power output previously determined form VO2max test (Jeukendrup *et al.*, 1997).

In order to make this test as similar to regular time-trial competitions as possible, we adopted this test with a few modifications. Subjects performed a maximal effort on a cycle ergometer for a total of one hour. Total work done after 1 hour (in kilojoules) was the performance variable of interest. During the test, the cycle ergometer was set to the hyperbolic mode (pedaling rate independent) and subjects did not receive any feedback (i.e. HR, power output, core temperature, etc.) except for total time elapsed. Subjects were allowed to modify power output as often as needed, but without knowing the absolute workload. The average power output during the 1 hour time-trial performance test has been shown to have a high correlation ($r = -0.88$) with 40 km time-trial performances in

highly trained competitive cyclists (Coyle *et al.*, 1991). Moreover, the same study has shown that a 1 hr power output is highly related to the VO_2 at the LT (r = 0.93). Therefore, the close association between performance during an actual 40 km time-trial and the 1-hr laboratory test indicates that the laboratory test simulated a time-trial reasonably well.

CHAPTER III

EXPLANATION OF THE METHODOLOGY

The experimental protocols that were part of this dissertation were approved by the Institutional Review Board of the University of Oregon (Protocol # A129-09F, # C1-96-10F) and were administered in accordance with the guidelines as set forth by the Office of Protection of Human Subjects of the University of Oregon and the Declaration of Helsinki.

Overview of the project

This project involved highly trained male and female cyclists (or triathletes), who were currently racing at the collegiate or professional level and trained a minimum of 5 days a week (VO_{2peak} > 60ml*kg^{-1} *min^{-1}). In brief, participants were put through a battery of physiological and performance tests, then went through a heat acclimation program, and then repeated the tests. The studies involved in this dissertation aimed to investigate performance variables (i.e. 1 hour time-trial, lactate threshold, VO_{2peak}) and other physiological variables related to cardiovascular control and thermoregulation. Our goal was not only to look at acclimation and effects on exercise in the heat as primary outcome variables per se, but also to look at whether heat acclimation can change performance (positively

or negatively) in a cool environment. Therefore, every exercise test in this dissertation was performed under hot (38°C, 30% RH, WBGT 33°C) and also cool conditions (13°C, 30% RH, WBGT 12°C).

Subjects were divided into two groups. One group (12 subjects) underwent a period of heat acclimation, while the other group (8 subjects) performed the same activity but under cool conditions, and thus served as a control group. The rationale for including a control group is so the potential adaptations seen in the experimental group can be attributed to the heat acclimation per se and not to a "training" effect (Gisolfi, 1973, Piwonka & Robinson, 1967, Piwonka *et al.*, 1965, Strydom *et al.*, 1966). During some studies done in the hot condition (38°C), subjects immersed in a water-filled tub (~41°C) for approximately 30 minutes to increase their rectal temperature by 0.8-1.0°C. On the protocols done under cool environmental conditions (13°C), subjects also immersed in a water-filled tub with thermoneutral water (~34°C) for 30 minutes to maintain the same resting rectal temperature. The water immersion allowed us to manipulate the subjects' rectal temperature without having to make them exercise prior to the studies, which can potentially act as a confounding variable. Furthermore, pilot work done in our environmental chamber demonstrated that even exercising at a very low power output (i.e. 125W) for 30 minutes in a cool environment (13°C 45% RH), resulted in an increase in rectal temperature of 0.9°C.

The study days for the experimental and control groups are displayed below:

Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Screening Health Check	Lactate Threshold and VO_{2max} 13°C	Leg kicking 13°C Leg kicking 38°C	Lactate Threshold and VO_{2max} 38°C	1-hr time trial 13°C	Skin study 23°C	1-hr time trial 38°C

Days 7-16	Days 17-22
Heat Acclimation or Control	Repeat Days 1-6

Subjects

A total of 17 subjects (13 men and 4 women) participated in this series of studies. Every subject (except for one) completed the entire study, which consisted of 22 study visits. One subject withdrew from the study due to insufficient time commitment. Every participant was classified as a "highly trained" competitive cyclist or triathlete currently competing at the college or professional level (maximal aerobic power > 60 ml kg^{-1} min^{-1}). All subjects were healthy with no allergies, non-smoking, normotensive, had no history of heat related disorders, and were free of cardiovascular disease. In addition, subjects were taking no medications, with the exception of oral contraceptives. All women who participated in the project had negative pregnancy tests prior to each of the study days. All data was collected in

the Evonuk Environmental Physiology Core at the University of Oregon, in Eugene, during the months of February and September. Each subject gave written informed consent before participating in the study.

Environmental stress

On the days when the studies were preformed under heat stress, the climatic chamber was set to 38°C and 30% relative humidity (RH) (WBGT = 33°C). These conditions were chosen based on the extensive literature on exercise-heat stress. This temperature and humidity has shown to adequately stress the cardiovascular system during intense exercise (Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005, Mortensen *et al.*, 2008).

On the days when the studies were carried out under cool conditions, the climatic chamber was set to 13°C and 30% RH. (WBGT = 12°C) Although there has been published research on exercise performance under cool conditions (Flore *et al.*, 1992, Therminarias *et al.*, 1989), no studies have explored heat acclimation effects on performance under moderate cold temperatures.

The heat acclimation exposures were set to 40°C and 30% RH. (WBGT = 35°C). The subjects who participated in this study were highly trained and thus, already partially heat acclimatized (Pandolf *et al.*, 1977). Therefore, these conditions ensured that the subjects were getting

adequate heat stress to maximize the heat acclimation adaptations. Furthermore, many studies have used similar heat acclimation protocols and reported successful heat acclimation responses (Machado-Moreira *et al.*, 2005, Nadel *et al.*, 1971b, Nielsen *et al.*, 1993).

The environmental conditions for the control group during the "control exposures" were set to 13°C and 30% RH to prevent the core temperature from increasing enough to elicit some heat acclimation adaptations.

Exercise equipment

Most of the studies performed in this dissertation were done on an electronically braked cycle ergometer (Lode Excalibur Sport™, Groningen, The Netherlands). In addition, the study that involved single-leg kicking exercise was performed with a custom built kicking ergometer.

Pharmacological interventions

The study compounds that have been used in this dissertation have been administered to human volunteers previously in the research setting in the exact format used here. In the next few paragraphs, the mechanisms of action as well as the theoretical basis for the use of each compound will be described.

Acetylcholine (Ach) is a neurotransmitter present in both, the peripheral nervous system and the central nervous system. Together with

an "unknown" cotransmitter, cholinergic nerves release acetylcholine to induce vasodilation. ACh has been used to determine endothelial function and dysfunction since Furchgott and Zawadzki introduced the concept of endothelium-dependent vasodilation using this amine (Furchgott & Zawadzki, 1980). Acetylcholine (Sigma-Aldrich, St. Louis, MO) has been used in this study at different concentrations to induce skin vasodilation as well as sweating. This compound has been used by many researches to investigate endothelium-dependent skin vasodilation as well as sweating (Kellogg *et al.*, 1995, Kimura *et al.*, 2007, Medow *et al.*, 2008). By locally stimulating the skin, we can isolate any potential peripheral adaptations that might occur after heat acclimation in the skin circulation as well as within the sweat glands.

Sodium nitroprusside (SNP; Nitropress, Ciba Pharmaceuticals) is a compound used as a nitric oxide donor that causes vasodilation. Different from Ach, it has been widely used as an endothelium-independent vasodilator (Lorenzo & Minson, 2007, McCord *et al.*, 2006, Medow *et al.*, 2008, Minson *et al.*, 2001, Minson *et al.*, 2002). Previous work in our lab have determined that a 28mM concentration of SNP adequately induces maximal skin vasodilation (Lorenzo & Minson, 2007, McCord *et al.*, 2006).

Measurements and techniques

Body weight

Dry, nude body weight was taken at the beginning and end of each study in this dissertation by a precision weighing balance to the nearest 5 g (Sartorius™ EB6CE-I, Precision Weighing Balances, Bradford, MA). The initial body weight was used to ensure body fluid balance remained constant during the 22 study visits. Body weights pre and post exercise, together with total liquid ingested during the study, were used to estimate total body water loss (and thus, sweat rate) using the following formula: (initial body weight – final body weight + oral fluid replacement). Acute changes in body weight during exercise can be used to calculate accurate total body sweat rates. Therefore, when a sensitive scale and proper techniques are used (i.e. dry, nude body weights) body weight can provide an accurate assessment of hydration status and total body water changes during exercise.

Heart rate

Heart rate (HR) was monitored continuously throughout each protocol using a Polar™ heart rate monitor (Model RS400, Polar Electro™, Lake Success, NY).

Arterial pressure

Measurements of systolic (SBP) and diastolic (DBP) blood pressure were done via brachial artery oscillometry (Cardiocap/5, Datex-Ohmeda™, Madison, WI). Mean arterial pressure was calculated as DBP + ((SBP-DPB/3).

Rating of perceived exertion

Indices of perceived exertion (RPE) was measured using the 6-20 Borg scale (Borg, 1970). This scale allows subjects to easily determine quantitatively the level of physical exertion that they are experiencing. The numbers in the lower end of the scale (i.e. 6 and 7) represent "very, very easy", while the higher numbers (i.e. 19 and 20) indicate "very, very hard".

Arterial oxygen saturation

Oxygen saturation (Sa_{O2}) was determined by forehead pulse oximetry (Nonin Medical, Inc. Minneapolis, MN). Pulse oximeters use a light source and photodiode light detector to measure the amount of light passing through an arteriolar bed. Sa_{O2} can be estimated noninvasively because the light-absorbing characteristics of hemoglobin differ between oxyhemoglobin and deoxyhemoglobin. Studies have shown that forehead sensor offered greater validity of Sa_{O2} measurements under exercise

conditions than the other locations (i.e earlobe or finger) (Yamaya *et al.*, 2002).

Gas exchange

Breath-by-breath measurements of oxygen consumption (VO_2), carbon dioxide production (VCO_2), and expired minute ventilation (V_E) were made by an automated system (KCBeck Physiological Consulting, St Paul, MN) modified to interface to a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services). The mass spectrometer sampling rate was 60 ml min^{-1}. Subjects breathed through a pneumotachograph (model 3700, Hans Rudolph, Kansas City, MO) that contained the mass spectrometer gas-sampling port. The pneumotachograph measured airflow by comparing impact and stagnation pressures in a region of slight narrowing of the flowing gas stream and was linearized by the technique of Yeh et. al. (1982). The pneumotachograph was connected to a Hans-Rudolph non-rebreathing valve (150 ml of total dead space) so that expired air could be collected into Douglas bags and subsequently analyzed for oxygen and carbon dioxide concentrations (mass spectrometer) and volumes (Tissot gasometer). Calculations of VO_2 and VCO_2 were performed using the Haldane transformation (Wilmore & Costill, 1973). This permitted the comparison of breath-by-breath (15 sec averages) and the Douglas bags determination of VO_2 and V_E. A low resistance filter (preVent, Medical

Graphics Corporation, St Paul, MN) was located between the pneumotachograph and the subject's mouth to prevent the pneumotachograph screens from getting dirty, especially during maximal physical efforts.

Calibration of the metabolic system was performed before each study by the standard computer program and precision-grade gas mixtures. The pneumotachograph was calibrated with a 3-liter calibration syringe (Hans Rudolph, Kansas City, MO). Each of the calibration was performed with the pneumotachograph attached to the non-rebreathing valve, exactly as configured during data collection.

Cardiac output

Cardiac output has been routinely measured by numerous direct and indirect techniques. Depending on the nature of the test (i.e. rest vs. exercise; submaximal vs. maximal) these methods compare well with one another. This dissertation measured cardiac output using an open-circuit acetylene washin method originally developed by Stout et. al. (1975), later modified by Gan et. al. (1993), and validated in humans during exercise against the direct Fick approach (Johnson *et al.*, 2000). Due to the high reproducibility and reliability of the cardiac output measurements using this technique, it has become widely accepted among the scientific community (Johnson *et al.*, 2000, Lynn *et al.*, 2009, Pricher *et al.*, 2004).

The mathematical model of this technique divides the lung structure into three-compartments consisting of serial dead space (anatomic dead space), parallel dead space (alveolar dead space), and the normal alveolar space. The acetylene washin technique holds a few physiological assumptions. First, pulmonary blood flow is constant during measurement cycle; and equilibration exists between partial pressures of the alveolar gas and capillary blood, and alveolar dead space is in parallel with an independent of the normal alveolar space (Gan *et al.*, 1993). Cardiac output can be estimated form the following equation of the Fick principle:

$$Q = VC_2H_2 / (C_vC_2H_2 - C_aC_2H_2)$$

where:

VC_2H_2 = rate of C_2H_2 uptake (ml min^{-1})

$C_vC_2H_2$ = C_2H_2 content in mixed venous blood (ml 100 min^{-1})

$C_aC_2H_2$ = C_2H_2 content of mixed arterial blood (ml 100 min^{-1})

There are many advantages of this washin technique that made it the best choice for the measurement of cardiac output in this dissertation. First, there has been a good correlation between this method and the direct Fick approach during submaximal and maximal exercise (Johnson *et al.*, 2000). Also, due to the open circuit nature of this technique (non-rebreathing), there is no buildup of carbon dioxide, especially during heavy exercise, and there is no need to match the rebreathe tidal volume to the subject's tidal volume, which could inhibit breathing. Furthermore, subjects do not need to alter their spontaneous breathing pattern. In fact, the use of

a large reservoir that contains the gas mixture does not affect the gas exchange or breathing pattern, authors reported that subjects were almost unaware when they were switched into the gas mixture during the exercise test (Johnson *et al.*, 2000). In summary, this method allows for a non-invasive, reliable, and valid estimation of cardiac output, even during maximal exercise.

During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services) and tidal volume was measured via pneumotachograph (model 3700, Hans Rudolph, Kansas City, MO) linearized by the technique of Yeh et. al. (1982) and calibrated by using the test gas before each study. Gas concentrations of inspired and expired helium and acetylene were measured for each breath. The difference of these concentrations equal the volume of the gas that is taken up by the pulmonary circulation. Cardiac output calculations have been described previously (Johnson *et al.*, 2000).

Alternative techniques

Other acceptable techniques to measure cardiac output include direct Fick method, dye-dilution method, and thermodilution method. These three methods are considered the “gold standard” and have been widely shown to give accurate and reliable determinations of cardiac output during

resting and submaximal exercise. Conversely, because of the invasive nature of these techniques plus some inaccuracy during the final stages of vigorous exercise, their use during maximal efforts have been questioned.

Alternative non-invasive measures of cardiac output have been developed such as foreign gas rebreathing method (N_2O and C_2H_2), and indirect Fick methods (Single-breath CO_2 and CO_2 rebreathe). Although the majority of these "conventional" techniques provide accurate and reliable estimations of cardiac output during rest and submaximal exercise, probably only the foreign gas rebreathe using acetylene might be suitable during maximal exercise.

Finally, new methodologies have been developed such as Doppler echocardiography, impedance cardiography, and radionuclide scintigraphy method. These techniques provide reasonably and accurate estimations of cardiac output. One of the major drawbacks with these methods is the cost of usage and technological limitations during maximal exercise have prevented these techniques from replacing the conventional measures. The alternative and novel techniques will be discussed in the following paragraphs.

The direct Fick method requires that measurements of O_2 (or CO_2) be taken from a systemic artery and form the pulmonary artery (mixed venous blood), along with a measure of O_2 consumption (or CO_2 elimination) by the lungs during steady-state conditions (Cournand *et al.*,

1945). This method is based on the principle that total uptake and release of any substance by an organ is the product of blood flow to the organ and the arteriovenous concentration difference of the substance (Fick, 1870), and is based on the following original equation $VO_2 = Q \times (C_aO_2 - C_vO_2)$. The invasive nature of this technique, plus the fact that measurements must be taken during steady state, make this method not very suitable for exercise physiologists.

The dye-dilution is similar to the Fick method with the exception that, instead of measuring O_2, the concentration of a dye (indocyanine green) is measured. A bolus dose of dye is injected into the venous circulation either through a pulmonary artery catheter or through a central venous line (i.e. vena cava). The downstream concentration of dye in arterial blood is sampled from a systemic artery at a constant flow rate, and a densitometer calculates the dye concentration over time (Ekblom *et al.*, 1968, Kopelman & Lee Gde, 1951). The average volume flow is inversely proportional to the area under the concentration-time curve. Its reliability and reproducibility have been found to be comparable with those observed for the direct Fick method (Cournand *et al.*, 1945, Grimby *et al.*, 1966b, Kopelman & Lee Gde, 1951). In addition, this technique is relatively less invasive than the direct Fick (no direct cardiac catherisation), although it does require a central venous line. However, repeated measures are limited as 50% of the dye is

cleared by the kidneys 10 min post-injection (Ehlers *et al.*, 1986), which makes it impractical for exercise testing.

Thermodilution is based on the same principle as dye-dilution except that a cold fluid is injected through the pulmonary artery catheter (instead of a dye). The fluid is usually saline or 5% dextrose. The resultant cooling of the blood is measured by a thermistor near the end of the pulmonary artery catheter. The amount of this cooling is inversely proportional to cardiac output. Some advantages include ability to perform multiple measurements due to the use of a harmless marker. Disadvantages consist of the use of heat as a marker and the unknown amount of coolant that may be lost during the handling of syringes, and in the catheter before it enters the circulation. Also, there is a possibility that heat could transfer from the circulation through the vessel wall. In addition, several authors reported consistent overestimation of cardiac output (Branthwaite & Bradley, 1968, Mackenzie *et al.*, 1986, Russell *et al.*, 1990, van Grondelle *et al.*, 1983), which makes this method questionable as a "gold standard" for measuring cardiac output.

Any of the foreign gas techniques that will be discussed in the following paragraphs are based on the fact that a series of inert soluble gases can enter or leave the bloodstream through the lungs (Driscoll *et al.*, 1989). These gases diffuse rapidly (i.e. perfusion limited) into the pulmonary circulation, they dissolve into the blood but they do not form

bonds with any elements from the blood (i.e. hemoglobin). Consequently, the rate of disappearance of the inert soluble gas is directly proportional to the blood flow through the lungs, which is equivalent to systemic cardiac output during equilibrium.

The most widely used foreign gases to estimate cardiac output are C_2H_2 and nitrous oxide (N_2O). The techniques involve both breath-holding (Cander & Forster, 1959) and rebreathing (Becklake *et al.*, 1962, Gledhill *et al.*, 1994, Krip *et al.*, 1997, Warburton *et al.*, 1998, Zeidifard *et al.*, 1976). For exercise physiologists the rebreathing maneuver post obvious advantages over the breath hold method and was the most implemented technique, especially during maximal exercise (Triebwasser *et al.*, 1977).

Nitrous oxide (N_2O) rebreathing can be used to measure cardiac output due to high solubility of this gas (Ayotte *et al.*, 1973). This technique uses a gas mixture containing N_2O, O_2, an insoluble gas (usually argon or helium) and the balance as nitrogen. The rebreathe maneuver rate is approximately 1 breath per second and the breath-by breath analysis of the expired gases is usually made by a mass spectrometer (or a rapid response nitrogen and N_2O analyzer), which is linked to a computer to allow for continuous monitoring of the concentration of gases. Recirculation of N_2O usually occurs after 15 seconds at rest and 8 seconds during exercise. The changes observed in the concentration of N_2O after adequate mixing and before recirculation are used to calculate cardiac output. A clear description

of the technique commonly used in exercise physiology is given by Becklake et. al. (1962). The hyperventilation required during resting conditions may elevate VO_2 or cardiac output, which makes this method unreliable at rest. During maximal exercise, however, this error due to hyperventilation decreases since an increase in exercise intensity naturally increases the subject's ventilation. In fact, the N_2O rebreathing maneuver has been shown to give accurate determinations of cardiac output during submaximal and maximal exercise (Andrew *et al.*, 1966, Becklake *et al.*, 1962, Becklake *et al.*, 1965, ZeidiFard & Davies, 1978, Zeidifard *et al.*, 1976).

The C_2H_2 rebreathe maneuver is similar to the N_2O rebreathing method. Many exercise physiologists, however, have chosen this foreign gas over N_2O because nitrous oxide is thought to be affected by blood lipid levels, whereas C_2H_2 is minimally affected (Gledhill *et al.*, 1994, Smyth *et al.*, 1984, Triebwasser *et al.*, 1977). In it, the subject rebreathes a gas mixture containing 35-45% O_2, 0.5-1.0% C_2H_2, 5-10% helium and a balance nitrogen. As with the N_2O maneuver, adequate mixing of the lung-bag rebreathing system is generally confirmed by a constant level of helium or argon, which usually occurs after the third breath of the rebreathe. C_2H_2 also mixes in the lung-bag system, after which it disappears in the blood in a linear fashion according to its solubility coefficient in the blood and cardiac output. Therefore, after the point of equilibration, the rate of decline in the

C_2H_2 is directly proportional to the flow of blood past the lungs and hence serves as a measure of cardiac output (Gledhill *et al.*, 1994, Hopkins *et al.*, 1996, Warburton *et al.*, 1998). The C_2H_2 rebreathing maneuver has been shown to overestimate arteriovenous oxygen difference at rest, which results in an underestimation of cardiac output (Chapman *et al.*, 1950, Werko *et al.*, 1949). Some limitations in using this foreign gas technique is that they are not useful in subjects with pulmonary abnormalities, where there is inefficient mixing of gases in the lungs (i.e. ventilation perfusion mismatch) and they are also insensitive to anatomical shunts. Finally, the cost of using a mass spectrometer might be the most practical limitation for many laboratories. In summary, despite the tendency to provide variable estimates of cardiac output at rest, the C_2H_2 maneuver has been shown to give accurate determinations during submaximal and maximal exercise (Asmussen & Nielsen, 1952, Liu *et al.*, 1997, Smyth *et al.*, 1984, Triebwasser *et al.*, 1977).

The single breath CO_2 method has the advantage of being noninvasive, very easy to perform and is reproducible over short periods. This method is based on the principle that during prolonged breath holding arterial CO_2 and alveolar CO_2 concentrations rise in a nonlinear fashion, while O_2 concentrations decrease linearly (Hlastala *et al.*, 1972, Kim *et al.*, 1966). Also the instantaneous respiratory exchange ration (R_{inst}) is calculated throughout the breath and is linearly related to the expired partial

pressure of CO_2 (pCO_2) (Hlastala *et al.*, 1972). Mixed venous CO_2 ($pmvCO_2$) and the arterial CO_2 ($paCO_2$) tensions are estimated using R_{inst} and pCO_2 (Inman *et al.*, 1985). When the arterial and mixed venous CO_2 contents match, the respiratory exchange ratio (R) is 0.32, since according to the Haldane effect, 0.32 volumes of CO_2 are released for every volume of oxygen taken up by hemoglobin of the venous blood without changing pCO_2 (Inman *et al.*, 1985, Kim *et al.*, 1966). The expired CO_2 tension at R = 0.32 is assumed to equal $pmvCO_2$ and $paCO_2$ is the tension of CO_2 determined by an average for R during the previous 6 breaths. The formula to determine cardiac output is the following:

$$Q = (VO_2 * (R - 0.32)) / (S * (pmvCO_2 - paCO_2))$$

where S is the slope of the CO_2 dissociation curve (4.7 ml CO_2 * L blood^{-1} * mmHg^{-1} of pCO_2)

Several investigators have shown that this method consistently underestimates cardiac output ranging from 12% at rest to 3% during moderate exercise (Hlastala *et al.*, 1972, Inman *et al.*, 1985). Thus, the reliability of the single-breath CO_2 method is similar to that of other measures of cardiac function.

CO_2 rebreathing to equilibrium can estimate cardiac output by using the indirect Fick method (Collier, 1956). In this technique, the subject rebreathes a gas mixture (containing 10-20% CO_2) of an appropriate volume (~1.5 times tidal volume) until an equilibration plateau is reached in

the CO_2 tracing. The CO_2 plateau is defined as an expiratory CO_2 level change of less than 0.1% from the preceding inspiratory CO_2 level. The Fick equation is the used to estimate cardiac output (Collier, 1956). As with the C_2H_2 rebreathe method, one drawback is the buildup of carbon dioxide as a result of the rebreathing as the subsequent dyspnea. This may be problematic at higher intensities of exercise or with longer rebreathing time in patients with ventilation mismatch problems such as aging and obstructive airway disease (Johnson *et al.*, 2000).

Doppler echocardiography has been recently suggested as being a suitable noninvasive means of estimating cardiac output (Christie *et al.*, 1987). The Doppler transducer is usually placed in the suprasternal notch to allow for an ultrasound signal to pass parallel through the flow of blood in the ascending aorta (Chandraratna *et al.*, 1984, Colocousis *et al.*, 1977, Hara & Floras, 1995). In this method, the velocity of the blood flow through the aorta, pulmonary artery or mitral valve is determined along with the diameter of the vessel allowing for estimation of cardiac output (Hara & Floras, 1995). This technique has a few limitations. For instance, the continuous-wave Doppler system assumes an angle of 180° between the sound beam and the direction of blood flow, which may be erroneous (Goldberg *et al.*, 1982, Nishimura *et al.*, 1984). Also, the large transducer used with the Doppler system makes it hard to place it in the suprasternal

notch and limits its usefulness during exercise conditions (Goldberg *et al.*, 1982).

Impedance cardiography is another novel noninvasive technique to measure stroke volume and thus, cardiac output. In this method, a small alternating current (4mA, 100kHz) is passed through the chest using 2 sets of band electrodes at the base of the neck and bottom of the chest. Changes in impedance are measured by other two recording electrodes.. The bioimpedance method assumes that changes in transthoracic electrical impedance during systole are representative of stroke volume (Jensen *et al.*, 1995). Some authors have found problems with the impedance cardiograms during strenuous exercise condition caused by movements associated with respiration and movement (Hetherington *et al.*, 1985, Miles *et al.*, 1981).

The radionuclide scintigraphy method consists of labeling the blood pool with a radioactive substance into the circulation of the subject and monitor its radioactivityby use of a γ-scintillation camera. Generally, technetium-99m (99mTc) is used as the radionuclide because of its ready availability, low cost, and physical short half-life of 6 hours (Bianco & Shafer, 1979). After an intravenous injection of a bolus of Tc-99m, data is collected by a scintillation detector for approximately 20 to 45 seconds. Time-radioactive curves are constructed, allowing the calculation of stroke volume (form left ventricular end diastolic volume and left ventricular end

systolic volume) (Urbanowicz *et al.*, 1990). Cardiac output can be calculated by multiplying stroke volume by heart rate or it can be determined using the area under the time-radioactivity curve similar to that used in the dye-dilution technique. Generally, authors have reported that the radionuclide method underestimated cardiac output approximately 20% at rest and throughout incremental to maximal exercise (Wijns *et al.*, 1985). Some of the problems using this technique include long acquisition periods because of the low doses of radionuclide (not good during maximal exercise), the background radioactivity from blood in non-cardiac structures surrounding the myocardium is often of the same magnitude as seen within the left ventricle, and some radio tracer techniques require the use of assumptions based on the normal geometry of the heart (Links *et al.*, 1982, Marshall *et al.*, 1977).

Core temperature

Core body temperature can be measured through a number of different techniques. In each of the studies presented in this dissertation, internal body temperature was assessed by measuring rectal temperature. Although there is no one true core temperature due to the differences among different sites in the body, it is known that temperatures at all core sites are within 1°C of central blood temperature at thermal steady state. Although blood temperature in the pulmonary artery is widely acknowledged

to accurately reflect hypothalamic temperature and is generally considered as the 'gold standard' (Holtzclaw, 1993), this method of core temperature measurement is invasive and not practical for exercising conditions.

Rectal temperature has been the most widely used method to measure core temperature among exercise and environmental physiologists (Aldemir *et al.*, 2000, Morris *et al.*, 2009, Waterhouse *et al.*, 2004, Waterhouse *et al.*, 2007). It's easy to use, and provides stable measurements during non-dynamic conditions. This location is considered the most practical and accurate for measuring core temperature. One limitation of this technique, however, is that is accuracy is questioned under conditions where rapid changes in core temperature occur (Lee *et al.*, 2000). In addition, this method can be influenced by changes in leg blood flow (Saltin & Hermansen, 1966). Nevertheless, compared to other methods that estimate core temperature (esophageal, tympanic, and intestinal), rectal temperature measurements are reliable, easy to instrument and very stable. In addition, rectal thermistors are relatively inexpensive, especially when using them on numerous subjects on multiple testing days. During this procedure, the thermistor was self-inserted approximately 10 cm past the anal sphincter, and the other end was connected to a patient monitor system (Cardiocap/5, Datex Ohmeda. GE, Buckinghamshire, United Kingdom) for real time temperature readings. Generally, reports have shown that rectal temperature tends to be slightly higher compared to

temperature measurements at different sites (Morris *et al.*, 2009, Sawka *et al.*, 2007).

Alternative methods

Tympanic temperature has been used as a surrogate measurement for core temperature (Briner, 1996). This location is appropriate since the tympanic membrane receives blood from the branches of the internal carotid artery and supplies blood to the thermoregulatory center in the hypothalamus. Furthermore, the ear canal is easily accessible for measuring temperature. However, some studies reported problems during measurement especially during exercise and heat stress that can lead to errors as a result of dirt, and inaccurate placement and thus it's not been a popular choice among exercise and environmental physiologists (Amoateng-Adjepong *et al.*, 1999, Briner, 1996).

Esophageal temperature measurements is preferred by many researchers as the site to measure core temperature because of its deep body location close to the left ventricle, the aorta and the blood flow to the hypothalamus. In addition, this measurement has a rapid response to quick changes in body temperature. However, the instrumentation involves inserting a thermistor through the nasal passages, which may cause discomfort, pain, irritation, and vomiting. Once in place, ingestion of fluids is difficult as well (Lee *et al.*, 2000).

The telemetry pill system monitors core temperature via a radio wave signal, transmitted from the ingested pill and sent to a small external receiver (Rav-Acha *et al.*, 2003). The pill needs to be swallowed between 6 and 10 hours prior to the measurements to allow for it to travel down the digestive system into the intestines. The telemetry pill has been shown to provide valid measurements of core temperature within the range of 36 and 38°C during rest and prolonged cycling in both warm and cold water immersion trials (O'Brien *et al.*, 1998). However, there is no published evidence to date that demonstrates that the telemetry pill system in accurate at the top end of the physiological range. As intense exercise during heat stress can induce a rise in core temperature greater than 40°C (Roberts, 2000), further validation of the telemetry pill during more severe exercise is required. This method has some limitations. Approximately 10-15% of the pills do not work properly due to electronic problems within the pill itself or with the signal being picked up by the receiver. This could prevent the planned study from being started on a given day since it takes 6-10 hours for the pill to give accurate readings after its ingested. Finally, this method is relatively expensive compared to other methods, especially with a large number of subjects and/or testing days.

Sublingual temperature is widely used as a clinical tool but less commonly in physiological research. The main limitation of this method is that sublingual temperature may be lowered due to evaporation when the

subject breathes through the mouth (Sawka *et al.*, 2007), which makes it impractical as a core temperature surrogate during exercise.

Therefore, for the purpose of the studies on this dissertation, rectal temperature was the chosen method to estimate core temperature because is inexpensive, reliable and widely accepted among exercise physiologists.

Skin temperature

Skin temperature was measured using thermocouples made of copper and constantan on selected body areas on the skin. The measurement of skin temperature is based on the non-linear relationship that exists between the flow of voltage between the two wires and their temperature. The voltage signal received from the thermocouples was linearized and then transferred to the data acquisition system for recording. Thermocouples are accurate to within 0.2°C of a standard thermometer when performing a two-point calibration in a water bath (Lund & Gisolfi, 1974). An estimate of mean ski temperature (T_m) was done using 7 body sites (Sawka & Wenger, 1988) (T_m = .021(face temperature) + 0.21(chest temperature) + 0.17(abdomen temperature) + 0.15(thigh temperature) + 0.08(calf temperature) + 0.12(upper arm temperature) + 0.06(forearm temperature).

Skin blood flow

This dissertation used laser-Doppler flowmetry (moorLab, Moor Instruments™, Devon, UK) to estimate blood flow through the cutaneous circulation. The theory behind this technique is based on the changes in wavelength (Doppler shift) of a beam of laser light after it hits moving blood cells in the cutaneous circulation to a depth of approximately 1mm. Although there is no direct measurement of blood flow, the output signal coming from the laser-Doppler unit is the result of red blood cell 'flux' in a single 1.0 mm^3 volume of tissue, and is linearly related to blood flow. Each integrated probe has one optic fiber emitting a laser light surrounded by 8 receiving fibers in a 2 mm ring. This technique has been validated against measurements of absolute skin blood flow during thermal stress, and correlations of 0.94 to 0.98 were observed between laser-Doppler flowmetry and venous occlusion plethysmography (Johnson *et al.*, 1984). The laser-Doppler probes were placed on the skin of the right ventral forearm. Skin blood flow measurements were expressed as cutaneous vascular conductance, calculated as laser-Dopper flux divided by mean arterial pressure and normalized to the maximal values achieved during local heating to 43.5°C or 28mM sodium nitroprusside (SNP) infusions at the end of the protocol (Lorenzo & Minson, 2007, Minson *et al.*, 2001).

Sweat rate

This dissertation estimated sweat rates from selected skin areas by resistance hygrometry (model HMP230, Vaisala™, Helsinki, Finland). Detailed explanation of the technique was previously described (Bullard, 1962). Briefly, air of known relative humidity is passed over a selected area of the skin through a capsule at a fixed flow rate. The inflow of air should be dry enough and flow rate should be adequate to assure complete and rapid evaporation of sweat. The change in water content of the air is then dependent on the sweating rate. Water content is calculated from the relative humidity change (ΔRH) in the air as it passes over the skin, the flow rate and the temperature. Therefore, the following equation was used:

*Sweating rate (mg/min) = air flow (in liter/min) * (ΔRH/100) * density of sat. steam (in mg/liter)*

The density of saturated stream at different temperatures was obtained from handbook tables.

Whole body heating

During selected protocols in this dissertation, subjects immersed in a water-filled tub (~41°C) for approximately 30 minutes to increase their rectal temperature by 0.8-1.0°C prior to the start of the test. On the protocols done under cool environmental conditions (13°C), subjects also immersed in a water-filled tub with thermoneutral water (~34°C) for 30 minutes to maintain

the same resting rectal temperature. Research has shown that high core and skin temperatures are necessary to adequately stress the cardiovascular system and alter some physiological variables associated with performance (i.e. VO_{2max}, and lactate threshold) (Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005, Tyka *et al.*, 2000, Tyka *et al.*, 2009). The water immersion allowed us to manipulate the subjects' rectal temperature without having to make them exercise prior to the studies, which can potentially act as a confounding variable. Furthermore, pilot work done in our environmental chamber demonstrated that even exercising at a very low power output (i.e. 125W) for 30 minutes in a cool environment (13°C 45% RH), resulted in an increase in rectal temperature of 0.9°C and the goal of the tests performed in the cool environment was to maintain the same resting core temperature at the beginning of the test.

Femoral blood flow

Femoral blood flow can be measured by a number of different techniques. Each of them will be discussed in the following paragraphs. A Doppler ultrasound machine (GE Vingmed™, Horton, Norway) equipped with a 10 Mhz linear-array transducer probe was used to measure mean blood velocity and vessel diameter of the right common femoral artery, distal to the inguinal ligament but above the bifurcation into the superficial and profunda femoral branches. The overall femoral blood flow was

calculated using mean blood velocity and artery diameter. The ultrasound probe generates a frequency-modulated constant voltage sine wave output and measures the returning sonic wave echoes. The difference is determined and a picture of the artery is generated for subsequent mean blood velocities and diameter measurements. For the velocity measurements, the artery was insonated at a constant angle of 60° with the sample volume adjusted to cover the entire with of the artery, while diameter measurements were obtained with the artery insonated perpendicularly. Diameter measurements were stored on VHS tape and posttest analysis was performed using edge-detection software. Femoral blood flow (FBF) was calculated as artery cross sectional area multiplied by femoral mean blood velocity (MBV) (FBF = MBV * π * (femoral diameter/2)^2 * 60). This technique requires an experienced researcher (or sonographer) in order to produce reproducible diameters and velocities. Much research using this technique has been published and is considered an excellent method to estimate leg blood flow in a non-invasive way (Parker *et al.*, 2007, Parker *et al.*, 2008, Proctor *et al.*, 2001, Ridout *et al.*, 2005).

Alternative methods

Thermodilution and venous occlusion plethysmography (VOP) are two common techniques currently accepted to measure arterial blood flow. Thermodilution was discussed in a previous section (see *Alternative*

Methods for cardiac output measurements), with the difference that in the case, the catheter is inserted in the femoral vein (instead of pulmonary artery). VOP uses a cuff that is placed on the proximal portion of the leg. The cuff pressure must be higher than the venous pressure but lower than the diastolic arterial pressure (to allow inflow of blood into the leg). Changes in the circumference of the limb are directly measured using mercury-in-silastic strain gauges. Venous collecting cuff pressure is measured using a pressure transducer attached to the collecting cuff and positioned in line with the venous collecting cuff and air source. The pressure transducer is calibrated using a mercury mamometer (Hiatt *et al.*, 1989). During the occlusion, the volume of the limb increases at a rate equal to arterial inflow.

Brachial artery blood flow

One novel approach in this dissertation is the use of Doppler ultrasound technique to estimate absolute maximal skin blood flow. This technique involved local warming of the left forearm in a cylindrical water spray device that sprayed heated water from jets encircling the suspended forearm (Martin *et al.*, 1995, Taylor *et al.*, 1984). At the same time, brachial artery diameters and blood velocity were measured using a Doppler ultrasound machine (Terason™, Burlington, MA) to calculate brachial artery blood flow. The forearm was heated for 45 minutes and 2-minutes

measurements were taken before forearm heating (baseline), and at 15, 30 and 45 minutes. During measurements, blood flow to the hand was occluded with a blood pressure cuff to prevent the hand circulation from being included in the calculations. This method if validated against VOP, would be an attractive alternative to measure maximal skin blood flow.

Changes in plasma volume and blood volume

The relative changes in plasma volume (PV) and blood volume (BV) were measured to assess adequate heat acclimation adaptations (Bass *et al.*, 1955, Nielsen *et al.*, 1993, Senay & Kok, 1977, Senay *et al.*, 1976). Changes in blood and plasma volume were estimated from changes in hematocrit (Hct) and hemoglobin (Hb) using the method of Dill & Costill (1974) using the following formula:

$$\%\Delta PV = [(Hb_1/Hb_2) \times [(100 - Hct_2)/(100 - Hct_1)] - 1] \times 100$$

$$\%\Delta BV = 100 \times [(Hb_1/Hb_2) - 1]$$

Where Hb is the hemoglobin concentration and Hct is the hematorcit concentration and the number values represent the different time point used for sampling. All samples were measured in duplicate. The underlying assumption when using these formulas to calculate the relative changes is that peripheral circulating erythrocyte volume does not change and is comparable to total erythrocyte volume (Bass *et al.*, 1958).

Catherizations for blood sampling

In selected studies, a catheter was introduced into a vein in the antecubital region of the subject's arm in order to draw blood samples. After disinfecting the area of the arm, an intravenous catheter was placed by inserting a 22 gauge needle into the vein. Once the catheter was advanced into the lumen of the vessel, the needle was removed and the catheter was secured with transparent medical dressing (3M Tegaderm™, Maplewood, MN). After every draw blood, the sampling line and catheter were cleared with non-lactated, non-dextrose saline solution (0.9%) in the exact volume that matched the blood draw. The sampled blood was kept in a sterile syringe, stored on ice or transferred into a vacuum-sealed (heparinized) test tube.

Blood analyses

Measurements of hemoglobin concentration were done spectrophotometrically using a diode-array spectrophotometer (OSM-3. Radiometer, Copenhagen, Denmark). This device measures absorption at several wavelengths and the hemoglobin concentration measurements were reported in grams per deciliter. Hematocrit was measured with a microcapillary method after 8 min of centrifuging at 9,500 *g* (Autocrit Ultra 3, Becton Dickson, USA).

Oxygen saturation (Sp_{O2}) was determined by forehead pulse oximetry (Nonin Medical, Inc. Minneapolis, MN). Pulse oximeters use a light source and photodiode light detector to measure the amount of light passing through an arteriolar bed. Sp_{O2} can be estimated noninvasively because the light-absorbing characteristics of hemoglobin differ between oxyhemoglobin and deoxyhemoglobin. Studies have shown that forehead sensor offered greater validity of Sp_{O2} measurements under exercise conditions than the other locations (i.e earlobe or finger) (Yamaya *et al.*, 2002).

Plasma osmolality measured by freezing point depression using an osmometer (3MO, Advance Instruments, Norwood, MA). Plasma osmolality measures of the concentration of ions in the blood such as sodium, chloride, potassium, urea, and glucose and is a good predictor of hydration status (Kenefick *et al.*, 2009, O'Brien *et al.*, 2005, Sawka *et al.*, 2007).

CHAPTER IV

EFFECTS OF HEAT ACCLIMATION ON MAXIMAL AEROBIC POWER AND LACTATE THRESHOLD IN HOT AND COOL ENVIRONMENTAL CONDITIONS

Introduction

It is well established that aerobic exercise performance is degraded by heat stress (Galloway & Maughan, 1997, Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005, Mortensen *et al.*, 2008). The mechanisms associated with degraded performance include cardiovascular strain, muscle glycogen depletion, and thermal discomfort (Sawka & Young, 2006), and all of which are abated with heat acclimation. The impact of heat acclimation to improve cardiovascular stability (lower heart rate, improved ability to sustain blood pressure and cardiac output) during exercise-heat stress has been particularly well studied (Bass *et al.*, 1955, Desai & Senay, 1984, Greenleaf & Greenleaf, 1970, Harrison *et al.*, 1981, Nadel *et al.*, 1974, Nielsen *et al.*, 1993, Roberts *et al.*, 1977, Rowell *et al.*, 1967, Rowell, 1974, Sawka *et al.*, 1983, Senay *et al.*, 1976, Wenger, 1988, Wyndham *et al.*, 1968). What is less well studied and understood is if heat

acclimation can mediate improved cardiovascular stability and improve maximal aerobic performance in temperate environments. Two of the most commonly used maximal performance tests are maximal oxygen consumption (VO_{2max}) and determination of lactate threshold. VO_{2max} tests are widely used to measure maximal aerobic performance (Arngrimsson *et al.*, 2004, Coyle *et al.*, 1990, Sawka *et al.*, 1985). Although the lactate threshold test is less 'standardized' than the VO_{2max} test, its importance in predicting performance is well established (Dumke *et al.*, 2006, Thomas *et al.*, 2008, Tyka *et al.*, 2000, Tyka *et al.*, 2009).

The effect of heat stress on short duration, intense exercise has been studied comprehensively. Rowell and colleagues (Rowell *et al.*, 1966) investigated the cardiovascular responses to unacclimated and sedentary men to short duration (15 min) exercise in the heat. They found that a high ambient temperature (43.3°C vs 25.6°C) caused significant reduction in the ability to achieve and sustain high cardiac output, which likely contributed to the degraded exercise capacity. These earlier findings were extended when a more recent study (Gonzalez-Alonso & Calbet, 2003) looked at the primary factors that limit VO_{2max} in trained men in temperate and hot conditions. The results showed a decrease in VO_{2max} and time to fatigue in the hot vs. temperate condition. In addition, cardiac output decreased before fatigue. The authors concluded that VO_{2max} decreases in the heat by accelerating the decline in cardiac output and mean arterial pressure,

ultimately leading to decreased leg blood flow, oxygen delivery and oxygen uptake. Recently, Mortensen and colleagues (2005) examined systemic and muscle oxygen delivery during maximal exercise involving large active muscle mass (cycling) and small active muscle mass (one-legged knee extensor exercise) in trained male subjects under temperate conditions. Only during the cycling trial was an attenuation in leg blood flow, leg oxygen delivery and VO_2 observed immediately preceding fatigue. The authors suggested that this is largely related to the inability of the cardiovascular system to continue to increase cardiac output to match the metabolic demands of the exercising muscles. Conversely, when exercising with a small muscle mass (and thus, no cardiac limitation), leg blood flow and leg oxygen delivery continued to increase until onset of fatigue (Mortensen *et al.*, 2005).

Heat acclimation improves performance in the heat largely due to enhanced cardiovascular and thermoregulatory adaptations (Armstrong & Maresh, 1991, Armstrong *et al.*, 1987, Gonzalez *et al.*, 1974, Greenleaf & Greenleaf, 1970, Wenger, 1988). Some of the physiological adaptations include an increase in plasma volume (Bass *et al.*, 1955, Harrison *et al.*, 1981, Senay *et al.*, 1976, Wyndham *et al.*, 1968), sweat rates and skin blood flow (Belding & Hatch, 1963, Chen & Elizondo, 1974, Collins *et al.*, 1965, Collins *et al.*, 1966, Fox *et al.*, 1963a, Mitchell *et al.*, 1976, Nadel *et al.*, 1974, Roberts *et al.*, 1977, Wyndham, 1967), and decreases in core

temperature, heart rate, and perceived exertion at a given level of intensity (Mitchell *et al.*, 1976, Nielsen *et al.*, 1993, Rowell, 1974, Wyndham *et al.*, 1968, Wyndham *et al.*, 1976). One question that has not been explored is whether heat acclimation will improve aerobic exercise performance in a temperate environment. In other words, can heat acclimation be used to improve exercise performance in non-heat stress conditions?

The effects of heat acclimation on maximal aerobic power (i.e. VO_{2max}) under hot (49°C) and temperate (21°C) conditions were first studied by Sawka *et al.* in 1985. Their data demonstrated that heat stress reduced VO_{2max} relative to the level achieved in a temperate environment, but heat acclimation significantly increased (4%) VO_{2max} in both environmental conditions (Sawka *et al.*, 1985). The authors attributed these changes to a "training effect" due to the heat acclimation program in part because subjects who participated in the study had VO_{2max} values ranging from 39-53 ml kg^{-1} min^{-1}, which corresponds with fair-to-high fitness levels (Astrand & Rodahl, 1977). However, a control group without any observed changes post acclimation would be necessary to confirm this assumption. The authors were unclear whether the subjects' core temperature was increased (and to what extent) prior to the VO_{2max} test in the heat. As previously discussed, high core (and skin) temperatures are necessary to have significant impact on VO_{2max} (Arngrimsson *et al.*, 2004, Mortensen *et al.*, 2005, Nybo *et al.*, 2001). To our knowledge, research has not been

published which attempts to investigate the influence of acute and chronic heat exposure (heat acclimation) on highly trained (VO_{2max} > 65 ml kg^{-1} min^{-1}) cyclists' maximal aerobic power in the heat and cool environment.

The primary goal of this study was to examine the effects of heat acclimation on maximal aerobic performance as measured by lactate threshold and VO_{2max} in endurance-trained cyclist in hot (38°C) and cool (13°C) environments. A secondary goal was to explore the dynamics between blood flow and oxygen delivery to active muscle during a single-leg knee extensor exercise. We hypothesized that a period of heat acclimation will improve lactate threshold and VO_{2max} values in both hot and cool conditions.

Methods

Study design

This manuscript is part of a larger project that investigated effects of heat acclimation on performance and also other physiological variables in endurance trained cyclists. In brief, participants were put through a battery of physiological and performance tests under two environmental conditions, then put through a heat acclimation or an exercising control program, and then the tests were repeated. The experiments consisted in a lactate threshold test followed by a VO_{2max} test. On a separate day, a single leg knee extensor exercise was performed in cool condition and followed by the

same protocol in a hot environment. On days the studies were preformed under heat stress, the climatic chamber was set to 38°C and 30% relative humidity (WBGT =33°C). On the days where the studies were performed in cool conditions, the climatic chamber was set to 13°C and 30% relative humidity. The order between heat and cool trials was randomized. The heat acclimation protocol consisted of 10 exposures of cycling exercise at a temperature of 40°C and 30% relative humidity (WBGT=35°C). Subjects performed two bouts of 45 minutes at 50% of their VO_{2max} with 10 minutes of rest in between. A matched control group exercised at the same intensity but with the chamber set at 13°C and 30% relative humidity (WBGT = 12°C).

On each study visit, subjects reported to the laboratory after a 2-hour fast, and well hydrated. Subjects were instructed to avoid consumption of alcohol or caffeine for at least 8 to 12 hours prior to the study. In addition, they were not allowed to exercise on the same day prior to the study and were told to avoid ingestion of non-prescription drugs for the entire duration of the multiple study visits.

Subjects

A total of sixteen subjects (13 men, 3 women) were used for these set of studies. Twelve highly trained endurance cyclists (10 men, 2 women), age 24 ± 6 (SD) completed the heat acclimation protocol (height 175 ± 6

cm, weight 67.7 ± 8.1 kg, body mass index 22.1 ± 3.9 kg m^{-2}). Eight subjects (7 men, 1 woman), age 26 ± 4 completed the control protocol (height 174 ± 6 cm, weight 70.2 ± 4.1 kg, body mass index 23.1 ± 3.1 kg m^{-2}). Of the sixteen total subjects used for these set of studies, four men age 28 ± 5 performed the control protocol followed by the heat acclimation exposures and experimental tests (height 176 ± 4 cm, weight 73.1 ± 1.5 kg, body mass index 23.5 ± 2.8 kg m^{-2}). A complete description of the subject groups is presented in Table 1. A minimum sample size of 11 subjects was calculated ($\alpha = 0.05$, $\beta = 0.20$) as sufficient to detect a 6% change (~.20 L min^{-1}) in maximal oxygen consumption post heat acclimation in a paired *t* test.

Measurements

Exercise was preformed while seated on an electronically braked cycle ergometer (Lode Excalibur Sport™, Groningen, The Netherlands). Heart rate (HR) was monitored continuously throughout each protocol via telemetry (model RS400, Polar Electro™, Lake Success, NY). Core temperature was measured using continuous measurements of rectal temperature by a thermistor (YSI 400 Series, Mallinckrodt Medical, St. Louis, MO) inserted 10 centimeters beyond the rectal sphincter. Dry, nude body weight was taken at the beginning of each study by a precision weighing balance to the nearest 5 g (Sartorius™ EB6CE-I, Precision

Weighing Balances, Bradford, MA). The initial body weight was used to ensure body fluid balance remained constant during the study visits.

Cardiac output was measured using an open-circuit acetylene washin method originally developed in 1975 (Stout *et al.*, 1975), modified in 1993 (Gan *et al.*, 1993), and validated in humans during exercise against the direct Fick approach (Johnson *et al.*, 2000). Femoral blood flow was measured with a Doppler ultrasound instrument (General Electric Vingmed™, Horton, Norway) equipped with a 10 Mhz linear-array transducer probe. Measurements of mean blood velocity and vessel diameter of the right common femoral artery were taken distal to the inguinal ligament but above the bifurcation into the superficial and profunda femoral branches. For the velocity measurements, the artery was isonated at a constant angle of 60° with the sample volume adjusted to cover the entire width of the artery, while diameter measurements were obtained with the artery isonated perpendicularly. Diameter measurements were stored on VHS tape and posttest analysis was performed using edge-detection software. Femoral blood flow was calculated as artery cross sectional area multiplied by femoral mean blood velocity.

Measurements of hemoglobin concentration were done spectrophotometrically using a diode-array spectrophotometer (OSM-3. Radiometer, Copenhagen, Denmark). Hemoglobin arterial oxygen saturation (Sp_{O2}) was determined by forehead pulse oximetry (Nonin

Medical, Inc. Minneapolis, MN). Leg oxygen delivery was estimated by multiplying the estimated arterial oxygen content (1.34 * hemoglobin concentration * arterial oxygen saturation) by leg blood flow). Changes in resting plasma volume between day 1 and day 10 of the heat acclimation exposures were estimated using hemoglobin and hematocrit values according to the equation from Dill & Costill (Dill & Costill, 1974).

Whole body heating

Prior to the start of the test (lactate threshold, VO_{2max}, and leg kicking), subjects immersed in a water-filled tub (~41°C) for approximately 30 minutes to increase their rectal temperature by 0.8-1.0°C. On the protocols done under cool environmental conditions (13°C), subjects also immersed in a water-filled tub with thermoneutral water (~34°C) for 30 minutes to maintain the same resting rectal temperature. The water immersion allowed us to manipulate the subjects' rectal and skin temperatures without having to make them exercise prior to the studies, which can potentially act as a confounding variable. Therefore, we could examine the impact of acclimation state on the different exercise protocols to a standardized heat stress condition. Furthermore, pilot work done in our environmental chamber showed that even exercising at a very low power output (i.e. 125W) for 30 minutes in a cool environment (13°C 45% relative humidity), resulted in an increase in rectal temperature of 0.9°C.

Lactate threshold

The protocol involved subjects exercising on a cycle ergometer continuously for 3-minutes stages. The initial power output was selected based on the subjects' height, weight, and their reported usual training workloads. Power output increments were selected so the test concluded after 4 to 7 stages. Gas exchange was continuously measured by open circuit calorimetry. During the last 30 seconds of each stage a capillary blood sample was taken from a fingertip and analyzed for lactate concentration (Lactate Pro. Arkray, Inc. Kyoto, Japan). Cardiac output measurements were taken during the last 30 seconds of each stage by open circuit acetylene washin method (Johnson *et al.*, 2000). Lactate threshold was determined using the point at which blood lactate increased 1mM above resting value (Coyle *et al.*, 1983).

Maximal oxygen uptake

Thirty to sixty minutes after the end of the lactate threshold test, subjects performed a VO_{2max} test. This time allowed the core temperature to return to baseline values. To elicit VO_{2max}, subject exercised to exhaustion in a cycle ergometer, with the power output increasing 20W every minute. The initial power output was chosen based on the subjects' lactate threshold to exhaust them in 8-15 minutes. Cardiac output measurements were taken every 3 minutes at the early stages of the test and then every

minute until fatigue to ensure that a maximal cardiac output was determined. Breath-by-breath measurements of oxygen uptake (VO_2), carbon dioxide production (VCO_2), and expired minute ventilation (V_E) were made by custom software (KCBeck Physiological Consulting, St Paul, MN) modified to interface to a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services). The mass spectrometer sampling rate was 60 ml min^{-1}. Subjects breathed through a pneumotachograph (model 3700, Hans Rudolph, Kansas City, MO) that contained the mass spectrometer gas-sampling port. The pneumotachograph was connected to a Hans-Rudolph non-rebreathing valve (150 ml of total dead space) so that expired air could be collected into Douglas bags and subsequently analyzed for oxygen and carbon dioxide concentrations (mass spectrometer) and volumes (Tissot gasometer). Calculations of VO_2 and VCO_2 were performed using the Haldane transformation (Wilmore & Costill, 1973). This permitted the comparison of breath-by-breath (15 sec averages) and the Douglas bags determination of VO_2 and V_E. A low resistance filter (preVent, Medical Graphics Corporation, St Paul, MN) was placed between the pneumotachograph and the subject's mouth to protect the pneumotachograph screens from saliva, especially during maximal physical efforts.

Single-leg knee extensor exercise

On a separate day subjects performed a leg kicking exercise. Subjects were introduced a catheter into a vein in the antecubital region of the subject's arm in order to draw blood samples. Blood was drawn from the left arm with the subjects sitting on the kicking ergometer. After every blood draw, the sampling line and catheter were cleared with non-lactated, non-dextrose saline solution (0.9%) in the exact volume that matched the blood draw. The sampled blood was kept in a sterile syringe, briefly stored on ice or transferred into a vacuum-sealed (heparinized) test tube.

The protocol involved subjects semi-reclined in custom-built leg kicking apparatus. The active leg was strapped to the leg-kicking attachment, while the inactive leg was allowed to hang free, but the subject was instructed not to swing or move the leg. After 1 minute of quiet rest the subject began to kick at 30 W for 3 min, after which resistance increased incrementally (10 W for women or 15 W for men) every 3 min until subject could no longer maintain cadence (40 kicks/min). Gas exchange (VO_2) and femoral blood flow measurements were taken between 0:00 and 2:30 minutes of each stage. Cardiac output, oxygen saturation, and hemoglobin concentration were measured between 2:30 and 3:00 minutes.

Data from each protocol were compared between pre and post acclimation trials by determining specific differences using a paired Student's *t*-tests and significance was set at $P < 0.05$, and values are presented as

mean and standard error (mean ± SE), unless otherwise indicated.

Results

Table 1 shows specific physiological characteristics of the control and heat acclimation groups. Although the control group showed a slight higher absolute VO_{2max} (4.9%) and maximal power output (3.2 %), no differences were found between groups for VO_{2max} and maximal power output per unit body weight. We suspect any differences were due to 2 women being in the heat acclimation group and 1 woman in the control group. In addition, the mean body weight in the control group was elevated compared to the heat acclimation group (70.2 ± 4.1 vs. 67.7 ± 8.1 kg, respectively).

Table 2 shows mean differences between day 1 and day 10 of the heat acclimation or exercise control period. Values shown are final heart rate and final core temperature at end of the second exercise bout, and changes in pre exercise resting plasma volume. All results are shown as mean and standard error. There was a statistically significant reduction in the final heart rate ($P < .001$), and core temperature ($P = .002$), in the heat acclimation group but not in the control group.

Table 1. Physiological characteristics of the heat acclimation and control groups. Values are shown as mean ± standard error for 12 subjects in the heat acclimation group and 8 subjects in the control group. Range values are shown in parentheses. Reported values of maximal oxygen consumption (VO_{2max}) and maximal power output were from VO_{2max} test done in cool (13°C) conditions.

	Heat Acclimation Group N= 12	Control Group N= 8
VO_{2max} (L min^{-1})	4.47 ± 0.21 (3.00-5.51)	4.70 ± 0.14 (4.25-5.51)
VO_{2max} (ml kg^{-1} min^{-1})	66.85 ± 2.07 (57.01-76.09)	66.80 ± 1.65 (59.06-76.60)
Maximal power output (W)	369. 17 ± 14.54 (260-430)	381.25 ± 10.76 (340-420)
Maximal power output (W kg^{-1})	5.45 ± 0.21 (4.69-6.04)	5.43 ± 0.15 (4.99-5.86)

Table 2. Mean differences between day 1 and day 10 of the heat acclimation or exercise control period. Values shown are final heart rate and final core temperature at end of the second exercise bout, and changes in pre exercise resting plasma volume. Values are shown as mean ± standard error for 12 subjects in the heat acclimation group and 8 subjects in the control group. Range values are shown in parentheses [a] $P < 0.05$ vs. Day 1. [b] $P < 0.05$ vs. Control group.

	Heat acclimation Group		Control Group	
	Day 1	Day 10	Day 1	Day 10
Final heart rate (bpm)	164.6 ± 2.3 (153-174)	150.1 ± 2.6 (134-164)[a]	129.9 ± 3.0 (121-146)	126.5 ± 5.1 (117-155)
Final T_c (°C)	39.3 ± 0.1 (38.6-40.1)	38.8 ± 0.1 (38.2-39.3)[a]	38.1 ± 0.1 (37.8-38.5)	38.1 ± 0.1 (37.8-38.5)
ΔPV (%)	6.5 ± 1.2 (-5.40-17.34)[b]		-4.6 ± 2.7 (-13.62-9.27)	

Effect of heat acclimation on maximal oxygen uptake

Figure 1a shows heat acclimation effects on VO_{2max} responses in cool (13°C) and hot (38°C) conditions. Heat acclimation increased VO_{2max} in the cool environment (66.85 ± 2.07 vs. 70.21 ± 2.35 ml kg^{-1} min^{-1}; P = 0.004) and hot condition (55.06 ± 2.43 vs. 59.61 ± 2.00 ml kg^{-1} min^{-1}; P = 0.006). No significant changes were found in the control group in the cool environment (66.80 ± 1.65 vs. 66.04 ± 1.65 ml kg^{-1} min^{-1}), or hot condition (54.32 ± 2.39 vs. 54.87 ± 2.31 ml kg^{-1} min^{-1}).

Figure 1b shows heat acclimation effects on maximal power output during VO_{2max} test in cool (13°C) and hot (38°C) conditions. Heat acclimation increased maximal power output in the cool environment (369.17 ± 14.54 vs. 380.83 ± 14.48 W; P = 0.026) and hot condition (327.50 ± 14.73 vs. 351.67 ± 13.70 W; P = 0.003). No significant differences were found in the control group in the cool environment (381.25 ± 10.76 vs. 382.50 ± 12.36 W) or hot condition (350.00 ± 12.25 vs. 347.50 ± 13.98 W).

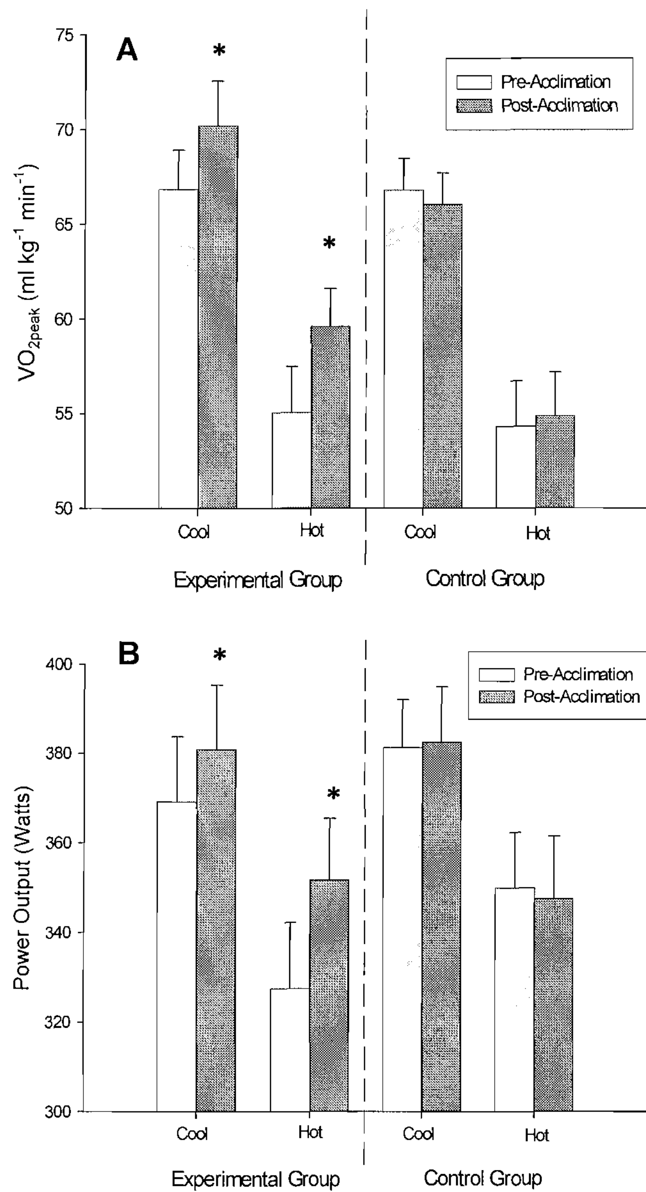


Figure 1. Effect of heat acclimation on maximal oxygen consumption (A) and maximal power output responses (B) in a cool (13°C) and hot (38°C) environment. Values are means ± SE for 12 heat acclimation subjects and 8 controls. * $P < 0.05$ vs. Pre-Acclimation within environmental condition.

Figure 2 shows the effect of heat acclimation on maximal cardiac output (A), and their corresponding stroke volume (B), and heart rate (C) during VO_{2max} test. Heat acclimation increased maximal cardiac output in the cool condition (24.64 ± 1.23 vs. 26.87 ± 0.82 L min^{-1}; P = 0.018), but not in the hot environment (22.02 ± 1.29 vs. 23.00 ± 1.32 L min^{-1}). Similarly, stroke volume during maximal cardiac output was increased after heat acclimation in the cool condition (137.9 ± 8.4 vs. 149.9 ± 5.2 ml; P = 0.032), but not in the hot environment (121.3 ± 7.5 vs. 124.1 ± 9.4 ml). Heat acclimation did not affect heart rate at maximal cardiac output in the cool [180.7 ± 4.5 vs. 180.0 ± 4.2 beats per minute (bpm)] or hot condition (184.0 ± 4.7 vs. 188.4 ± 4.6 bpm). No significant differences were found in cardiac output, stroke volume or heart rate in the control group in the cool environment (25.17 ± 1.16 vs. 24.83 ± 1.06 L min^{-1}; 135.9 ± 5.6 vs. 135.2 ± 5.3 ml; 185.0 ± 2.2 vs. 183.5 ± 3.8 bpm) or hot condition (23.82 ± 1.02 vs. 22.71 ± 1.46 L min^{-1}; 127.2 ± 5.6 vs. 123.1 ± 8.3 ml; 187.6 ± 3.6 vs. 185.0 ± 3.9 bpm).

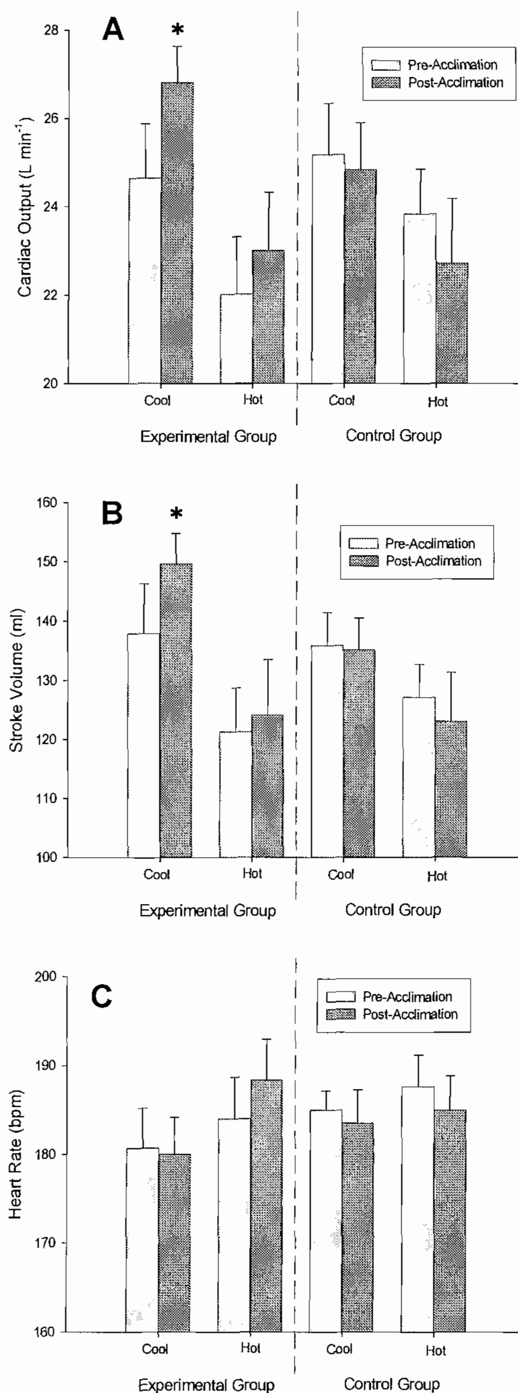


Figure 2. Heat acclimation effects on maximal cardiac output (A), and their corresponding stroke volume (B), and heart rate (C) during VO_{2max} test in a cool (13°C) and hot (38°C) environment. Values are means ± SE for 12 heat acclimation subjects and 8 controls. * $P < 0.05$ vs. Pre-Acclimation within environmental condition.

Effects of heat acclimation on lactate threshold

Figure 3 shows the effect of heat acclimation on the lactate threshold responses (in Watts). Heat acclimation increased lactate threshold in the cool environment (263.0 ± 16.1 vs. 277.1 ± 14.9 W; $P = 0.002$), and hot condition (233.3 ± 16.3 vs. 244.0 ± 16.1 W; $P < 0.001$). No significant differences were found in the control group in the cool environment (289.2 ± 12.9 vs. 287.1 ± 12.8 W) or hot condition (251.5 ± 12.8 vs. 249.8 ± 13.5 W).

Figure 4 presents individual data for pre and post acclimation trials in different performance variables of both groups and both environmental conditions. Note that in the heat acclimation group there is a consistent increase of these performance variables post acclimation. On the other hand, there are no clear trends in the control group.

Figure 5 summarizes the cardiorespiratory changes induced by acclimation trials in hot and cool environment for both groups. The heat acclimation group showed significant improvements in every variable (except for the maximal cardiac output in the hot condition). On the other hand, there was no significant difference in the control group in any of the cardiorespiratory variables.

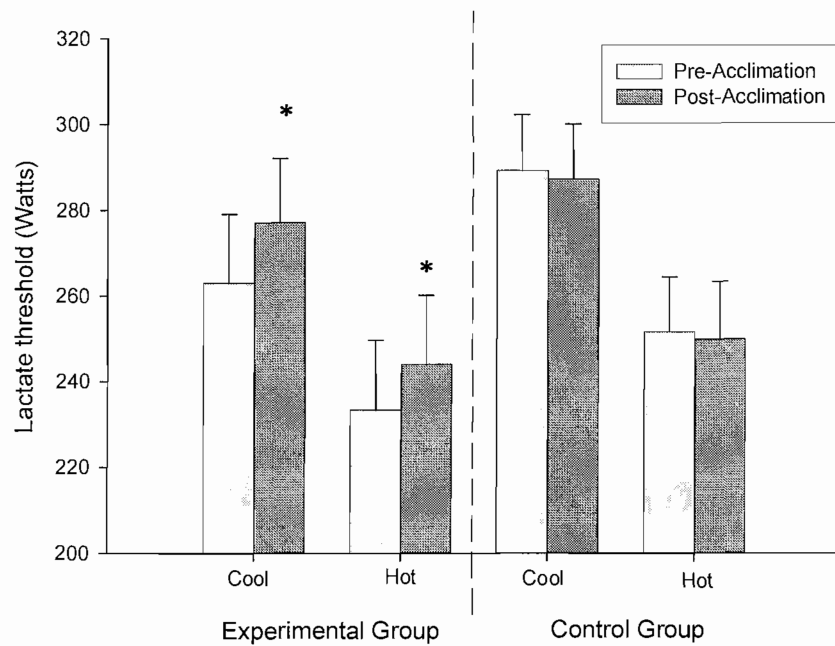


Figure 3. Effect of heat acclimation on lactate threshold responses in a cool (13°C) and hot (38°C) environment. Values are means ± SE for 12 heat acclimation subjects and 8 controls. ∗ $P < 0.05$ vs. Pre-Acclimation within environmental condition.

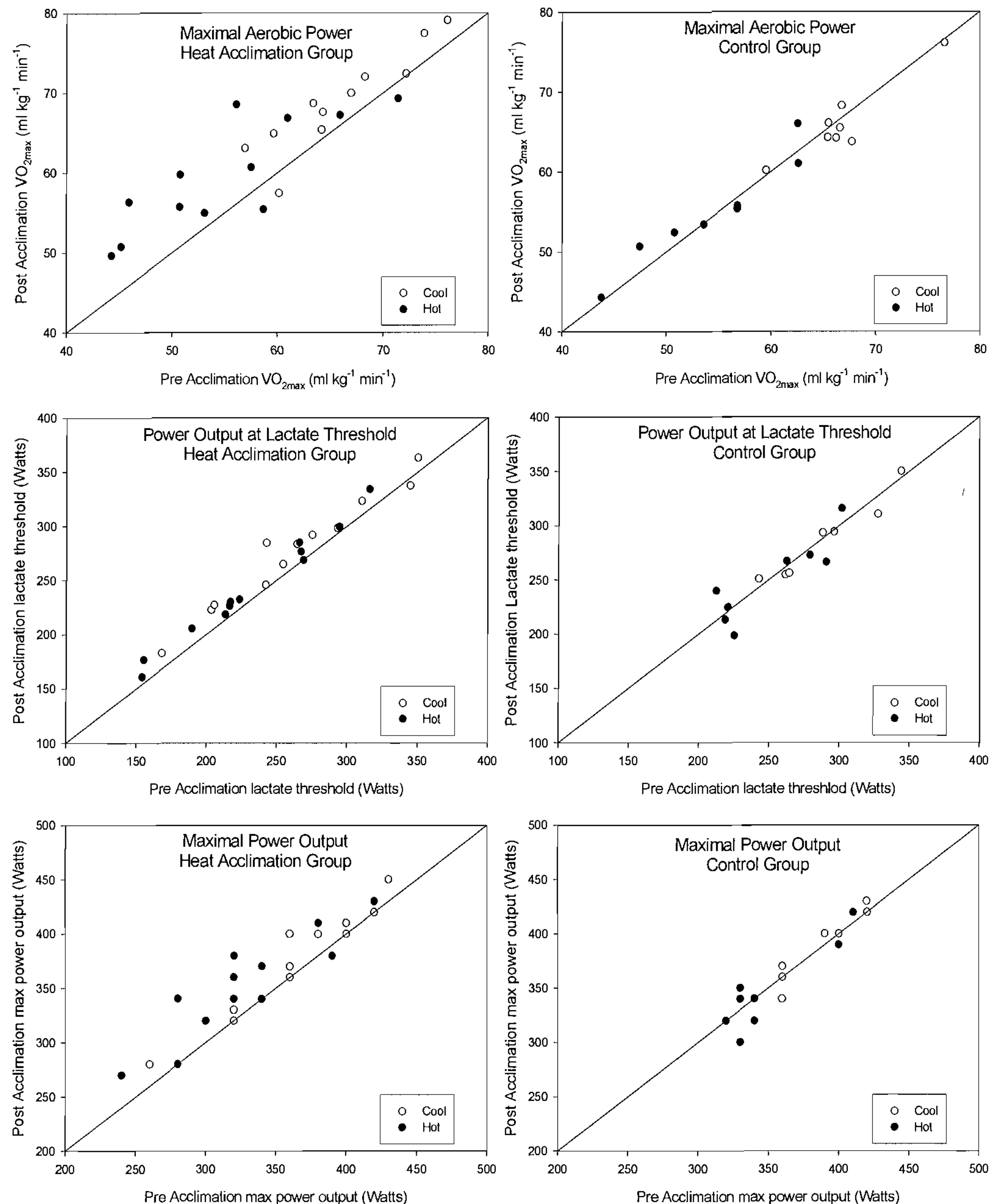


Figure 4. Individual data for relationship between pre and post acclimation in performance variables of heat acclimation and control groups under hot and cool condition. Maximal aerobic power is shown in the top panels (A and B), lactate threshold in the middle panels (C and D), and maximal power output in the bottom panels (E and F). Straight line represents line of equality.

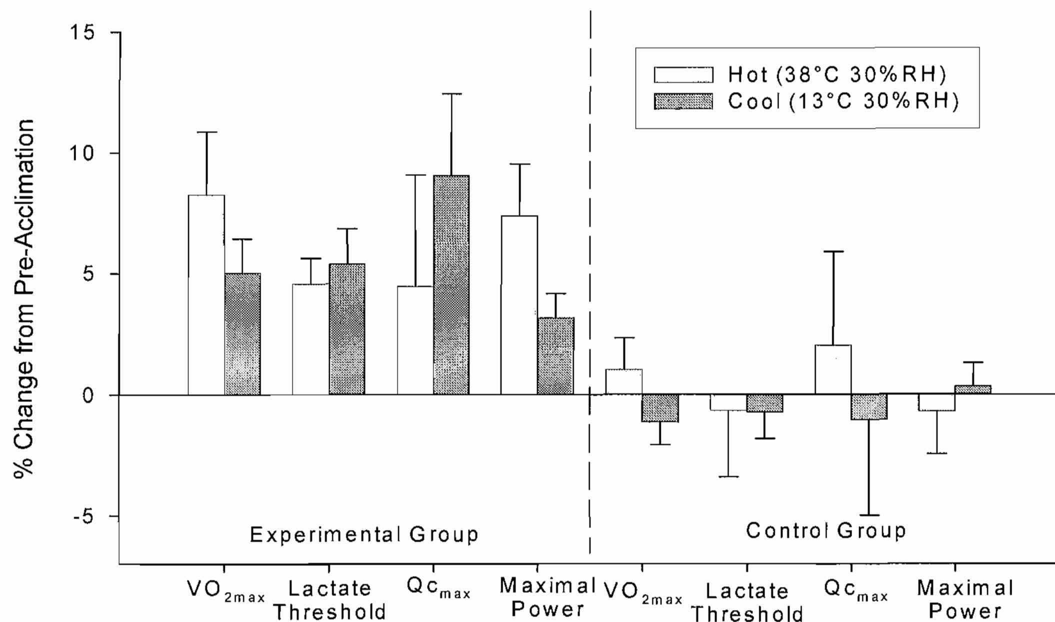


Figure 5. Cardiorespiratory changes as a percent change from the pre-acclimation trials in both environmental conditions. Values are means ± SE for 12 heat acclimation subjects and 8 controls. ∗ $P < 0.05$ vs. Pre-Acclimation within environmental condition.

Effect of heat acclimation on hemodynamics during leg kicking exercise

Table 3 shows the leg blood flow and oxygen delivery during single-leg kicking exercise in the hot and cool conditions. No statistical difference were as seen in the heat acclimation or control group in either temperature condition.

Table 3. Effects of heat acclimation on leg hemodynamics during incremental single-leg kicking exercise in the hot and cool environmental conditions. Values are means ± SEM for 11 subjects in the experimental group and 8 subjects in the control group. [a] $P < 0.05$ vs. Pre-acclimation trials within workload and environmental condition. [b] $P < 0.05$ vs. O_2 delivery within workload and environmental condition. [c] $P < 0.05$ vs. Blood flow within workload and environmental condition.

	Rest		30W		45W		60W	
	Pre Accl	*Post Accl*	*Pre Accl*	*Post Accl*	*Pre Accl*	*Post Accl*	*Pre Accl*	*Post Accl*
Blood flow at 13°C (L min^{-1})								
Experimental	0.36 ± 0.04	0.32 ± 0.03	1.79 ± 0.12	1.68 ± 0.14	1.85 ± 0.14	1.82 ± 0.20	2.40 ± 0.17	2.13 ± 0.22
Control	0.35 ± 0.02	0.38 ± 0.05[a]	1.44 ± 0.16	2.09 ± 0.19[a]	1.91 ± 0.18	2.12 ± 0.17[a]	1.99 ± 0.23	2.28 ± 0.17
O_2 delivery at 13°C (ml O_2 min^{-1})								
Experimental	62.85 ± 8.29	54.89 ± 5.52	312.78 ± 22.54	284.90 ± 22.60	323.03 ± 25.29	308.38 ± 35.23	432.88 ± 28.00	367.72 ± 37.74
Control	62.06 ± 4.17	65.98 ± 9.70	244.28 ± 27.98	355.72 ± 30.30[a]	319.42 ± 41.56	360.61 ± 31.27	349.64 ± 36.09	398.64 ± 34.86
Blood flow at 38°C (L min^{-1})								
Experimental	0.95 ± 0.10[c]	0.81 ± 0.10[c]	1.74 ± 0.15	1.77 ± 0.15	1.89 ± 0.18	1.86 ± 0.15	2.14 ± 0.02	1.96 ± 0.08
Control	0.98 ± 0.08[c]	1.20 ± 0.12[c]	1.66 ± 0.13	1.90 ± 0.17	1.66 ± 0.14	2.13 ± 0.14	1.90 ± 0.18	2.04 ± 0.05
O2 delivery at 38°C (ml O_2 min-1)								
Experimental	165.54 ± 17.36[b]	139.43 ± 15.95[b]	297.33 ± 26.84	291.97 ± 22.54	327.55 ± 34.07	320.23 ± 23.81	376.37 ± 4.28	342.17 ± 8.70
Control	169.80 ± 14.75[b]	202.08 ± 20.05[b]	285.80 ± 23.23	318.14 ± 29.41	289.89 ± 23.73	362.69 ± 20.27[a]	333.93 ± 38.41	341.09 ± 20.74

Discussion

This study is the first to delineate the impact of heat acclimation on improving maximal aerobic performance in temperate conditions. These findings have direct implications for athletes and military personnel employing heat stress to optimize improvements from physical training programs. We employed highly trained cyclists because they would provide more consistent maximal efforts and the heat acclimation supplementation is being considered to aid competitive athletes further improve their performance beyond using traditional training approaches. Our data indicate that heat acclimation improved lactate threshold and VO_{2max} in the heat. Importantly, heat acclimation also increased lactate threshold and VO_{2max} in a cool environment.

Effect of heat acclimation on hemodynamics and VO_{2max}

Although previous research has shown improvements in VO_{2max} in thermoneutral environments after heat acclimation, such changes were attributed to a "training effect" due to the heat acclimation program (Nadel *et al.*, 1974, Sawka *et al.*, 1985). Our study used highly trained cyclists (average VO_{2max} > 66 ml kg^{-1} min^{-1}), and the low exercise intensity used during the heat acclimation protocol (~50% of VO_{2max}) makes a training effect very unlikely (Pollock, 1973). Furthermore, it has been shown that heat acclimation programs at low intensity (50% of VO_{2max} or less) have no

impact on cardiovascular fitness (Desai & Senay, 1984, Roberts *et al.*, 1977). Finally, we used a control group and these subjects did not show an improvement in VO_{2max}. Thus, we are confident that the results obtained in our study are due to heat acclimation effects per se and not due to training.

After heat acclimation subjects increased their resting plasma volume by about 6.5% and this is in agreement with others (Aoyagi *et al.*, 1994, Nielsen *et al.*, 1993, Patterson *et al.*, 2004, Senay *et al.*, 1976). Controversy exists as to whether hypervolemia (i.e. plasma volume expansion) has the potential to increase maximal cardiac output, and how this potential change affects VO_{2max}. Hypervolemia has been shown to either have no effect (Robinson *et al.*, 1966) or increase maximal cardiac output during exercise in thermoneutral environments (Kanstrup & Ekblom, 1982, Spriet *et al.*, 1980). Much of the conflicting findings could be attributed to different methodologies used to induce hypervolemia (i.e. dextran or whole blood infusions, physical training, or chronic heat stress), degree of hypervolemia, and fitness level of the population used. For example, one study showed that plasma volume expansion via 500-700 ml dextran increased maximal cardiac output, however, such change had no effect on VO_{2max} in moderately active subjects (Kanstrup & Ekblom, 1982). The significant increase in maximal cardiac output after plasma volume expansion compensated for the reduced arterial oxygen content induced by hemodilution. However, when hypervolemia was induced by whole blood

infusions (1,200 ml) in highly trained athletes, significant increases in maximum stroke volume, cardiac output, and VO_{2max} were observed (Spriet *et al.*, 1980). Another study also supports the theory that a slight increase in plasma volume (400 ml) causes improvements in stroke volume and thus cardiac output (Hopper *et al.*, 1988), although this research involved untrained subjects. There has also been evidence that plasma volume expansion may be responsible for slight, but significant increases in VO_{2max} (Coyle *et al.*, 1990, Nadel *et al.*, 1974, Sawka *et al.*, 1985). Coyle *et. al.* (1990) showed that plasma volume expansion by 200-300 ml of 6% dextran significantly increased VO_{2max} in untrained subjects (Coyle *et al.*, 1990). The authors measured an increased cardiac output after plasma volume expansion at submaximal exercise intensities. More importantly, they suggested that the potential for plasma volume expansion to increase VO_{2max} depends on the tight balance between the extent to which maximal cardiac output is increased compared to the reduction in hemoglobin concentration and thus, arterial oxygen content. The authors observed that a slight plasma volume expansion caused increases in stroke volume by 10-15% with only a small amount (4%) of hemodilution, and as a result VO_{2max} increased slightly (4%). Similar to Coyle's findings, we observed a moderate increase in plasma volume (6.5%) with a small degree of hemodilution (3.3%), which resulted in a 9% increase in the maximal cardiac output and a VO_{2max} increase of 5%. Furthermore, maximal power

output was also significantly increased by 3% and 8% in the cool and hot, respectively.

The improved cardiac performance following a period of chronic heat stress is also supported by several animal studies, which show evidence that heat acclimation induces a number of mechanical and metabolic adaptations in the rat heart (Horowitz *et al.*, 1986a, Horowitz *et al.*, 1986b, Horowitz *et al.*, 1993, Levy *et al.*, 1997). For instance, heat acclimation increases left ventricular compliance and pressure generation and decreases oxygen consumption (Horowitz *et al.*, 1986b, Horowitz *et al.*, 1993). In addition, the improved myocardial efficiency is a result of a transition from fast myosin (V_1) to mainly slow myosin isoforms (V_3) (Horowitz *et al.*, 1986a). Finally, another study that investigated the combined effects of heat acclimation and exercise training in a rat heart have shown additive effects on the mechanical and metabolic properties of rat hearts compared with the effects of exercise training or heat acclimation alone (Levy *et al.*, 1997). In addition, these authors also suggest that augmented force generation (i.e. contractility) post heat acclimation is associated with elevation of cytosolic calcium concentration on contraction. Together, these reports are in agreement with our observations and suggest that heat acclimation may enhance mechanical adaptations and improve metabolic efficiency of the heart in highly trained individuals.

The increased maximal cardiac output in the cool could increase active muscle blood flow, which leads to potential increases in oxygen delivery and maximal oxygen uptake. In support of this theory, one study showed that a small increase in plasma volume (and thus total blood volume) increased exercise VO_2 in dogs (Sarelius & Sinclair, 1981). The authors suggested that the increased central blood volume induced by hypervolemia and decrease venous capacitance due to exercise (Bevegard & Shepherd, 1967) could lead to greater pulmonary and cardiac blood volumes than those normally seen during exercise (Braunwald & Kelly, 1960). Increased pulmonary venous, left atrial and left ventricular volume would cause an increase cardiac output, increased oxygen delivery, and increased perfusion of skeletal muscle (Sarelius & Sinclair, 1981). In addition, the heat acclimation-induced plasma volume expansion, together with the decreased skin blood flow demand driven by the cool temperature, may increase central blood volume (Wood & Bass, 1960). Augmented central blood volume has been considered to have permissive function in the regulation of cardiac function (Rowell *et al.*, 1966, Sjostrand, 1953, Thauer, 1962), leading to increases in stroke volume, cardiac output and potentially leg blood flow.

Contrary to the cool condition, we did not observe an increase in the maximal cardiac output in the heat, although there was a significant increase in VO_{2max}. Therefore, other cardiovascular adaptations must

account for the increased VO_{2max}. One possibility could be that at maximal efforts in the heat there is redistribution of the cardiac output. More specifically, there could be a greater degree of vasoconstriction in inactive vascular beds (i.e. splanchnic area and inactive muscles), allowing for an increased cardiac output directed to the exercising muscles. One study suggested that after heat acclimatization, distribution of cardiac output not only to skin but also to other organs, probably changes (Rowell *et al.*, 1967). Also, there is evidence that hepatic-splanchnic (Rowell *et al.*, 1965) and renal blood flows (Radigan & Robinson, 1949) are decreased when exercising in the heat compared to cool environments and as work progresses, there are further reductions in visceral blood flow (Rowell *et al.*, 1965). Furthermore, the enhanced evaporative cooling due to improved sweating capabilities that result from heat acclimation (Chen & Elizondo, 1974, Yamazaki & Hamasaki, 2003) may lower skin temperature and reduce skin blood flow, and thus, allowing for an increased cardiac output directed to active muscles.

Effect of heat acclimation on lactate threshold

The lower lactate levels during exercise observed after heat acclimation is in agreement with previous research. At a given exercise intensity, heat acclimation has been shown to reduce the blood lactate concentration (Febbraio *et al.*, 1994, Young *et al.*, 1985). In addition to the

lower blood lactate levels measured after heat acclimation in both environments, we showed that the threshold at which blood lactate levels begin to rise also is delayed. Some studies suggested that heat acclimation induces metabolic adaptations during exercise by reducing the aerobic metabolic rate (Aoyagi *et al.*, 1994, Sawka *et al.*, 1983, Young *et al.*, 1985), or decreasing the rate of glycogenolysis (Febbraio *et al.*, 1994, Febbraio *et al.*, 1996, Kirwan *et al.*, 1987). Alternatively, the increased plasma volume (and thus, total blood volume) (Bass *et al.*, 1955, Harrison *et al.*, 1981, Senay *et al.*, 1976, Wyndham *et al.*, 1968) could have an effect in blood lactate concentration in two ways. First, it may allow for an increased blood flow through the splanchnic circulation, enhancing lactate removal (Rowell *et al.*, 1968) and thus delaying blood lactate accumulation. Or, the decreased blood lactate concentration may be a result of an increased total blood volume per se, so that absolute blood lactate levels remain constant. Nevertheless, the relationship between heat acclimation and the physiological effects on lactate threshold need to be further explored.

The literature on anaerobic threshold and cold exposure is conflicting, although it seems that cooler temperatures (10-15°C) may delay the lactate threshold compared to thermoneutral or warm environments (Flore *et al.*, 1992, Therminarias *et al.*, 1989, Tyka *et al.*, 2009). The approximately 30 Watt difference between the lactate threshold in the heat and cool condition observed on this investigation (see figure 3) are

consistent with these earlier findings. Moderate cold exposure (10°C) has been shown to decrease the plasma lactate levels compared to neutral conditions (Flore *et al.*, 1992), although these changes were less marked than those observed during severe cold stress (Therminarias *et al.*, 1989). These discrepancies may be attributed to differences in methodologies, degree of cold stress, fitness of subjects and type of exercise. A novel finding from this study is that heat acclimation increased lactate threshold in a cool environment. To our knowledge, there have been no studies that have explored this relationship. The previously discussed increase in cardiac output to active muscles, together with the decrease aerobic metabolic rate (Aoyagi *et al.*, 1994, Sawka *et al.*, 1983, Young *et al.*, 1985), may account for the delay in blood lactate accumulation. In addition, the possibility of an increased lactate removal by increases in splanchnic blood flow (Rowell *et al.*, 1968) cannot be discarded. One alternative, however, is that the change in thermoregulatory drive induced by heat acclimation (Nadel *et al.*, 1974, Yamazaki & Hamasaki, 2003), together with the improved cardiovascular stability (Nielsen *et al.*, 1993) may have allow for an enhanced muscle perfusion.

At first glance, the speculations about the dynamics of cardiac output, leg blood flow and oxygen delivery might be conflicting with our observations from the single-leg knee extensor exercise. We failed to observe any changes in the dynamics of muscle blood flow and oxygen

delivery in the heat acclimation group. However, this study consisted of exercising with a small muscle mass, as supposed to whole body dynamic exercise (i.e. cycling). The difference in the amount of muscle mass activated during maximal exercise has major effects on the cardiovascular system (Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005, Mortensen *et al.*, 2008). It has been reported that dynamic exercise with a small muscle mass might result in intramuscular tension that exceeds perfusion pressure and thereby effectively decreases the vascular cross sectional area perfused, which may limit oxygen delivery (Sawka *et al.*, 1981, Sawka, 1986). Reports have shown that during maximal whole body dynamic exercise cardiac output failed to continue to increase and match the muscle metabolic demands and thus, leg blood flow and oxygen delivery decreased before the onset of fatigue (Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005). On the other hand, during a maximal exercise involving a small muscle mass cardiac output was not limited and continued to increase together with leg blood flow and oxygen delivery until onset of fatigue (Mortensen *et al.*, 2005). This difference has been attributed to the inability of the cardiovascular system to continue to increase cardiac output and match the metabolic demands of exercising muscle. Therefore, we cannot conclude that the observations from the single-leg kicking exercise can be extrapolated to predict what might happen between cardiac output, leg blood flow and oxygen delivery during

a whole body dynamic exercise. Further studies investigating the specific effects of heat acclimation on the dynamics of leg blood flow and oxygen delivery during large muscle mass exercise are warranted.

We observed a slight reduction in plasma volume in the control group. To ensure the subjects were properly hydrated, nude body weight and plasma osmolality were measured. Euhydrated subjects were considered if their nude body mass was within 1% of their 5-day average and plasma osmolality <290mOsmol kg^{-1} H_2O (Sawka *et al.*, 2007). Therefore, the possibility that subjects in the control group were dehydrated was eliminated. This plasma volume reduction could be explained by the fact that this group exercised in a cool environment (13°C). One of the most recognized effects of cold exposure is an increase in urine output, or cold-induced diuresis, resulting in an iso-osmotic reduction in plasma volume. One study have shown that the increased systemic and renal blood pressure associated with cold-induced vasoconstriction may increase filtration and reduce reabsorption of water and solutes by the kidneys (Freund & Young, 1996). Another possibility for the plasma volume reduction in the control group could be related to a slight detraining and/or de-acclimatization. Although all subjects were encouraged to continue their normal training routine during the entire study, the amount of time devoted to the study made it difficult for the subject to continue with their normal rides outside the lab. Therefore, the total volume and intensity of training

during these periods might have been lower than normal, which may account for the slight plasma volume reduction.

Limitations

With most of the heat acclimation protocols that involve exercise there is a chance that the changes observed post heat acclimation could be due to a training effect (Sawka *et al.*, 1985, Young *et al.*, 1985). As discussed before, we believe this is not the case in our study due to several reasons. The combination of low exercise intensity during the heat acclimation process (50% of their VO_{2max}), plus the subjects' high fitness level (mean VO_{2max} of 66 ml kg^{-1} min^{-1}) make the training effect unlikely (Pollock, 1973). In addition, we did not observe these changes in the control group, who exercised at the same exercise intensity as the heat acclimation group.

The data collection was carried between the months of February and August so there is a possibility that subjects may be naturally acclimatized during the early months of summer. It's important to note that due to their heavy training routines, research has shown that highly trained cyclists are already "partially" heat acclimatized, even during the winter months (Gisolfi & Robinson, 1969). Although cyclists were encouraged to continue their normal training during the duration of the entire study, they were not allowed to train on hot days or at the peak heat on any given day. In

addition, the warmest months in the city of Eugene are July and August with an average high temperature of less than 82°F, and the degree of heat stress that subjects were exposed to in the study was well above, and for a more sustained time period, than what they would typically get during a training session outside during that time. In addition, we tested subjects in the control group over the same time period as the heat acclimation group throughout the data collection period and we failed to see any differences. Moreover, we did not observe any differences in responses between the subjects studied during the winter and those studied during the summer. In any case, any possible "partial acclimation" from the subjects would underestimate potentially larger differences in the heat acclimation group post acclimation.

Effects of core temperature at the beginning of the test performed in the hot condition can be ruled out as a potential factor affecting VO_{2max} or lactate threshold since in both tests (pre and post acclimation) the subjects had their rectal temperature raised 1°C above resting levels and rectal temperature at the start of each test was not different between pre-acclimation and post-acclimation trials (38.34°C vs. 38.14°C, respectively). In addition, although resting core temperature slightly decreased post acclimation, it was statistically not significant (37.21°C vs. 37.07°C, $P = 0.21$).

There are numerous approaches currently used to determine

anaerobic threshold using blood lactate levels during incremental exercise. Some involve simple subjective observations (Kindermann *et al.*, 1979), while other methods use complex mathematical algorithms (Beaver *et al.*, 1985). For the purpose of manuscript, we used the 1 mM lactate threshold method, which determines the power output or oxygen uptake at which blood lactate increases 1 mM above resting values (Coyle *et al.*, 1983). This method, and some of its variations (i.e. blood lactate increases of 0.5 mM or 0.75 mM above baseline values), has been commonly used to estimate anaerobic threshold (Dumke *et al.*, 2006, McGehee *et al.*, 2005, Thomas *et al.*, 2008), due to the relative ease and non subjective nature in discerning the anaerobic threshold. Furthermore, this approach takes into account individual variations in the subjects' resting steady state lactate levels.

To achieve the desired rectal temperature prior to the start of each test done in the hot condition, subjects rested inside a water-filled tub (~41°C) for approximately 30 minutes. Research has shown that high core and skin temperatures are necessary to adequately stress the cardiovascular system and alter some physiological variables associated with performance (i.e. VO_{2max}, and lactate threshold) (Gonzalez-Alonso & Calbet, 2003, Mortensen *et al.*, 2005, Tyka *et al.*, 2000, Tyka *et al.*, 2009). The water immersion allowed us to manipulate the subjects' rectal temperature without having to make them exercise prior to the studies, which can potentially act as a confounding variable. By controlling the

temperature of the water inside the tub we were able to either increase the subjects' rectal temperature in the hot condition, or maintain the same resting temperature during the testing in the cool condition. Furthermore, pilot work done in our climatic chamber demonstrated that even exercising at a very low power output (i.e. 125W) for 30 minutes in a cool environment (13°C 45% relative humidity), resulted in an increase in rectal temperature of 0.9°C. Finally, other investigators have successfully used this approach to manipulate the subject's core temperature prior to an exercise test (Gonzalez-Alonso *et al.*, 1999).

Perspectives

The results from this study have important theoretical and practical application in the field of exercise and performance. To our knowledge, this is the first study that has shown direct benefits of a period of heat acclimation on cool weather performance. As small as it seems, a 5% increase in the VO_{2max}, and most importantly a 5% (or approximately 14 Watts) increase in the lactate threshold in already highly trained cyclist could make a big difference in competitions. In fact, Chapter V of this dissertation showed that a period of heat acclimation significantly increased time trial performance under hot and cool environmental conditions. The competitions at the highest level have become so specific that a 5% boost

in a cyclist time trail performance can make the difference between wining a race or not.

As being the first published study demonstrating performance and physiological effects of heat acclimation on cool temperatures, many questions arise. Studies investigating heat acclimation effect on the dynamics of central cardiac function, active muscle blood flow, and oxygen delivery during whole body dynamic exercise in cool weather is warranted. Furthermore, the role of skin blood flow and skin temperatures must be investigated to discern the interaction between the thermoregulatory and cardiovascular systems during maximal efforts under moderate cold stress after heat acclimation.

CHAPTER V

EFFECTS OF HEAT ACCLIMATION ON ONE HOUR TIME TRIAL PERFORMANCE AND PACING STRATEGY IN HOT AND COOL ENVIRONMENTAL CONDITIONS

Introduction

Warm weather degrades aerobic exercise capabilities as evidenced by Marathon running performance slowing as a function of environmental heat stress (Ely *et al.*, 2007). Experimental studies have supported this observation by demonstrating shorter duration degraded aerobic exercise performance in hot environments compared cool and temperate temperatures (Galloway & Maughan, 1997, Parkin *et al.*, 1999, Tatterson *et al.*, 2000). Although endurance exercise in the heat results in major alterations in the cardiovascular, thermoregulatory, metabolic and neuromuscular systems, hyperthermia has recently been argued to be a major determinant of aerobic endurance performance in the heat (Gonzalez-Alonso *et al.*, 1999, Nybo *et al.*, 2001), but that notion is not supported by all (Ely *et al.*, 2009, Kenefick *et al.*, 2009). The primary cardiovascular perturbation from heat stress is sustaining increased skin

blood flow for heat loss (Sawka & Wenger, 1988, Sawka & Young, 2006) and recent evidence shows that despite no difference in core temperature that elevated skin temperature (reflective of elevated skin blood flow) will degrade aerobic endurance (time-trial) performance in the heat (Altareki *et al.*, 2009, Ely *et al.*, 2009). Heat acclimation improves thermoregulatory responses, reduces cardiovascular strain and improves exercise-heat performance (Eichna *et al.*, 1945, Fox *et al.*, 1967, Nielsen *et al.*, 1993, Shvartz *et al.*, 1972).

Although there have been previous reports on the effect of different ambient temperatures on exercise capacity, Galloway and Maughan (1997) were the first investigators to systematically measure the effects of different ambient temperature on exercise endurance capacity in a laboratory setting, and quantify the effects of different ambient temperatures on exercise capacity at a constant power output (Galloway & Maughan, 1997). They observed exercise duration was longest at 11°C. Below this temperature (at 4°C) and above this temperature (at 21°C and 31°C), a reduction in exercise capacity was observed. The authors attribute the detriments in performance at the higher ambient temperatures mainly to a reduced central venous pressure, secondary to a large peripheral pooling of blood combined with the large evaporative fluid loss, although hyperthermia also may have limited exercise capacity (Galloway & Maughan, 1997). At the colder ambient temperature (4°C) the authors suggested that earlier

onset of fatigue was caused by altered muscle temperature which may reduce mechanical efficiency and increase total energy cost.

Some sports medicine scientists have argued that aerobic performance during exercise to fatigue at a constant power output in temperate or hot environments appears to be related to the attainment of an upper limit in body core temperature (Febbraio *et al.*, 1996, Gonzalez-Alonso *et al.*, 1999, Nielsen *et al.*, 1993, Tatterson *et al.*, 2000). These studies reported that subjects ceased to exercise at the same core temperature, regardless of hydration status (Febbraio *et al.*, 1996), glucose availability (Febbraio *et al.*, 1996), heat acclimation status (Nielsen *et al.*, 1993), initial core temperature (Gonzalez-Alonso *et al.*, 1999) or rate of body heat storage (Gonzalez-Alonso *et al.*, 1999). However, in those studies high cardiovascular strain frequently provides an alternative explanation to high core temperature (hyperthermia). Another study performed under hot (32°C) and thermoneutral (23°C) conditions reported that during a self paced time trial, highly trained cyclists selected power output relative to changes in core temperature (Tatterson *et al.*, 2000). The reduced power output in the hot trial compared to the thermoneutral environment was accompanied by reductions in blood lactate, although rectal temperature was almost identical in both conditions. Based on these observations the authors suggested that exercise performance is related to factors associated with thermoregulation and not limited by metabolic

capacity (Tatterson *et al.*, 2000). Contrary to those reports, Ely and colleagues (Ely *et al.*, 2009) reported that outdoor running performance is independent of core temperature or rate of heat storage. These same investigators employing laboratory time-trial performance tests demonstrated that performance degradation was related to skin and not core temperature (Kenefick *et al.*, 2009). The work of Ely is supportive of traditional physiological viewpoints regarding multiple mechanisms of reduced performance (Sawka & Young, 2006) revolving around the cardiovascular penalty of sustaining high skin blood flow (Sawka *et al.*, 1996).

Heat acclimation improves exercise performance in hot environments (Eichna *et al.*, 1945, Fox *et al.*, 1967, Nielsen *et al.*, 1993, Shvartz *et al.*, 1972). In addition, some reports have shown that heat acclimation induces metabolic and cardiovascular adaptations that reduce the blood lactate concentration at a given level of intensity (Febbraio *et al.*, 1994, Young *et al.*, 1985). These metabolic adaptations may be caused by reduced aerobic metabolic rate (Sawka *et al.*, 1983, Young *et al.*, 1985), or decreased the rate of glycogenolysis (Febbraio *et al.*, 1994, Febbraio *et al.*, 1996, Kirwan *et al.*, 1987). Alternatively, exercise post-heat acclimation may be improved by the increased plasma volume (and thus, total blood volume) (Bass *et al.*, 1955, Harrison *et al.*, 1981, Senay *et al.*, 1976, Wyndham *et al.*, 1968), which may allow for an increased blood flow through the

splanchnic circulation, enhancing lactate removal (Rowell *et al.*, 1968) thereby delaying blood lactate accumulation. Although there are many reports on the effect of hot environments on exercise performance and the changes that follow a period of heat acclimation, there is no published research on the effects on heat acclimation on performance in cool weather in highly trained cyclists. The improved thermoregulatory and cardiovascular adaptations that result from a period of heat acclimation could potentially enhance exercise performance in cool environments.

Currently, no uniform laboratory endurance performance test exists, but a wide variety of different exercise protocols are used (Anantaraman *et al.*, 1995, Below *et al.*, 1995, Clark *et al.*, 2000, Coyle *et al.*, 1991, el-Sayed *et al.*, 1997, Hickey *et al.*, 1992, Jeukendrup *et al.*, 1996, Jeukendrup *et al.*, 1997, Krebs & Powers, 1989). Many investigators assessed exercise performance using a time trial approach in which either a fixed amount of work is performed as quickly as possible or as much work as possible is done in a set time (Carter *et al.*, 2004, Jeukendrup *et al.*, 1996, Tatterson *et al.*, 2000). This method may be more suitable to assess performance in competitive cyclists. Although done in a research laboratory, this approach better resembles a “real life” time trial competition than a constant power test and can be used to assess pacing strategies as well. Kenefick *et* al. have demonstrated the reliability and sensitivity of time-trial and pacing data to evaluate exercise-heat performance (Kenefick *et al.*, 2009).

The primary aim of the present study was to investigate the effect of heat acclimation in highly trained cyclists on performance during a 1-hr high-intensity cycle time trial in both hot (38°C) and cool (13°C) environments. A secondary objective was to explore the effects of heat acclimation on the pacing strategy chosen by the cyclists. We hypothesized that heat acclimation would increase performance during a 1-hr time trial in both environmental conditions without altering the pacing strategy.

Methods

Study design

This manuscript is part of a larger project that investigated effects of heat acclimation on performance and other physiological variables in endurance trained cyclists. In brief, participants were put through a battery of physiological and performance tests under two environmental conditions, then put through a heat acclimation or an exercising control program, and then the tests were repeated. On the days when the time trial was performed under heat stress, the climatic chamber was set to 38°C and 30% relative humidity (RH) (WBGT = 33°C). On the days where the time trial was carried under cool conditions, the climatic chamber was set to 13°C and 30% RH. The order of heat and cool trials was randomized across subjects. The heat acclimation protocol consisted of 10 exposures of cycling exercise at a temperature of 40°C and 30% relative humidity

(WBGT = 35°C). Subjects performed two bouts of 45 minutes at 50% of their VO_{2max} with 10 minutes of rest in between. The control group exercised at the same intensity and time but with the chamber set at 13°C and 30% RH (WBGT = 12°C).

Subjects

A total of sixteen subjects (13 men, 3 women) were used for these set of studies. Twelve highly trained endurance cyclists (10 men, 2 women), age 24 ± 6 (SD) completed the heat acclimation protocol (height 175 ± 6 cm, weight 67.7 ± 8.1 kg, body mass index 22.1 ± 3.9 kg m^{-2}). Eight subjects (7 men, 1 woman), age 26 ± 4 completed the control protocol (height 174 ± 6 cm, weight 70.2 ± 4.1 kg, body mass index 23.1 ± 3.1 kg m^{-2}). Of the sixteen total subjects used for these set of studies, four men age 28 ± 5 performed the control protocol followed by the heat acclimation exposures and experimental tests (height 176 ± 4 cm, weight 73.1 ± 1.5 kg, body mass index 23.5 ± 2.8 kg m^{-2}). A complete description of the subject groups is presented in table 4.

Measurements

Exercise was performed on an electronically braked cycle ergometer (Lode Excalibur Sport™, Groningen, The Netherlands). Heart rate (HR) was monitored continuously throughout each protocol via telemetry (model RS400, Polar Electro™, Lake Success, NY). Core temperature was

estimated using continuous measurements of rectal temperature by a thermistor (YSI 400 Series, Mallinckrodt Medical, St. Louis, MO) inserted 15 centimeters beyond the rectal sphincter. Skin temperature was measured using thermocouples made of copper and constantan on selected body areas on the skin. An estimate of mean skin temperature was calculated using 7 body sites (forehead, chest, abdomen, upper arm, forearm, upper thigh, and calf) (Sawka & Wenger, 1988). Skin blood flow requirements (SKBF) were estimated based on core temperature (Tc), skin temperature (Tsk), specific heat of the blood (SH, ~1 Kcal per ^{o}C) and heat production (Hp in Kcal min^{-1}) using the following formula: SKBF = 1/SH × Hp / (Tc – Tsk) (Sawka & Young, 2006). These estimates assume that blood entering and leaving the cutaneous circulation is equal to core and skin temperatures, respectively (REF). Dry, nude body weight was taken at the beginning and conclusion of each study visit by a precision weighing balance to the nearest 5 g (Sartorius™ EB6CE-I, Precision Weighing Balances, Bradford, MA). The initial body weight was used to ensure body fluid balance remained constant during the study visits.

Cardiac output was measured using an open-circuit acetylene washin method originally developed in 1975 (Stout *et al.*, 1975), modified in 1993 (Gan *et al.*, 1993), and validated in humans during exercise against the direct Fick approach (Johnson *et al.*, 2000). Breath-by-breath measurements of oxygen consumption (VO_2), carbon dioxide production

(VCO_2), and expired minute ventilation (V_E) were made by custom software (KCBeck Physiological Consulting, St Paul, MN) modified to interface to a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services). Expired air was also collected into Douglas bags and subsequently analyzed for oxygen and carbon dioxide concentrations (mass spectrometer) and volumes (Tissot gasometer). Calculations of VO_2 and VCO_2 were performed using the Haldane transformation (Wilmore & Costill, 1973). This permitted the comparison of breath-by-breath (15 sec averages) and the Douglas bags determination of VO_2 and V_E.

Specific protocol

On each study visit, subjects reported to the laboratory after a 2-hour fast and well hydrated. Subjects were instructed to avoid consumption of alcohol or caffeine for at least 8 to 12 hours prior to the study. In addition, they were not allowed to exercise on the same day prior to the study and were told to avoid ingestion of non-prescription drugs for the entire duration of the multiple study visits.

Dry, nude body weight was taken, and a rectal thermistor was inserted. Once seated on the cycle ergometer, subjects were instrumented with the skin thermocouples. After a brief warm-up (5 minutes at 40% of maximal power) subjects were asked to perform a maximal effort for a total of one hour. Total work done after 1 hour (in kilojoules) was the

performance variable of interest. During the test, the cycle ergometer was set to the hyperbolic mode (pedaling rate independent) and subjects did not receive any feedback (i.e. HR, power output, core temperature, etc.) except for total time elapsed. Subjects were allowed to modify power output as often as needed, but without knowing the absolute workload. Every 5 minutes measurements of power output, cadence, work performed, heart rate, rate of perceived exertion (RPE), and rectal temperature were taken. A capillary blood sample was taken from a fingertip and analyzed for lactate concentration (Lactate Pro. Arkray, Inc. Kyoto, Japan) at 10, 25, 40 and 55 minutes. Finally, oxygen consumption and cardiac output data were collected at 20, 40 and 60 minutes. Skin temperature at each site was recorded continuously and mean skin temperature was estimated using the formula from Sawka & Wenger (1988). Mean body temperature was calculated using weighed coefficients for rectal temperature (T_{re}) and mean skin temperature (T_{sk}) [body temperature = $0.8(T_{re}) + 0.2(T_{sk})$]. A percent change in power output (pace) was calculated every 5 minutes by the following equation: (true power output – average power output over the entire time trial duration) / (average power output) × 100. At the end of the time trial, subjects were toweled off and nude body weight was recorded. On a following day, the subjects returned and repeated the time trial in the cool or hot condition.

Data from each protocol were compared between pre and post

acclimation trials by determining specific differences using a paired Student's *t*-tests and significance was set at $P < 0.05$, and values are presented as mean and standard error (mean ± SE), unless otherwise indicated.

Results

Table 4 shows specific physiological characteristics of the control and heat acclimation groups. Although the control group showed a slight higher absolute VO_{2max} (4.9%) and maximal power output (3.2 %), no differences were found between groups for VO_{2max} and maximal power output per unit body weight. We suspect any differences were due to 2 women being in the heat acclimation group and 1 woman in the control group. In addition, the mean body weight in the control group was elevated compared to the heat acclimation group (70.2 ± 4.1 vs. 67.7 ± 8.1 kg, respectively).

Table 5 shows mean differences between day 1 and day 10 of the heat acclimation or exercise control period. Values shown are final heart rate and final core temperature at end of the second exercise bout, and changes in pre exercise resting plasma volume. All results are shown as mean and standard error. There was a statistically significant reduction in the final heart rate ($P < 0.001$), and core temperature ($P = 0.002$), in the heat acclimation group but not in the control group.

Table 4. Physiological characteristics of the heat acclimation and control groups. Values are shown as mean ± standard error for 12 subjects in the experimental group and 8 subjects in the control group. Range values are shown in parentheses. Reported values of maximal oxygen consumption (VO_{2max}) and maximal power output were from VO_{2max} test done in cool (13°C) conditions.

	Heat Acclimation Group N= 12	Control Group N= 8
VO_{2max} (L min^{-1})	4.47 ± 0.21 (3.00-5.51)	4.70 ± 0.14 (4.25-5.51)
VO_{2max} (ml kg^{-1} min^{-1})	66.85 ± 2.07 (57.01-76.09)	66.80 ± 1.65 (59.06-76.60)
Maximal power output (W)	369. 17 ± 14.54 (260-430)	381.25 ± 10.76 (340-420)
Maximal power output (W kg^{-1})	5.45 ± 0.21 (4.69-6.04)	5.43 ± 0.15 (4.99-5.86)

Table 5. Mean differences between day 1 and day 10 of the heat acclimation or exercise control period. Values shown are final heart rate and final core temperature at end of the second exercise bout, and changes in pre exercise resting plasma volume. Values are shown as mean ± standard error for 12 subjects in the heat acclimation group and 8 subjects in the control group. Range values are shown in parentheses [a] $P < 0.05$ vs. Day 1. [b] $P < 0.05$ vs. Control group.

	Heat acclimation Group		Control Group	
	Day 1	Day 10	Day 1	Day 10
Final heart rate (bpm)	164.6 ± 2.3 (153-174)	150.1 ± 2.6 (134-164)[a]	129.9 ± 3.0 (121-146)	126.5 ± 5.1 (117-155)
Final T_c (°C)	39.3 ± 0.1 (38.6-40.1)	38.8 ± 0.1 (38.2-39.3)[a]	38.1 ± 0.1 (37.8-38.5)	38.1 ± 0.1 (37.8-38.5)
ΔPV (%)	6.5 ± 1.2 (-5.40-17.34)[b]		-4.6 ± 2.7 (-13.62-9.27)	

Figure 6 shows heat acclimation effects on total work (in kilojoules) completed during the time trial. The experimental group showed significant increases in total work done in both the cool (879.8 ± 48.5 vs. 934.7 ± 50.9 kJ, P = 0.005) and hot conditions (718.7 ± 42.3 vs. 776.2 ± 50.9 kJ, P = 0.014). No significant changes were found in the control group in either environmental condition (897.1 ± 41.0 vs. 905.3 ± 49.48 kJ; 752.8 ± 43.2 vs.722.7 ± 43.6 kJ, respectively).

Figure 7 shows the individual and mean (±SE) time trial results (in kJ). Responses from the heat acclimation group in the cool (A) and hot (B) environments are shown in the top panels. Responses from the control group in the cool (C) and hot (D) environments are shown in the bottom panels. Due to equipment malfunction, data from two time trials (one in the HA hot and one in the control cool) were removed. Note that every subject increased total work done after heat acclimation except for one in each condition.

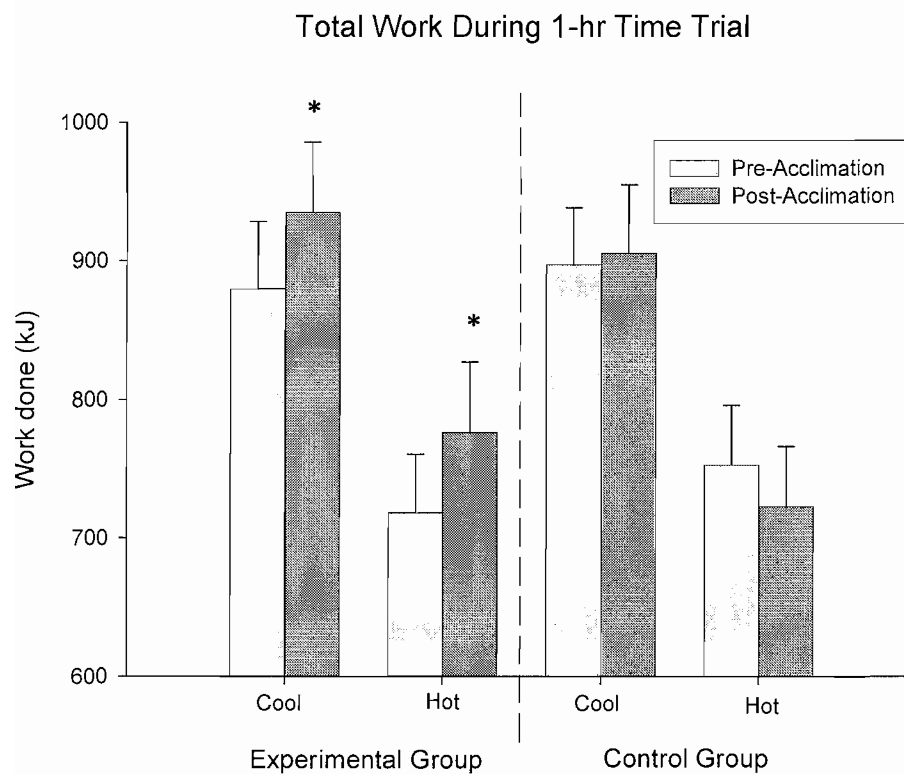


Figure 6. Effect of heat acclimation on time trial performance in kilojoules (kJ). Values shown are means ± SE.∗P < 0.05 vs. Pre-Acclimation within environmental condition.

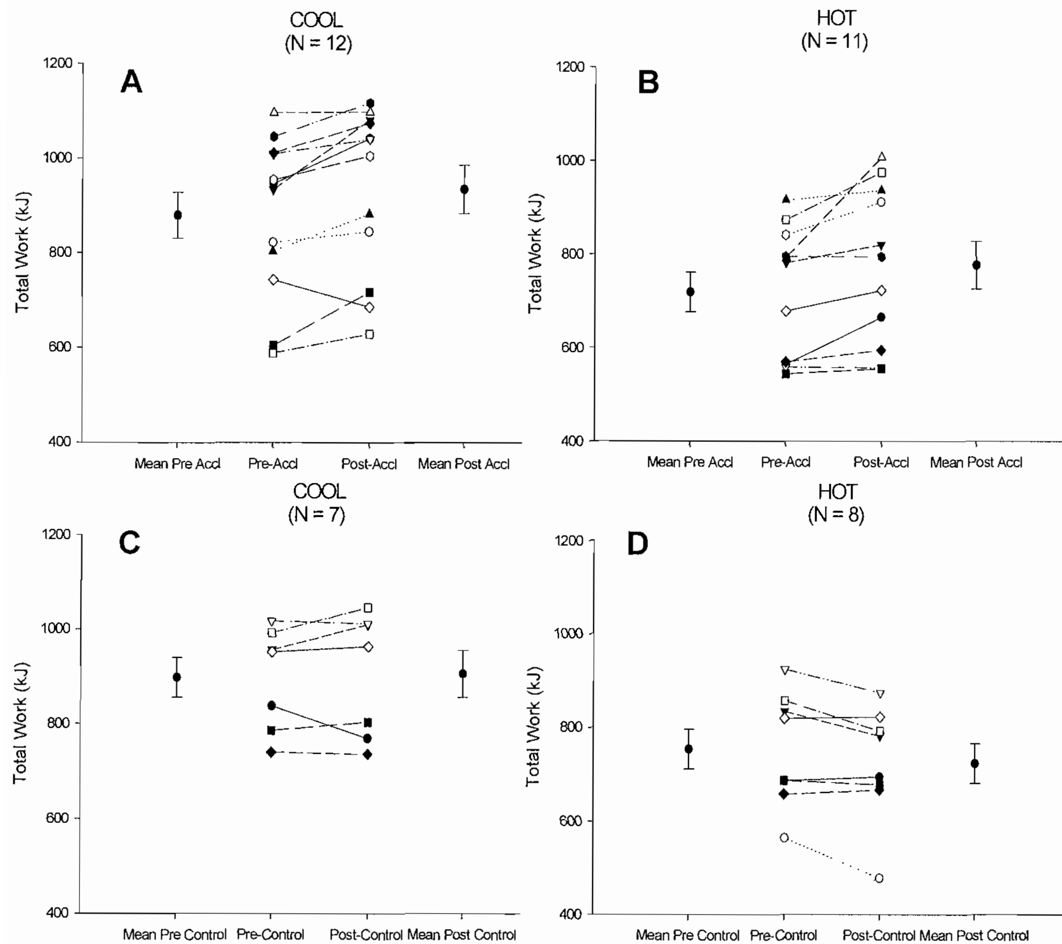


Figure 7. Individual and mean (±SE) time trial results (in kJ). Responses from the heat acclimation group in the cool (A) and hot (B) environments are shown in the top panels. Responses from the control group in the cool (C) and hot (D) environments are shown in the bottom panels.

Figure 8 shows the effect of heat acclimation on absolute power output and pacing strategy normalized to the average power output in 5-min time blocks in the heat acclimation group (circles) and control group (triangles). Responses from the cool trials are shown in the top panels. Responses from the hot trials are shown in the bottom panels.

Table 6 displays mean responses during the 1 hr time trial in the experimental group and control group before and after the heat acclimation or control period. All results are show as mean and standard error.

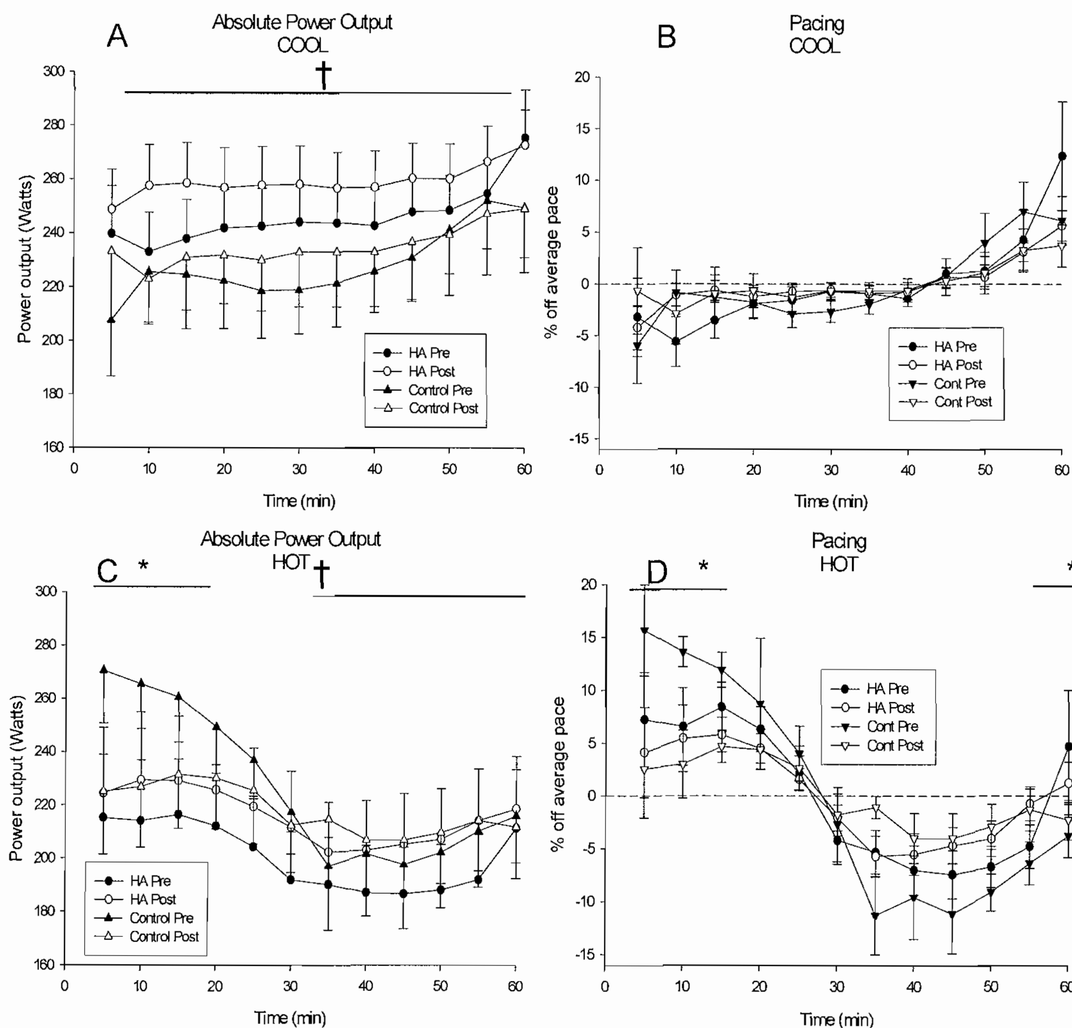


Figure 8. Effect of heat acclimation on absolute power output and pacing strategy normalized to the average power output in 5-min time blocks in the heat acclimation group (circles) and control group (triangles). Responses from the cool trials are shown in the top panels. Responses from the hot trials are shown in the bottom panels. Values shown are means ± SE. † Statistical difference ($P < 0.05$) between Pre and Post in the heat acclimation group. * Statistical difference ($P < 0.05$) between Pre and Post in the control group.

Table 6. Mean responses during the 1 hr time trial pre and post acclimation in the experimental and control groups. Values are mean± SE for 12 subjects in the experimental group and 8 subjects in the control group.* P < 0.05 vs. pre-acclimation trial

	Hot condition (38°C)		Cool condition (13°C)	
	Pre Accl.	*Post Accl.*	*Pre Accl.*	*Post Accl.*
Ending rectal temperature (°C)				
Experimental	39.5 ± 0.1	39.4 ± 0.2	38.8 ± 0.2	38.8 ± 0.1
Control	39.3 ± 0.2	38.9 ± 0.1*	38.9 ± 0.2	38.7 ± 0.2
Ending mean skin temperature (°C)				
Experimental	34.77 ± 0.28	33.87 ± 0.24*	24.13 ± 0.47	23.04 ± 0.26*
Control	34.72 ± 0.39	34.60 ± 0.23	23.69 ± 0.45	25.14 ± 0.68
Core-to-skin gradient (°C)				
Experimental	4.70 ± 0.38	5.53 ± 0.27*	14.66 ± 0.53	15.81 ± 0.33*
Control	4.55 ± 0.25	4.38 ± 0.16	15.03 ± 0.43	13.30 ± 0.64*
Skin blood flow (L min^{-1})				
Experimental	2.33 ± 0.22	2.33 ± 0.23	1.01 ± 0.07	1.03 ± 0.06
Control	3.00 ± 0.25	2.77 ± 0.24	1.08 ± 0.06	1.29 ± 0.08*
Ending mean body temperature (°C)				
Experimental	38.70 ± 0.14	38.29 ± 0.15	35.86 ± 0.14	35.68 ± 0.11
Control	38.50 ± 0.14	38.10 ± 0.11	35.89 ± 0.10	36.00 ± 0.13
Total body water loss (L)				
Experimental	1.77 ± 0.18	2.19 ± 0.20*	1.10 ± 0.10	1.34 ± 0.13*
Control	1.94 ± 0.18	2.02 ± 0.18	1.09 ± 0.10	1.05 ± 0.14
Mean Power Output (W)				
Experimental	200.8 ± 12.0	215.9 ± 14.1*	246.0 ± 13.3	259.2 ± 13.8*
Control	207.9 ± 12.0	201.4 ± 12.2	248.7 ± 11.6	253.6 ± 14.0
Mean blood lactate (Mmol)				
Experimental	3.0 ± 0.3	3.2 ± 0.2	4.2 ± 0.4	4.7 ± 0.5
Control	3.3 ± 0.4	2.3 ± 0.4	3.9 ± 0.3	3.6 ± 0.5
Mean cardiac output (L min^{-1})				
Experimental	20.1 ± 1.6	20.5 ± 1.4	22.3 ± 1.4	24.3 ± 1.5*
Control	22.6 ± 1.6	22.9 ± 1.8	25.0 ± 1.6	25.4 ± 1.9

Table 6 continued

	Hot condition (38°C)		Cool condition (13°C)	
	Pre Accl.	*Post Accl.*	*Pre Accl.*	*Post Accl.*
Mean VO_2 (L min^{-1})				
Experimental	2.79 ± 0.22	2.90 ± 0.23*	3.50 ± 0.20	3.78 ± 0.21*
Control	3.11 ± 0.16	2.97 ± 0.15	3.81 ± 0.19	3.73 ± 0.20
Mean rate of perceived exertion (RPE)				
Experimental	15.7 ± 0.2	15.6 ± 0.3	15.8 ± 0.3	15.7 ± 0.1
Control	15.6 ± 0.4	15.7 ± 0.3	15.2 ± 0.4	15.8 ± 0.4

Discussion

Our major finding is that heat acclimation improves time-trial performance in hot and cool environmental conditions. In addition, there were no changes in pacing strategy in those individuals who went through a period of heat acclimation in either environmental condition. In this group, overall performance was improved by self-selecting higher power outputs post-heat acclimation (see Fig. 8).

Although there are several reports documenting improvements in exercise capacity in the heat after a period of heat acclimation (Eichna *et al.*, 1945, Fox *et al.*, 1967, Nielsen *et al.*, 1993, Shvartz & Benor, 1971, Shvartz *et al.*, 1972, Stolwijk *et al.*, 1977), to our knowledge no attempts have been made to document the effect of heat acclimation on maximal cycling time trial performance in hot or cool ambient temperatures. This investigation employed a time trial approach to explore and quantify the

changes in performance of highly trained cyclists, which is a measure more specific to the demands of athletic competitions. Our observation that total work done in kilojoules is improved after a period of heat acclimation agrees with our hypothesis. Although the time trial method seems more appropriate when the main concerns are to investigate "real-life" time trial performance and changes in pacing strategy, one challenge is that it becomes difficult to compare specific physiological responses (i.e. cardiac output) due to the "self-selected power output" nature of this approach. Nevertheless, the observation that heat acclimation improves lactate threshold in the heat by about 6% (see Chapter IV of this dissertation), is well related to the approximate 8% improvement in total work performed in 1 hour during this study. Furthermore, lactate threshold in the cool environment was also improved approximate 6% post heat acclimation, while time trial was improved 6.5%.

Some investigators suggest that the ability to exercise in the heat is limited to the attainment of an upper limit in core temperature (Galloway & Maughan, 1997, Gonzalez-Alonso *et al.*, 1999, MacDougall *et al.*, 1974, Nielsen *et al.*, 1990, Nybo *et al.*, 2001, Walters *et al.*, 2000), and heat acclimation does not seem to alter this relationship (Nielsen *et al.*, 1993, Nielsen *et al.*, 1997). In contrast to this theory, a very recent study showed evidence against the attainment of a critical core temperature threshold for fatigue in humans (Ely *et al.*, 2009). In addition, novel research (Nielsen *et*

al., 2001, Nybo & Nielsen, 2001a) has demonstrated that arousal levels (a surrogate for motivation or “drive”) decrease progressively as hyperthermia develops, rather than simply failing after core temperature reached a critical limit (i.e. 40°C). Furthermore, arousal level (examined as changes in electroencephalographic brain signal) was strongly correlated with the increase in core temperature and the increase in the rating of perceived exertion (RPE) (Kayser *et al.*, 1994, Rasmussen *et al.*, 2004). Studies that employed constant exercise at a fixed work rate until exhaustion would mask the progressive reductions in motivation or arousal as hyperthermia develops, until the arousal/motivation declines to levels that cause subjects to terminate the test. Our investigation showed a strong correlation between core temperature and RPE (r =0 .95), which supports the theory that as body temperature increases, arousal or motivation is reduced. Furthermore, our observations also agree with the growing body of evidence for anticipatory regulation of exercise (Marino, 2004), which suggests that during exercise or competitions in which force output is selected by the athlete and is free to vary, motor command and voluntary activation are reduced incrementally as core temperature rises. Although our results support the concept of hyperthermia influencing self selection of power output, the mechanisms influencing the development of fatigue are much more complex and influenced by a delicate interplay between peripheral and central factors (Nybo, 2008) that need to be further explored.

Consistent with the idea that changes in body core temperature influences selected power output during self paced exercise, our results agree with a report from Tatterson *et al.* (2000), who had highly trained cyclists perform a 30 min time trial under hot (32°C) and thermoneutral (23°C) conditions and found that power output was selected in relation to changes in rectal temperature. Although rectal temperature was almost identical in both conditions, the reduction in power output in the hot trial compared to the thermoneutral environment was accompanied by reductions in blood lactate. Therefore, the authors suggested that muscle metabolic capacity was not limiting the time trial performance, instead, factors associated with thermoregulation was more influential on exercise performance (Tatterson *et al.*, 2000). In agreement with this study, we observed a reduction in power output and blood lactate levels in the hot environment compared to the cool condition (see table 6).

The difference in pacing between environmental conditions in our study agrees with a previous report in with strategy was not changed in a hot vs. thermoneutral environments, but the absolute power was decreased in the heat (Tatterson *et al.*, 2000). We further advance this knowledge by showing that heat acclimation did not alter the pacing strategy in either ambient condition. In other words, the pattern of selected power output pre and post-heat acclimation "paralleled" each other, with the post-heat acclimation tracing being shifted to higher power outputs (see Figure 8).

Subjects who were heat acclimated started out at a higher absolute power output, but relative to their average power output throughout the time trial their pace selection was not different form the pre acclimation trial. However, we did not expect the control group to drop power output the first 15 minutes in hot condition post-acclimation. Based on our measurements (i.e. lower heart rate and core temperature post-acclimation), we suspect that this is not physiological but from memory of past experience. The average power output in the heat pre and post-heat acclimation was approximately 201W and 216W, respectively, while in the cool condition the average power output was 246W pre and 259W post acclimation. This absolute difference in power output of 15W in the heat and 13W in the cool environment between acclimation states remarkably resembles the improvements in lactate threshold post acclimation of 11W and 14W, respectively (see Chapter IV).

Several observations from the present investigation may also suggest that the improved ability of the human body to thermoregulate after heat acclimation plays a major role in exercise performance. Core temperature was not statistically different throughout the entire time trial with respect to acclimation state in the experimental group. However, heat acclimation increased mean power output approximately 15 watts in both hot and cool environments ($P = 0.017$ and $P = 0.002$, respectively), which would indicate an increase in endogenous heat production during the post

heat acclimation time trial. Therefore, the observation that core temperature was not greater despite an increased metabolic heat production supports the theory that heat acclimation improves thermoregulatory responses, and consequently enhances exercise performance. The improved ability to dissipate heat after heat acclimation may be attributed to several factors. Onset of sweating has been shown to occur at lower core and skin temperatures after heat acclimation, as well as increased maximal sweat rates have been reported post heat acclimation (Henane & Valatx, 1973, Nadel *et al.*, 1974, Roberts *et al.*, 1977, Wyndham, 1967). Although we did not measure sweat rates directly, we estimated sweating capacity by calculating total body water loss, and we found that heat acclimation significantly increased total body sweat rates in both cool and hot conditions ($P = 0.003$ and $P = 0.001$, respectively). Moreover, skin blood flow is believed to be elevated at a given core (and skin) temperature after heat acclimation (Fox *et al.*, 1963b, Roberts *et al.*, 1977). This adaptation might permit the skin arterioles to dilate more to allow for a better heat transfer between the body and the environment (Wenger, 1988). Alternatively, the increase in evaporative cooling induced by enhanced sweating after heat acclimation might widen the core-to-skin temperature gradient for heat loss and allow for a lower skin blood flow, thus reducing cardiovascular strain. After heat acclimation we observed a significant decrease in the mean skin temperature at the end of the time trial in both environments, which resulted

in an increased core-to-skin temperature gradient because core temperature remained essentially unchanged (see table 6). In addition, although there was an increased metabolic heat production due to the higher power output post acclimation, estimated skin blood flow did not change. Therefore, the increased cardiac output observed after heat acclimation could be directed to other vascular beds (i.e. splanchnic or active muscles). Consequently, the elevated core-to-skin temperature gradient may reflect a heat acclimation adaptation to reduce cardiovascular strain to sustain thermal balance and improve exercise performance in highly trained cyclists.

Another alternative explanation for the increase in time trial performance in the heat acclimation group may come from the effects of heat acclimation on metabolism. Some studies suggested that heat acclimation induces metabolic adaptations during exercise by reducing the aerobic metabolic rate (Sawka *et al.*, 1983, Young *et al.*, 1985), or decreasing the rate of glycogenolysis (Febbraio *et al.*, 1994, Febbraio *et al.*, 1996, Kirwan *et al.*, 1987). Thus, at a given absolute workload there is a decrease in oxygen consumption post-heat acclimation. Consequently, these adaptations would allow subjects to maintain higher power outputs, reduce relative intensity and improve the time trial performance.

Methods to evaluate aerobic and anaerobic performance in athletes have been studied extensively, although there is only scarce literature with

regard to the reproducibility of endurance performance tests. Currently, no uniform laboratory endurance performance test exists, but a wide variety of different exercise protocols are used (Anantaraman *et al.*, 1995, Below *et al.*, 1995, Clark *et al.*, 2000, Coyle *et al.*, 1991, el-Sayed *et al.*, 1997, Hickey *et al.*, 1992, Jeukendrup *et al.*, 1996, Jeukendrup *et al.*, 1997, Krebs & Powers, 1989). Traditionally, these methodologies were based on submaximal performance rides at a fixed percentage of VO_{2max} or maximal power output (Galloway & Maughan, 1997, Hinckson & Hopkins, 2005, McLellan *et al.*, 1995, Nielsen *et al.*, 1993). An alternative performance test recently used involves a time trial in which either a fixed amount of work is performed as quickly as possible or as much work as possible is done in a set time (Carter *et al.*, 2004, Jeukendrup *et al.*, 1996, Tatterson *et al.*, 2000). There are advantages and disadvantages when using one method or the other. Some investigators suggested that the large error of measurement in “constant power or VO_2” tests (coefficient of variation up to ~ 30%) would disguise any changes in endurance performance of a few percentage points, which may be of great importance to elite athletes (McLellan *et al.*, 1995). The “time-trial” method, on the other hand, has many characteristics that may be more attractive to assess performance in competitive cyclists. Although done in a research laboratory, this test better resembles a “real life” time trial competition than the constant power approach and can be used to assess pacing strategies as well.

Furthermore, published research reported that time trials are highly reproducible and have a lower coefficient of variation (~1-3%) (Jeukendrup *et al.*, 1996), which would unmask any changes in performance of a few percentage points.

We observed a slight reduction in plasma volume in the control group. To ensure the subjects were properly hydrated, nude body weight and plasma osmolality were measured. Euhydrated subjects were considered if their nude body mass was within 1% of their 5-day average and plasma osmolality <290mOsmol kg^{-1} H_2O (Sawka *et al.*, 2007). Therefore, the possibility that subjects in the control group were dehydrated was eliminated. This plasma volume reduction could be explained by the fact that this group exercised in a cool environment (13°C). One of the most recognized effects of cold exposure is an increase in urine output, or cold-induced diuresis, resulting in an iso-osmotic reduction in plasma volume. One study have shown that the increased systemic and renal blood pressure associated with cold-induced vasoconstriction may increase filtration and reduce reabsorption of water and solutes by the kidneys (Freund & Young, 1996). Another possibility for the plasma volume reduction in the control group could be related to a slight detraining and/or de-acclimatization. Although all subjects were encouraged to continue their normal training routine during the entire study, the amount of time devoted to the study made it difficult for the subject to continue with their normal

rides outside the lab. Therefore, the total volume and intensity of training during these periods might have been lower than normal, which may account for the slight plasma volume reduction.

Limitations

With most of the heat acclimation protocols that involve exercise there is a chance that the changes observed post heat acclimation could be due to a training effect (Sawka *et al.*, 1985, Young *et al.*, 1985). In addition, there could also be the possibility that the multiple time trials performed during this investigation had some “learning effect” that allowed subjects to improve their time trial performance, regardless of the heat acclimation status. We believe this is not the case in our study due to several reasons. First, the combination of low exercise intensity during the heat acclimation process (50% of their VO_{2max}), plus the subjects’ high fitness level (mean VO_{2max} of 66 ml kg^{-1} min^{-1}) make the training effect unlikely (Pollock, 1973). The “learning effect” would also be unlikely because all the subjects who participated on this study were well trained and had previous experiences performing similar time trials competitions. Also, the higher power output selected post-heat acclimation at the beginning of the time trial would have dropped quickly if not physiologically supported. In addition, we did not observe significant changes in the control group, who exercised at the

same exercise intensity as the experimental group and performed the same amount of time trials.

The data collection was carried between the months of February and August so there is a possibility that some subjects may be naturally acclimatized during the early months of summer. It's important to note that due to their heavy training routines, research has shown that highly trained cyclists are already "partially" heat acclimatized, even during the winter months (Gisolfi & Robinson, 1969). Therefore, this possibility cannot be discarded. Although cyclists were encouraged to continue their normal training during the duration of the entire study, they were not allowed to train on hot days or at the peak heat on any given day. In addition, the warmest months in the city of Eugene are July and August with an average high temperature of less than 82°F, and the degree of heat stress that subjects were exposed to in the study was well above what they would typically be exposed to during a training session outside during that time. In addition, we tested subjects in the "control group" over the same time period as the experimental group throughout the data collection period and we failed to see any differences. Moreover, we did not observe any differences in responses between the subjects studied during the winter and those studied during the summer. In any case, any possible "partial acclimation" from the subjects would underestimate potentially larger differences in the experimental group post acclimation.

In summary, data from the present investigation demonstrate that heat acclimation improves exercise performance during a 1 hour self paced time trial in hot and also in cool environments. In addition, we found to be no difference in the pacing strategy post acclimation in either condition.

CHAPTER VI

HEAT ACCLIMATION INDUCES PERIPHERAL MODIFICATIONS IN CUTANEOUS VASCULAR FUNCTION IN HUMANS

Introduction

Increased skin blood flow and sweating are the two most important thermoregulatory responses of humans to an increase in core temperature. It is well established that individuals who undergo a period of chronic heat exposure (i.e. heat acclimation) have improved ability to thermoregulate, especially while exercising in a hot environment (Eichna *et al.*, 1950, Nielsen *et al.*, 1993, Roberts *et al.*, 1977, Rowell *et al.*, 1967). An increased ability of the cardiovascular system to perfuse the skin microcirculation, together with enhanced evaporative cooling due to higher sweat rates at a given core temperature, may widen the core-to-skin thermal gradient (Eichna *et al.*, 1950, Rowell *et al.*, 1967) and allow heat dissipation from the body core to the environment. Although there has been some research on the effects of heat acclimation on skin blood flow, the findings on the specific mechanism by which skin blood flow is increased after heat acclimation remain inconsistent (Roberts *et al.*, 1977, Takeno *et al.*, 2001,

Yamazaki & Hamasaki, 2003). For example, Roberts *et al.* (1977) reported that a 10-day period of heat acclimation lowered the internal temperature threshold for cutaneous vasodilation, without a significant change in the slope of the relations. The authors postulated that these changes were caused by a central mechanism. Conversely, other studies showed that heat acclimation increases the slope of the relation of forearm vascular conductance or sweat rate to internal temperature during exercise in the heat (Sawka *et al.*, 1989, Takeno *et al.*, 2001). Differences in the heat acclimation protocols (ambient temperature, intensity and duration of exercise) and the type of heat test (i.e. rest or during exercise) can account for such inconsistencies. However, in all these studies the changes in cutaneous blood flow were induced by an increased internal temperature. Therefore, it remains uncertain if these changes are centrally mediated or if there are local structural changes occurring within the cutaneous vasculature. More specifically, skin blood flow may be augmented by an increased ability of the skin vessels to vasodilate (i.e., increased maximal skin blood flow), or there may be an improved vasodilatory response (i.e., increased sensitivity) for a specific local stimulus.

Due to the improvements in cardiovascular and thermoregulatory function that follow a period of heat acclimation, sweat rate is higher at a given exercise intensity or core temperature (Fox *et al.*, 1963b, Nielsen *et al.*, 1993, Senay *et al.*, 1976, Wyndham *et al.*, 1976). The current thinking is

that this is a predominantly centrally mediated response (Colin & Houdas, 1965, Kuno, 1956, Nadel *et al.*, 1974, Roberts *et al.*, 1977, Shvartz *et al.*, 1979, Wyndham *et al.*, 1976). Roberts *et al.* (1977) showed that heat acclimation increased sweat rate by lowering the internal temperature threshold for sweating, and also by increased slope of the sweat rate: internal temperature relationship. A very well designed study by Chen & Elizondo (1974) showed evidence that the increased sweat output following heat acclimation is due primarily to an increased sweating capacity of the sweat gland apparatus. In other words, there might be some underlying adaptations that can modify sweating independent of a central drive. A few studies have observed an increased sweat rate during exogenous administration of sudorific agents (methacholine or acetylcholine) after heat acclimation in humans (Collins *et al.*, 1966, Inoue *et al.*, 1999). Interestingly, it has also been demonstrated that if local skin temperature is maintained at a cool temperature throughout the heat acclimation period, sweat responses at that location were not modified by heat acclimation (Chen & Elizondo, 1974, Fox *et al.*, 1964). The authors suggested that increased sweat rate observed after a period of heat acclimation was due to physiological changes at the level of the sweat gland apparatus. Therefore, improved peripheral sweat gland function following heat acclimation can be a result of: 1) increased periglandular concentrations of acetylcholine, 2)

increased cholinergic sensitivity of the eccrine sweat gland, or 3) increased glandular hypertrophy (Sato & Sato, 1983).

Therefore, our aim was to further investigate if the skin blood flow and sweating adaptations that follow a period of heat acclimation were peripheral. We accomplished this by locally stimulating the skin with specific concentrations of the endothelium-dependent vasodilator acetylcholine infused via microdialysis and measured the skin blood flow responses and sweating, thus eliminating any central stimulation (i.e. exercise or increased core temperature). Also, we assessed the skin blood flow response to a standard local heating protocol (Holowatz *et al.*, 2005, Kellogg *et al.*, 1999, McCord & Minson, 2005). Finally, we measured maximal skin blood flow by two methods: 1) by infusing endothelium-independent vasodilator sodium nitroprusside and measured skin blood flow via laser-Doppler flowmetry; 2) by locally heating the forearm with a warm water spray device and measure brachial artery blood flow via Doppler ultrasound technique. We hypothesized that, to a given acetylcholine concentration, skin blood flow and sweat rates will be higher after heat acclimation; and that the local heating response and absolute maximal skin blood flow will remain unchanged.

Methods

Study design

This manuscript is part of a larger project that investigated effects of heat acclimation on performance and also other physiological variables in endurance trained cyclists. In brief, participants were put through a battery of physiological and performance tests under two environmental conditions, then put through a heat acclimation or an exercising control program, and then the tests were repeated. The heat acclimation protocol consisted of 10 exposures of cycling exercise at a temperature of 40°C and 30% relative humidity (WBGT = 35°C). Subjects performed two bouts of 45 minutes at 50% of their VO_{2max} with 10 minutes of rest in between. A control group exercised at the same intensity but with the chamber set at 13°C and 30% RH (WBGT = 12°C).

Subjects

A total of sixteen subjects (13 men, 3 women) were used for these set of studies. Twelve highly trained endurance cyclists (10 men, 2 women), age 24 ± 6 (SD) completed the heat acclimation protocol (height 175 ± 6 cm, weight 67.7 ± 8.1 kg, body mass index 22.1 ± 3.9 kg m^{-2}). Eight subjects (7 men, 1 woman), age 26 ± 4 completed the control protocol (height 174 ± 6 cm, weight 70.2 ± 4.1 kg, body mass index 23.1 ± 3.1 kg m^{-2}). Of the sixteen total subjects used for these set of studies, four men

age 28 ± 5 performed the control protocol followed by the heat acclimation exposures and experimental tests (height 176 ± 4 cm, weight 73.1 ± 1.5 kg, body mass index 23.5 ± 2.8 kg m^{-2}). A complete description of the subject groups is presented in table 7.

Subjects monitoring

On each study visit, subjects reported to the laboratory after a 2-hour fast, and well hydrated. Studies were performed in an air-conditioned laboratory (22-24°C) with the subjects in a supine position and the experimental arm extended at the right side at heart level. Subjects were instructed to avoid consumption of alcohol or caffeine for at least 8 to 12 hours prior to the study. In addition, they were not allowed to exercise on the same day prior to the study and were told to avoid ingestion of non-prescription drugs for the entire duration of the multiple study visits. Blood pressure was monitored continuously throughout the entire experiment (Cardiocap, Datex Ohmeda). In order to rule out changes in red blood cell (RBC) flux due to pressure changes, subject's blood pressures were measured via auscultation (in the left arm) every 5-7 minutes throughout the entire protocol.

Skin blood flow and sweat rate measurements

As an index of skin blood flow (SkBF), RBC flux was measured by using non-invasive laser-Doppler flowmetry (moorLab, Moor Instruments,

Devon, UK). Two probes were used in conjunction with 2 sweat rate capsules, to continuously monitor RBC flux at each site. In addition, 2 probes were combined with local skin heating devices and placed on the forearm to investigate skin blood flow responses to a local skin heating protocol.

Sweat rates were quantitatively measured by the resistance hygrometry technique (Bullard, 1962). In brief, dry nitrogen was supplied to the sweat capsules (0.5 cm^2 area) at a fixed rate of 0.2 L min^{-1}. The humidity of the air flowing out of the capsules was measured with capacitance hygrometers (model HMP230, Vaisala, Helsinki, Finland). Sweat rate was calculated based on relative humidity, air temperature and airflow.

Specific protocol

Two microdialysis fibers (MD 2000, Bioanalytical Systems) with a membrane length of 10 mm and a 20-kDa membrane cutoff were placed at least 5 cm apart in the forearm skin of the right arm of the subjects. Placement of the microdialysis fibers was achieved by inserting a 25-gauge needle through the skin with entry and exit points ~2.5 cm apart. The microdialysis fiber was then threaded though the lumen of the needle. The needle was withdrawn from the skin, leaving the microdialysis membrane in place.

After the needle insertion, a period of 90-120 minutes allowed the trauma response to resolve. During this time, the microdialysis fibers were continuously perfused with Ringer solution at a rate of 2.0 μl min^{-1}. Following, integrated laser-Doppler probes and sweat rate capsules were placed directly over the microdialysis membranes to continuously measure RBC flux and sweat rate. Both sites were monitored continuously until a stable 10-min baseline was recorded before the first acetylcholine concentration infusion. Subjects then received perfusate containing 1.0, 10, and 100 mM of acetylcholine dissolved in Ringer solution. The concentrations used were determined based on previous research done in human skin utilizing microdialysis delivery of this agonist (Medow *et al.*, 2008, Stewart *et al.*, 2007). Each infusion lasted for a minimum of 20 minutes, or until there was a clear plateau in the skin blood flow and sweat rate recordings. Finally, maximal RBC flux was achieved by infusing 28 mM sodium nitroprusside (SNP; Nitropress, Ciba Pharmaceuticals) known to result in maximal dilation of skin sites (Kellogg *et al.*, 1998).

The local skin heating devices were turned on and held constant at 33°C for 10 min during baseline data collection. After the baseline period, the temperature of the local heaters was increased at a rate of 0.5°C every 5 seconds to a temperature of 42°C. This rate of local heating does not result in any pain sensation (Minson *et al.*, 2001). The local heaters were held constant at 42°C until skin blood flow reached a stable 10-minute

plateau. The temperature of the local heaters was then raised to 43.5°C to elicit maximal cutaneous vasodilation.

During part of the study, the subjects' left forearm was locally heated in a cylindrical water spray device that sprayed heated water from jets encircling the suspended forearm (Taylor *et al.*, 1984). At the same time, brachial artery diameters and blood velocity were measured using a Doppler ultrasound (Terason™, Burlington, MA) to calculate brachial artery blood flow. The forearm was heated for 45 minutes and measurements were taken for 2 minutes before forearm heating (baseline), and at 13, 28 and 43 minutes. During each measurement, blood flow to the hand was occluded with a blood pressure cuff placed around the wrist distal to the spray device to prevent the hand circulation from being included in the calculations of brachial blood flow.

Data analysis

Data were digitized and saved on a computer at 40Hz using Windaq data acquisition software (Dataq Instruments, Akron, OH). Data were analyzed off-line using signal-processing software. RBC flux values from the laser-Doppler units were divided by mean arterial pressure (MAP) to yield a value of cutaneous vascular conductance (RBC flux ÷MAP = CVC). RBC flux values were then calibrated to 100% during maximal blood flow (SNP infusion). Expression of data in this manner takes into account any

changes in blood flow due to changes in blood pressure and also better reflects changes in SkBF. Thus, data are presented as a percentage of maximal CVC (%CVC_{max}). Because of the transient nature of the initial peak, a 5- to 10-s period of skin blood flow was used for analysis. For the plateau during local heating and drug infusions, a stable 5- to 7-min period of skin blood flow was used for subsequent analyses. Sweat rate was calculated based on relative humidity, air temperature, skin surface area, and airflow and are expressed as mg cm^{-2} min^{-1}.

Data from each protocol were compared between pre and post acclimation trials by determining specific differences using a paired Student's *t*-tests and significance was set at $P < 0.05$, and values are presented as mean and standard error (mean ± SE), unless otherwise indicated.

Results

Table 7 shows specific physiological characteristics of the control and heat acclimation groups. Although the control group showed a slightly higher absolute VO_{2max} and maximal power output, no statistical differences were found between the experimental and control groups ($P > 0.05$). We suspect that these differences were due to the number of female subjects (2 women in the experimental group and 1 woman in the control group). In addition, the mean body weight in the control group was elevated compared

to the experimental group (70.2 ± 4.1 vs. 67.7 ± 8.1 kg, respectively). These slight differences disappeared when values were expressed relative to body mass.

Figure 9 shows heat acclimation effects on cutaneous vascular responses to specific concentrations of acetylcholine. The experimental group showed significant increases in the cutaneous vascular responses to 1, 10, and 100 mM of acetylcholine (43.53 ± 3.44 vs. 52.56 ± 2.59 $\%CVC_{max}$, 67.75 ± 3.44 vs. 78.05 ± 3.06 $\%CVC_{max}$, 80.99 ± 3.76 vs. 88.45 ± 1.05 $\%CVC_{max}$, respectively; all $P < 0.05$). No significant changes were found in the control group in sweat rate responses to all concentrations of acetylcholine (40.50 ± 5.61 vs. 45.67 ± 6.88 $\%CVC_{max}$; 65.28 ± 2.82 vs. 67.70 ± 5.55 % CVC_{max}; 83.17 ± 2.03 vs. 80.42 ± 1.85 $\%CVC_{max}$).

Figure 10 shows heat acclimation effects on local sweat rate responses to specific concentrations of acetylcholine. The experimental group showed significant increases in sweating responses to 1, 10, and 100 mM of acetylcholine (0.13 ± 0.02 vs. 0.18 ± 0.02 mg cm^{-2} min^{-1}, 0.21 ± 0.03 vs. 0.31 ± 0.03 mg cm^{-2} min^{-1}, 0.45 ± 0.05 vs. 0.67 ± 0.06 mg cm^{-2} min^{-1}, respectively; all $P < 0.05$). No significant changes were found in the control group in sweat rate responses to the same concentrations of acetylcholine (0.13 ± 0.02 vs. 0.14 ± 0.02 mg cm^{-2} min^{-1}; 0.18 ± 0.03 vs. 0.20 ± 0.04 mg cm^{-2} min^{-1}; 0.42 ± 0.08 vs. 0.45 ± 0.08 mg cm^{-2} min^{-1}).

Table 7. Physiological characteristics of the heat acclimation and control groups. Values are shown as mean ± standard error for 12 subjects in the experimental group and 8 subjects in the control group. Range values are shown in parentheses. Reported values of maximal oxygen consumption (VO_{2max}) and maximal power output were from VO_{2max} test done in cool (13°C) conditions.

	Heat Acclimation Group N= 12	Control Group N= 8
VO_{2max} (L min^{-1})	4.47 ± 0.21 (3.00-5.51)	4.70 ± 0.14 (4.25-5.51)
VO_{2max} (ml kg^{-1} min^{-1})	66.85 ± 2.07 (57.01-76.09)	66.80 ± 1.65 (59.06-76.60)
Maximal power output (W)	369. 17 ± 14.54 (260-430)	381.25 ± 10.76 (340-420)
Maximal power output (W kg^{-1})	5.45 ± 0.21 (4.69-6.04)	5.43 ± 0.15 (4.99-5.86)

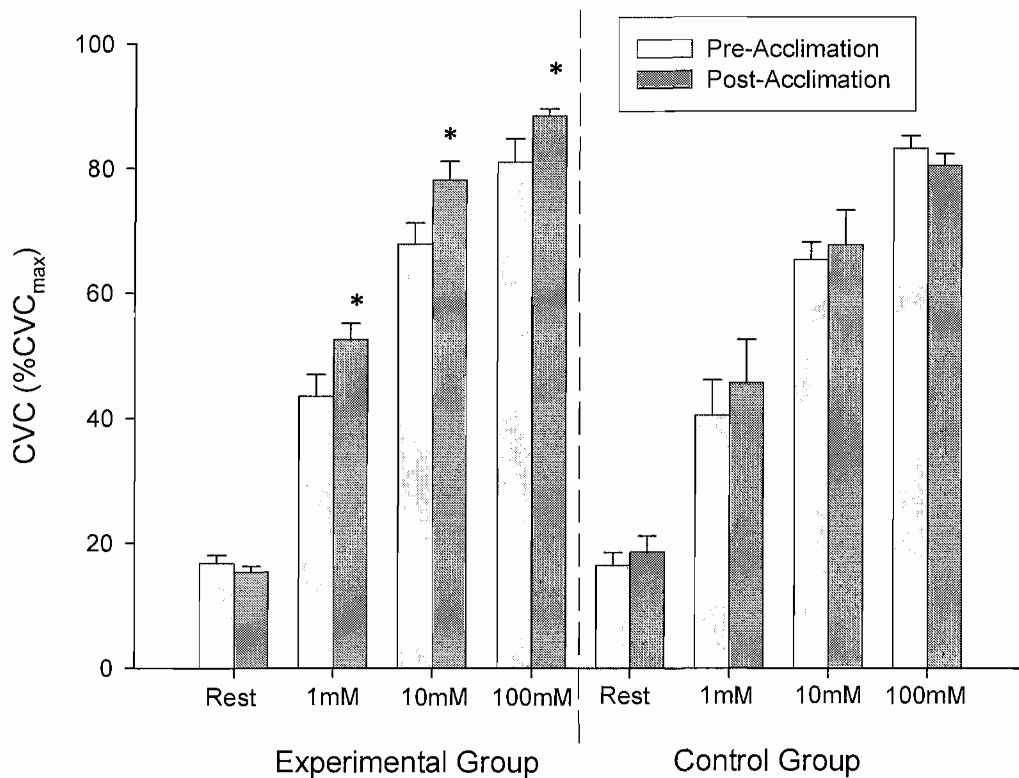


Figure 9. Effect of heat acclimation on cutaneous vascular conductance in response to specific concentrations of acetylcholine. Values are means ± SE for 12 experimental subjects and 8 controls.∗P < 0.05 vs. Pre-Acclimation within concentration.

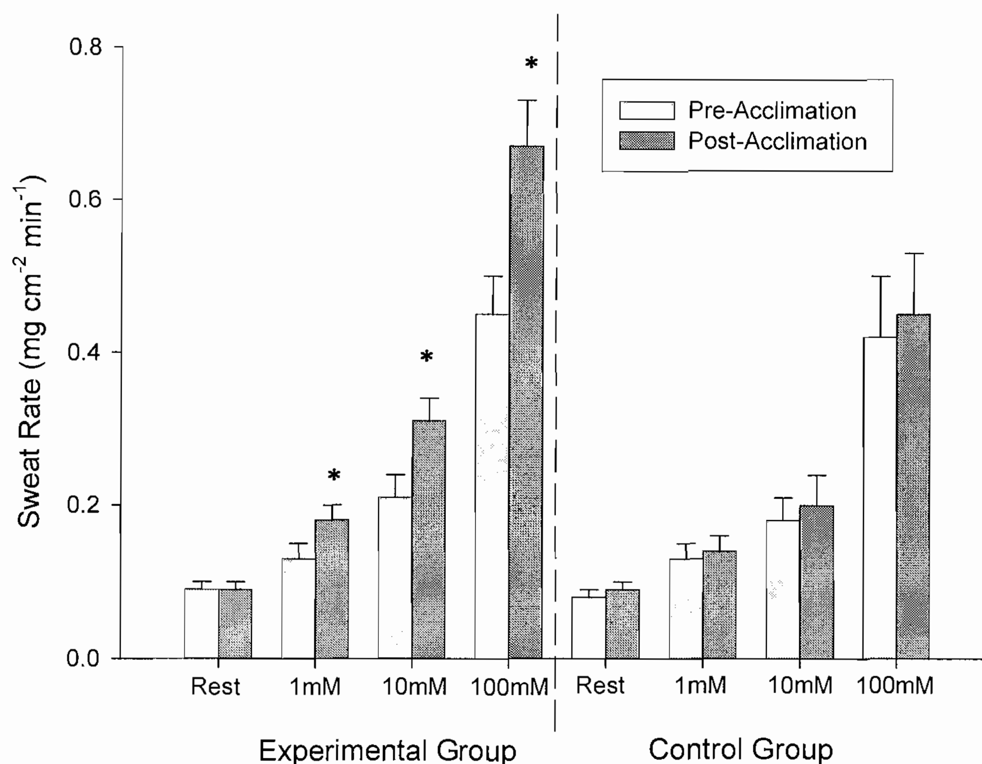


Figure 10. Effect of heat acclimation on sweat rate responses to specific concentrations of acetylcholine. Values are means ± SE for 12 experimental subjects and 8 controls.*P < 0.05 vs. Pre-Acclimation within concentration.

Table 8 shows heat acclimation effects on vascular responses during skin local heating and forearm heating protocols. All results are show as mean and standard error. There were no significant changes in any of the variables in the control or experimental group.

Table 8. Vascular responses from skin local heating protocol (initial peak, plateau and maximal skin blood flow), and from forearm heating protocol (brachial blood flow). Values are shown as mean ± standard error for 12 subjects in the experimental group and 8 subjects in the control group. Range values are shown in parentheses. There were no significant changes in any of the variables in the control or experimental group.

	Experimental Group		Control Group	
	Pre-Acclimation	Post-Acclimation	Pre-Acclimation	Post-Acclimation
Local heating initial peak (%CVC_{max})	66.36 ± 2.14 (49.35-75.90)	68.17 ± 2.45 (54.25-81.14)	68.71 ± 1.99 (61.75-74.25)	66.60 ± 2.72 (51.31-73.49)
Local heating plateau (%CVC_{max})	79.64 ± 2.02 (64.29-90.36)	80.94 ± 1.73 (72.37-96.52)	77.93 ± 2.33 (68.24-89.12)	77.83 ± 1.74 (70.87-87.41)
Maximal skin blood flow (RBC flux)	286 ± 24 (131-464)	302 ± 15 (222-393)	310 ± 24 (198-397)	308 ± 16 246-383)
Brachial artery blood flow ml min^{-1}	290.9 ± 12.7 (226.5-329.4)	269.9 ± 23.6 (211.0-407.1)	301.5 ± 31.0 (195.0-474.6)	291.9 ± 14.2 (238.7-362.3)

Discussion

To our knowledge, this is the first study that used microdialysis technique to investigate potential peripheral adaptations in the thermoregulatory responses (i.e. skin blood flow and sweating) after a period of heat acclimation in highly trained athletes. The major findings of this study are as follows. First, local adaptations within the skin microcirculation and sweat gland apparatus play major roles in the increased thermoregulatory responses induced by heat acclimation in highly trained athletes. Second, absolute maximal skin blood flow

(estimated from maximal brachial artery blood flow) does not appear to change following a period of heat acclimation.

Much of the research performed on heat acclimation effects on skin blood flow responses are conflicting. For example, there is some evidence that the increased skin blood flow observed after a period of heat acclimation are caused by a central mechanism (Fox *et al.*, 1963b, Roberts *et al.*, 1977, Yamazaki & Hamasaki, 2003). One study observed that there was a reduction on the internal temperature threshold for forearm vasodilation, without any changes in the slope of the forearm blood flow:internal temperature relation (Roberts *et al.*, 1977). Another study showed that threshold temperatures for vasodilation were reduced after heat acclimation (Yamazaki & Hamasaki, 2003). Therefore, it is speculated that heat acclimation modifies thermoregulatory responses in the skin by central mechanisms. These studies, however, used increases in internal temperature to elicit skin vasodilation, therefore any potential peripheral adaptations cannot be excluded. In addition, skin blood flow was estimated from forearm blood flow measurements via venous occlusion plethysmography. By using the microdialysis technique we were able to administer fixed concentrations of an endothelium dependent agonist (acetylcholine) in a small area of the skin, adequately stimulating muscarinic receptors. In addition, with laser-Doppler flowmetry we were able to directly measure changes in blood flow that occurs only within the

skin microcirculation. Therefore, the utilization of these combined methodologies and expression of values as $\%CVC_{max}$, a more consistent comparison between subjects and drug concentrations was achieved. On the basis of this, our results agreed with our hypotheses and provide evidence that heat acclimation significantly increased the blood flow to the skin when stimulated with acetylcholine, suggesting that there are local adaptations within the skin microcirculation.

Although the specific pathways leading to cutaneous vasodilation in response to increases in body temperature remain enigmatic, this mechanism is believed to be effected by a cholinergic co-transmitter system, with acetylcholine contributing up to 20% of the vasodilation and some other substance(s), co-released from cholinergic terminals, responsible for the rest (Kellogg *et al.*, 1995). In addition, studies have shown that nitric oxide may contribute up to 40-50% of active vasodilation (Kellogg *et al.*, 1998, Shastry *et al.*, 1998, Wilkins *et al.*, 2003), while there is another study that suggests that prostanoids also play a role in active cutaneous vasodilation (McCord *et al.*, 2006). Potential co-transmitters that are believed to contribute to the cutaneous active vasodilation are vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP), and substance P (Bennett *et al.*, 2003, Morris *et al.*, 2001, Wong & Minson, 2006, Wong *et al.*, 2005). Recently Kellogg *et al.* demonstrated that much of active cutaneous vasodilation may be due to nNOS (Kellogg *et al.*, 2008).

One study showed that in vitro stimulation of vasodilator nerves in animal models have caused the release of neuropeptides, notably VIP and CGRP (Morris *et al.*, 2001). In addition, another study provided evidence in support of a role for VIP in active cutaneous vasodilation (Bennett *et al.*, 2003), although another study was not able to verify these findings (Wilkins *et al.*, 2005). Co-transmitter systems are believed to have redundancies where a lack of one neurotransmitter can be compensated for by another (Bartfai *et al.*, 1988, Lundberg *et al.*, 1982) and whether this occurs in the cutaneous vasodilator system remains unknown. More studies investigating the effects of heat acclimation on these specific mechanisms are warranted.

Our observations on the control of skin blood flow following heat acclimation are consistent with other reports (Fox *et al.*, 1963b, Roberts *et al.*, 1977, Yamazaki & Hamasaki, 2003), and we have extended their results by demonstrating that there is increased sensitivity in the cutaneous vascular conductance to a local stimulus. That this study used local stimulation and therefore no central mechanism was activated, supports the theory that peripheral adaptations to the cutaneous circulation play a role in the enhanced skin blood flow observed after heat acclimation. It is generally agreed that acetylcholine mediates increases in skin blood flow by activating muscarinic receptors on endothelial cells of cutaneous blood vessels, inducing skin vasodilation via multiple potential pathways including nitric oxide (Holowatz *et al.*, 2005, Kellogg *et al.*, 2005, Medow *et al.*, 2008),

prostaglandins (Holowatz *et al.*, 2005, Kellogg *et al.*, 2005, Medow *et al.*, 2008), and endothelium-derived hyperpolarizing factor (EDHF) mechanisms (Palmer *et al.*, 1987). One study showed evidence that acetylcholine mediated vasodilation involves cholinergic-muscarinic receptor activation of nitric oxide and prostaglandins by endothelial cells (Kellogg *et al.*, 2005). However, the possibility for EDHF vasodilatory role could not be excluded. Therefore, the increase vasodilation to acetylcholine infusions observed post-heat acclimation may be caused by up-regulating some of these pathways via exercise or heat stress (or both). Future studies investigating the effects of heat acclimation on the specific nitric oxide, prostaglandins and EDHF pathways leading to cutaneous vasodilation are warranted.

To our knowledge, there has not been published research on the effect of heat acclimation on maximal skin blood flow in highly trained cyclists. Previous studies have shown that locally heating the forearm to 42°C with a warm water spray device for 35-45 minutes successfully achieves maximal skin vasodilation (Martin *et al.*, 1995, Taylor *et al.*, 1984). In addition, increases in local skin temperature to 43.5°C failed to further increase skin blood flow, suggesting that maximal skin blood flow was achieved at 42°C. Our data from the brachial artery ultrasound suggest that the maximal ability of the skin vessels to vasodilate is not altered with heat acclimation. In addition, the maximal RBC flux values also remained unchanged post heat acclimation (see Table 8). Together, these

observations provide evidence that heat acclimation does not alter maximal skin blood flow. Instead, the increase sensitivity of the skin microvasculature to dilate in response to fixed acetylcholine doses play a role in the augmented skin blood flow observed post heat acclimation.

Observations from previous research focused on the effects of heat acclimation on sweating responses are also conflicting. Differences in the methodology for estimation of sweat rate, heat acclimation protocol used, and fitness level of the subject may explain some of the inconsistencies reported. Research has shown that heat acclimation lowers the internal temperature threshold for sweating (Nadel *et al.*, 1974, Roberts *et al.*, 1977, Sawka *et al.*, 1989, Yamazaki & Hamasaki, 2003), suggesting a role for central mechanisms. There are several studies that propose that heat acclimation induces thermoregulatory changes at the level of the sweat gland (Buono *et al.*, 2009, Chen & Elizondo, 1974, Collins *et al.*, 1966, Inoue *et al.*, 1999, Sato *et al.*, 1990), although there are some methodological concerns in a few of these studies. Chen & Elizondo (1974) compared electrically stimulated sweat rate forearm sweat production before and after 9 days of heat acclimation and showed evidence that the increased sweat output following heat acclimation is due primarily to an increased sweating capacity of the sweat gland apparatus. However, only 4 subjects completed the protocol and there were no statistical analyses on the data. Also, the electrical current used to elicit sweating was different

between subjects and ranged between 1.0 and 1.6 mA. In addition, others investigators have observed increased sweating during exogenous administration of sudorific agents (i.e. methacholine, acetylcholine, or pilocarpine) after heat acclimation (Buono *et al.*, 2009, Collins *et al.*, 1966, Inoue *et al.*, 1999, Sato *et al.*, 1990), but the methodologies used during these studies also raise some concerns. For example, the photographic method used to estimate sweat gland output does not provide an accurate quantitative value (Inoue *et al.*, 1999); large doses of tranquilizer needed to sedate the animals (Sato *et al.*, 1990); and uncertainty of fitness level of subjects (Buono *et al.*, 2009) limit their results' applicability to highly trained athletes.

Our results showed further evidence that a period of heat acclimation increased sweat rate to acetylcholine doses, suggesting increased cholinergic sensitivity of the eccrine sweat gland, or increased glandular hypertrophy (Sato & Sato, 1983). However, our study cannot exclude the possibility that a central mechanism may also play a role in affecting sweat rate after heat acclimation. On the basis of this, some studies provided evidence that both, core and skin temperatures are necessary to alter sweat rates post-heat acclimation. Local cooling of the forearm during the heat acclimation exposures prevented any significant increases in sweating whereas the area on the contralateral control arm demonstrated a marked increase after heat acclimation (Chen & Elizondo, 1974, Fox *et al.*, 1964).

Moreover, they reported that local training of the sweat gland by repeated local heating of the skin to approximately 41°C failed to induce any significant increase in thermal sweating (Chen & Elizondo, 1974). Together, these reports and our results suggest that a central stimulus (i.e. increased core temperature) and a peripheral stimulus (i.e. increase skin temperature) need to be present in order to maximize sweat rate adaptations during heat acclimation.

With most of the heat acclimation protocols that involve exercise there is a chance that the changes observed post heat acclimation could be due to a training effect (Sawka *et al.*, 1985, Young *et al.*, 1985). We believe this is not the case in our study due to several reasons. The combination of low exercise intensity during the heat acclimation process (50% of their VO_{2max}), plus the subjects' high fitness level (mean VO_{2max} of 66 ml kg^{-1} min^{-1}) make the training effect unlikely (Pollock, 1973). In addition, we did not observe significant changes in the control group, who exercised at the same exercise intensity as the experimental group.

Our results provide evidence in support of a role for peripheral adaptations in the thermoregulatory apparatus in response to heat acclimation. By using multiple acetylcholine doses administered by microdialysis we were able to stimulate specific areas of the skin, avoiding activation of a central mechanism. Thus, our study suggests that heat

acclimation per se improves thermoregulatory function in part via peripheral mechanisms.

CHAPTER VII

CONCLUSIONS

The human body has a high degree of plasticity. For thousands of years, humans have developed specific adaptations that allowed them to survive almost anywhere in the world. In fact, the human body's ability to adapt to thermal stress has tremendous potential. The "heat acclimation" phenomenon has been studied since the beginning of the 20th century and its applications are potentially endless. For instance, heat acclimation improves working capacity of miners, helps older individuals to cope better in hot environments, and makes athletes more heat tolerant so they can improve performance when they have to compete under hot environmental conditions. Many of the heat acclimation adaptations are well documented and include increased ability to thermoregulate via improved sweat rates and skin blood flow, and enhanced cardiovascular support in part due to plasma volume expansion. These adaptations have been linked to improvements in performance under hot environments in highly trained individuals but the effects of heat acclimation on performance under cool conditions have not been investigated, until now.

The main questions addressed in this dissertation were: 1) can we use heat acclimation to improve exercise performance? and 2) are there peripheral adaptations in the thermoregulatory system following a period of heat acclimation? The short answer to these questions is *YES*. We observed that heat acclimation improved performance under both environmental conditions when compared to the control group. We also found that heat acclimation induced functional thermoregulatory adaptations that take place within the skin that allow for an increased sweat rate and skin blood flow. Importantly, we used a control group that went through the same testing procedures but instead of exercising in the heat during the heat acclimation exposures, they exercised at the same intensity but in a cool condition. We did not observe these changes in the control group, which allowed us to determine that the changes observed in the heat acclimation group were due to the heat exposure and not a training adaptation. In addition, the combination of low exercise intensity during the heat acclimation process (50% of their VO_{2max}), plus the subjects' high fitness level (mean VO_{2max} of 66 ml kg^{-1} min^{-1}) make any adaptation due to training very unlikely.

Although we observed consistent improvements in performance post-heat acclimation, the specific mechanisms remain unknown. Unfortunately, careful examination of the relationships between individual responses failed to give us any further insight in determining mechanisms

of induction (see appendix A for detailed analyses). Thus, the improvements in performance observed post heat acclimation could be potentially explained by the interaction of several key adaptations, including plasma volume expansion, maximal cardiac performance, increased active muscle blood flow and oxygen delivery. Coyle *et. al.* (1990) showed that increases in plasma volume similar to the degree observed in this dissertation significantly increased VO_{2max} (Coyle *et al.*, 1990). Furthermore, the authors measured an increased cardiac output after plasma volume expansion at submaximal exercise intensities. Therefore, the potential for plasma volume expansion to increase VO_{2max} might depend on the tight balance between the extent to which maximal cardiac output is increased compared to the reduction in hemoglobin concentration and thus, arterial oxygen content. Similar to Coyle's findings, we observed a moderate increase in plasma volume (6.5%) with a small degree of hemodilution (3.3%), which resulted in a 9% increase in the maximal cardiac output and a VO_{2max} increase of 5%. In addition to plasma volume expansion, the increased cardiac function observed post heat acclimation may also be explained by animal studies which showed that heat acclimation induces a number of mechanical and metabolic adaptations in the rat heart (Horowitz *et al.*, 1986a, Horowitz *et al.*, 1986b, Horowitz *et al.*, 1993, Levy *et al.*, 1997). For instance, heat acclimation increased left ventricular compliance and pressure generation and decreased myocardial oxygen consumption

(Horowitz *et al.*, 1986b, Horowitz *et al.*, 1993). Another study observed that heat acclimation increased cardiac contractility in rats and this augmented force generation is associated with elevation of cytosolic calcium concentration on contraction (Levy *et al.*, 1997). These observations suggest that heat acclimation may enhance mechanical adaptations and improve metabolic efficiency of the heart, which could lead to improvements in cardiac function in highly trained individuals.

The increased cardiac performance observed in this dissertation could increase active muscle blood flow, which may lead to potential increases in oxygen delivery and maximal oxygen uptake. One study showed that a small increase in plasma volume (and thus total blood volume) increased exercise VO_2 in dogs (Sarelius & Sinclair, 1981). The authors suggested that the increased central blood volume induced by hypervolemia would cause an increase cardiac output, increased oxygen delivery, and increased perfusion of skeletal muscle. In addition, augmented central blood volume has been considered to have permissive function in the regulation of cardiac function (Rowell *et al.*, 1966, Sjostrand, 1953, Thauer, 1962), leading to increases in stroke volume, cardiac output and potentially leg blood flow.

Key observations from Chapter V and Chapter VI may also be able to help decipher the possible mechanisms that result in improved performance after heat acclimation. Results from Chapter V showed

significant decreases in mean skin temperature post heat acclimation, which resulted in an increased core-to-skin temperature gradient because core temperature remained essentially unchanged (see table 6 in Chapter V). Therefore, the increased core-to-skin temperature gradient caused a reduction in the estimated skin blood flow requirements to achieve thermal balance (Sawka & Young, 2006) so the increased cardiac output observed after heat acclimation (see table 6 in Chapter V) could be directed to other vascular beds (i.e. splanchnic or active muscles). Consequently, the elevated core-to-skin temperature gradient may reflect a heat acclimation adaptation to reduce cardiovascular strain to sustain thermal balance and improve exercise performance in highly trained cyclists. The decrease in mean skin temperature observed post heat acclimation could be explained from our observations in Chapter VI. Our findings from this chapter may suggest that the improved evaporative cooling due to the increased sweat rates post heat acclimation could be responsible for the lower skin temperatures, which would reduce skin blood flow requirements to achieve thermal balance.

In Chapter VI we also found that heat acclimation increased skin blood flow response to a given acetylcholine concentration but the response to local heating remained unchanged. It is generally agreed that acetylcholine mediates increases in skin blood flow via multiple potential pathways including nitric oxide (Holowatz *et al.*, 2005, Kellogg *et al.*, 2005,

Medow *et al.*, 2008), prostanoids (Holowatz *et al.*, 2005, Kellogg *et al.*, 2005, Medow *et al.*, 2008), and endothelium-derived hyperpolarizing factor (EDHF) mechanisms (Palmer *et al.*, 1987). The skin blood flow response to local heating has been shown to be mediated mostly by nitric oxide (Kellogg *et al.*, 1999, Minson *et al.*, 2001). On the other hand, there is evidence against roles for either prostanoids (Gooding *et al.*, 2006, McCord *et al.*, 2006) or histamine (via H_1 receptors) (Gooding *et al.*, 2006, Wong *et al.*, 2006) in the skin vasodilation in response to local heating. Taken together, it seems the augmented skin blood flow response to acetylcholine after heat acclimation may be due to upregulation of the COX pathway leading to the production of prostanoids, or by an enhanced EDHF mechanism.

Figure 11 summarizes the potential mechanisms by which heat acclimation adaptations might enhance performance by effects on the cardiovascular and thermoregulatory systems.

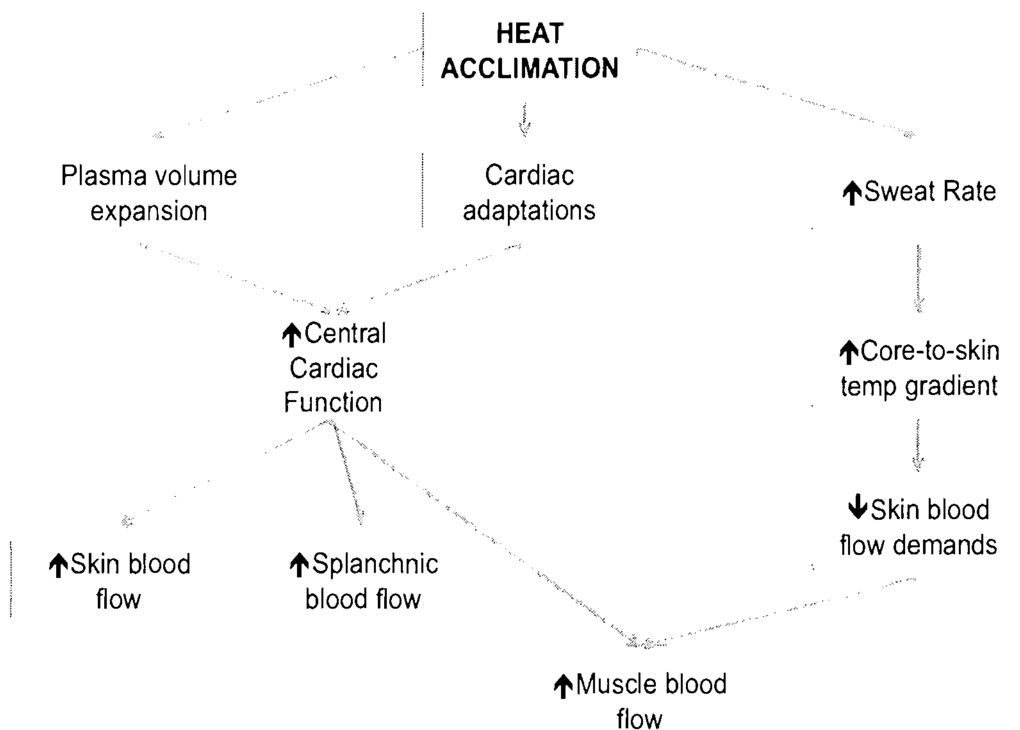


Figure 11. Possible mechanisms through which heat acclimation enhances performance by effects on the cardiovascular and thermoregulatory systems.

Implications and future directions

This being one of the first set of studies demonstrating performance and physiological effects of heat acclimation on cool temperature exercise, many questions arise. We found consistent improvements across all performance variables, but the specific mechanisms remain to be elucidated. Our results combined with previous studies may suggest that the plasma volume expansion and increased cardiac contractility after heat acclimation may increase central cardiac function, which could improve

active muscle blood flow and oxygen delivery. Therefore, studies investigating heat acclimation effect on the dynamics of central cardiac function, active muscle blood flow, and oxygen delivery during whole body dynamic exercise is warranted. In other words, the specific mechanisms by which heat acclimation improves performance may result from a combination of increases in central cardiac function (observed on this dissertation) and improvements in the leg blood flow and oxygen delivery.

The decreased mean skin temperatures and subsequent increase in the core-to-skin temperature gradient observed post acclimation during the time trial and the effects on skin blood flow may play a fundamental role in the mechanism/s of increased exercise performance. As previously discussed, the improved cardiac function and reduced requirements for skin blood flow may allow for a greater cardiac output to be directed to active muscle or to the splanchnic circulation. Therefore, the role of skin blood flow and skin temperatures must be investigated to discern the interaction between the thermoregulatory and cardiovascular systems during maximal efforts after heat acclimation. In addition, the possible contributions of prostanoids and EDHF need to be further explored to elucidate the specific pathway that augments skin blood flow post heat acclimation.

Our experimental model has some similarities with the theory of “live high- train low” developed by Levine & Stray-Gundersen. These authors found that competitive athletes who lived at moderate altitude but continued

their regular training at a low altitude improved their performance when they competed at sea level (Levine & Stray-Gundersen, 1997). The authors suggested that the most important “live high” adaptation that would improve sea-level performance is an increase in the red blood cell mass and oxygen-carrying capacity. Furthermore, the “train low” allowed the cyclists to maintain their running velocities, oxygen delivery and overall fitness levels. They also found that at submaximal running speeds the increase in oxygen-carrying capacity allowed a lower cardiac output and therefore more peripheral diffusion time and oxygen extraction (increased a-vO_2 difference), as well as providing for additional cardiac flow reserve. Similar to Levine & Stray-Gundersen’s findings, the results from our project showed that a period of low intensity exercise in a hot environment (i.e. heat acclimation), plus regular training at a non-heat stress condition, improves cool weather performance in well-trained cyclists. More specifically, the cyclists who participated on this project and supplemented their regular training with a low-intensity exercise under heat stress, showed consistent improvements in athletic performance under cool environmental conditions. We also found improved central cardiac and thermoregulatory function, which would increase cardiovascular support during maximal efforts. In summary, the heat acclimation portion and the regular training done by the subjects in our study would symbolize the “live high” and “train low” phases of Levine & Stray-Gundersen’s approach, respectively. The runners who

underwent the "live high-train low" protocol improved their VO_{2max} by approximately 5%, which is the same magnitude of increased VO_{2max} in the cool condition for those cyclists who went through the heat acclimation protocol in our study. In addition, the "live high-train low" runners improved their 5000km time by approximately 13 seconds (1.5%) while the "heat acclimation" cyclists improve their 1 hour time trial performance by ~6%.

To our knowledge, this is the first study that measured similar improvements in both, aerobic power and time-trial performance post heat acclimation. The fact that the improvements were comparable in magnitude but not highly related shows the importance of this dual approach to determine aerobic capacity and exercise performance. Therefore, both of these tests may need to be considered to measure a physiological variable (i.e. VO_{2max}) and a performance outcome (i.e. time trial). Furthermore, this study directly measured heat acclimation effects on athletic performance *per se* rather than assess physiological strain during a given task (i.e. exercise for 100 minutes or until exhaustion).

As mentioned earlier, this study is the first to delineate the impact of heat acclimation on improving maximal performance in temperate conditions. These findings have direct implications on many fronts. For instance, athletes and military personnel could employ heat stress to optimize improvements from their regular physical training programs. In addition, heat acclimation supplementation could be considered to aid

competitive athletes to improve their performance beyond using traditional training approaches. In perspective, a 5% change in performance for a highly trained cyclist could make the difference between a wining a gold medal or not. For instance, the 2009 Tour de France the winner of the 40km time trial was Alberto Contador with a time of 48min 30sec, edging Fabian Cancellara by only 3 seconds. A 5% increase in Contador's time trial result would have put him outside the top 25 finishers.

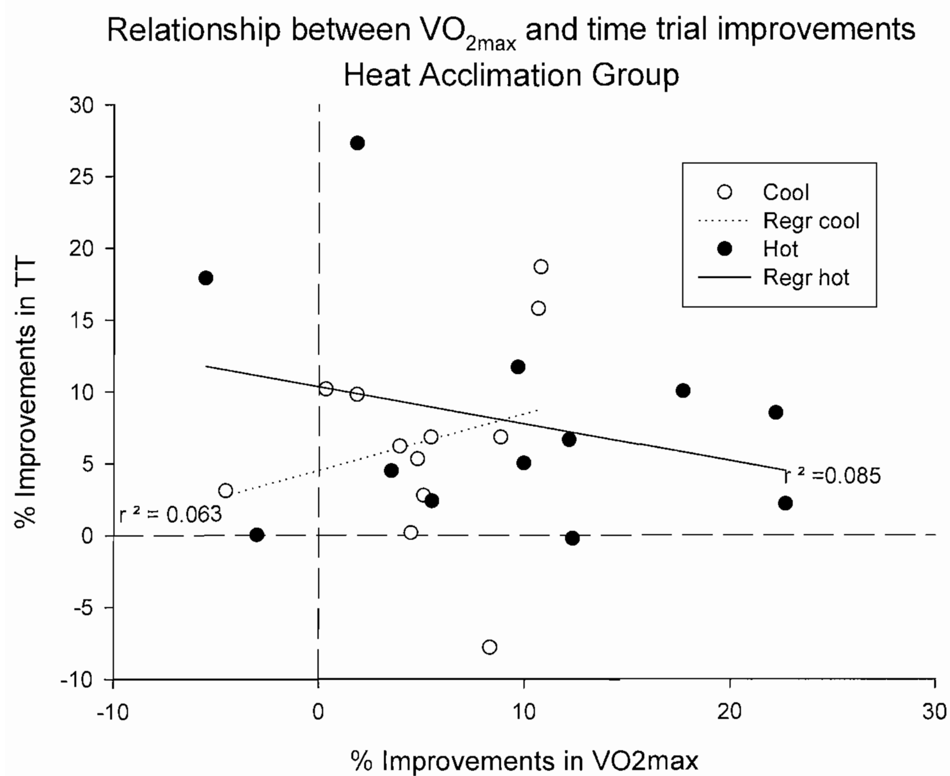
The observation that heat acclimation improved central cardiac function in temperate conditions could have clinical implications as well. Traditionally, the thermal load associated with training in the heat decreases the capacity to do work so it is generally recommended to train in cool conditions so individuals can sustain higher metabolic rate and get more fitness improvements. However, this study showed that supplementing the regular workout routines with low intensity heat training can induce improvements in cardiac output. Therefore, this may have an application for cardiac failure patients. Heart failure is a common, costly, disabling and eventually deadly condition that may affect up to 10% of people over 65 (Dickstein *et al.*, 2008). This occurs most commonly when the cardiac output is low (often termed "congestive heart failure"). Patients with cardiac failure could exercise at lower intensities in the heat and improve central cardiac function. Based on our findings and others, these improvements may come from benefits to systolic function (i.e contractility

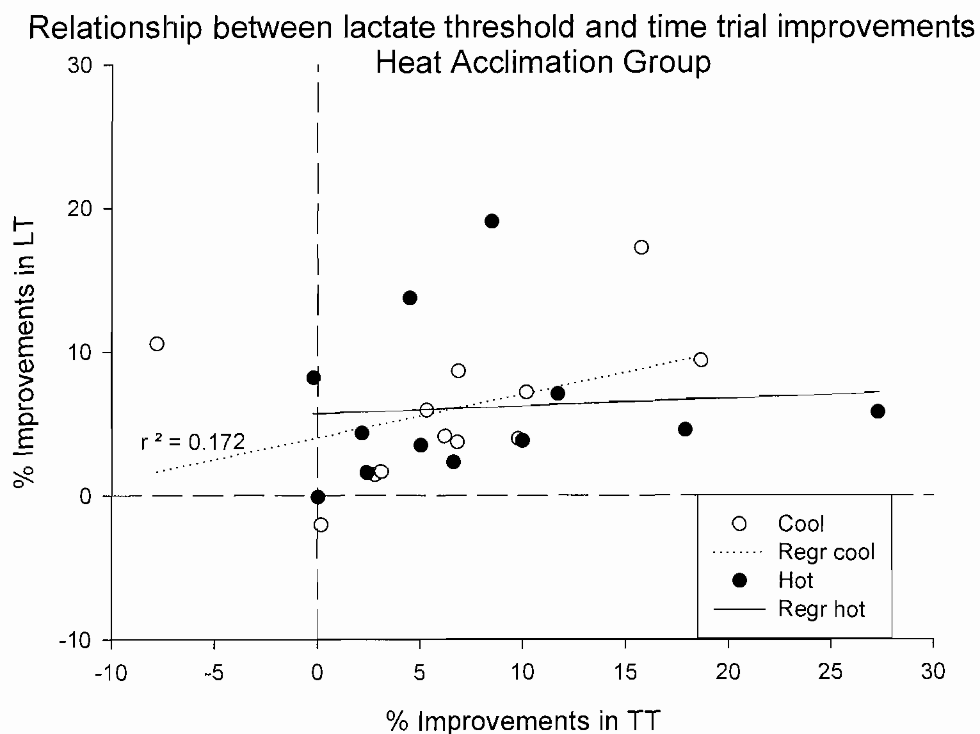
and stroke volume) or diastolic function (i.e. relaxation and compliance). More specific research on this area remains to be done before this idea could be implemented.

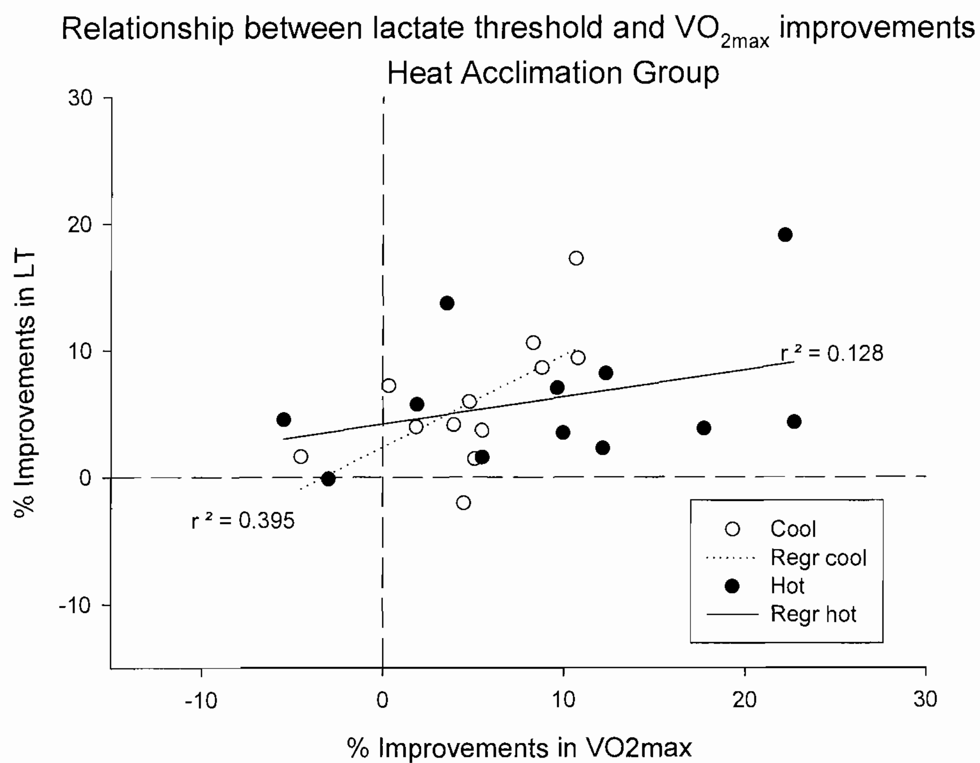
In summary, a novel finding from this dissertation is that heat acclimation consistently enhanced performance in highly trained cyclists. We believe that this concept will have great impact in the field of exercise performance at the elite level.

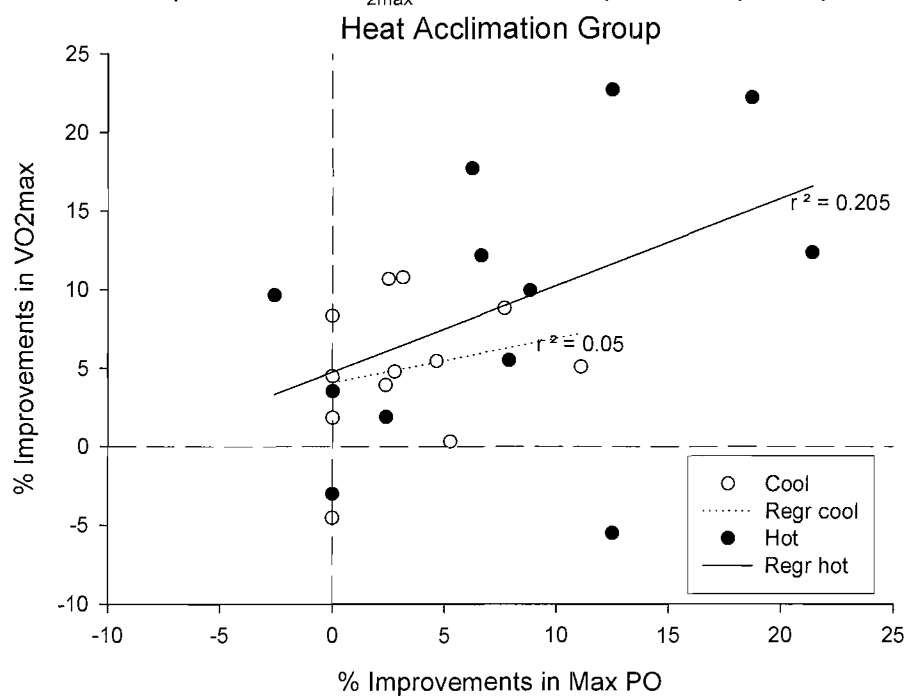
APPENDIX A

INDIVIDUAL DATA FROM SUBJECTS SHOWING RELATIONSHIPS BETWEEN PHYSIOLOGICAL RESPONSES AND PERFORMANCE VARIABLES

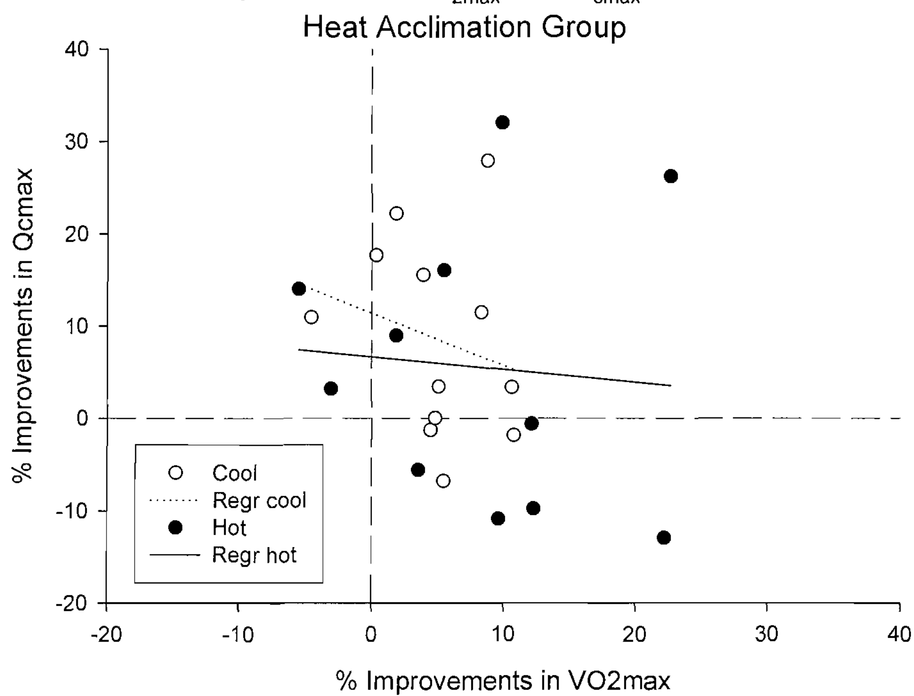



Relationship between lactate threshold and time trial improvements
Heat Acclimation Group
% Improvements in LT
% Improvements in TT
r ² = 0.172
Cool
Regr cool
Hot
Regr hot
30
20
10
0
-10
-10
-5
0
5
10
15
20
25
30

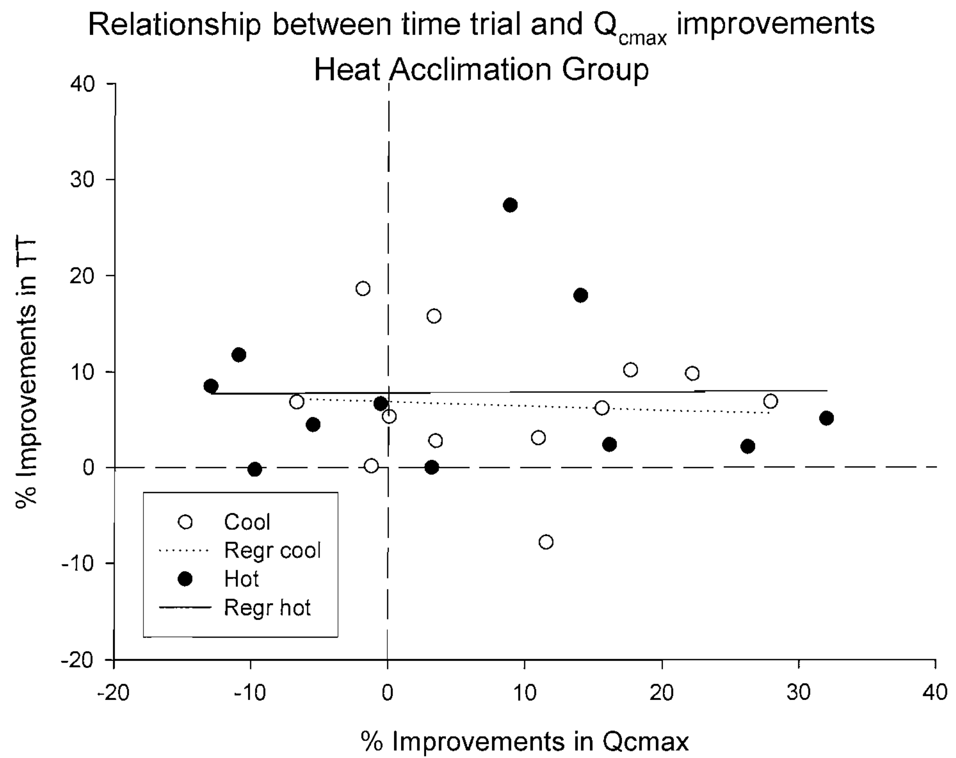

Relationship between lactate threshold and VO2max improvements
Heat Acclimation Group
% Improvements in LT
% Improvements in VO2max
r ² = 0.128
r ² = 0.395
Cool
Regr cool
Hot
Regr hot
30
20
10
0
-10
-10
0
10
20
30



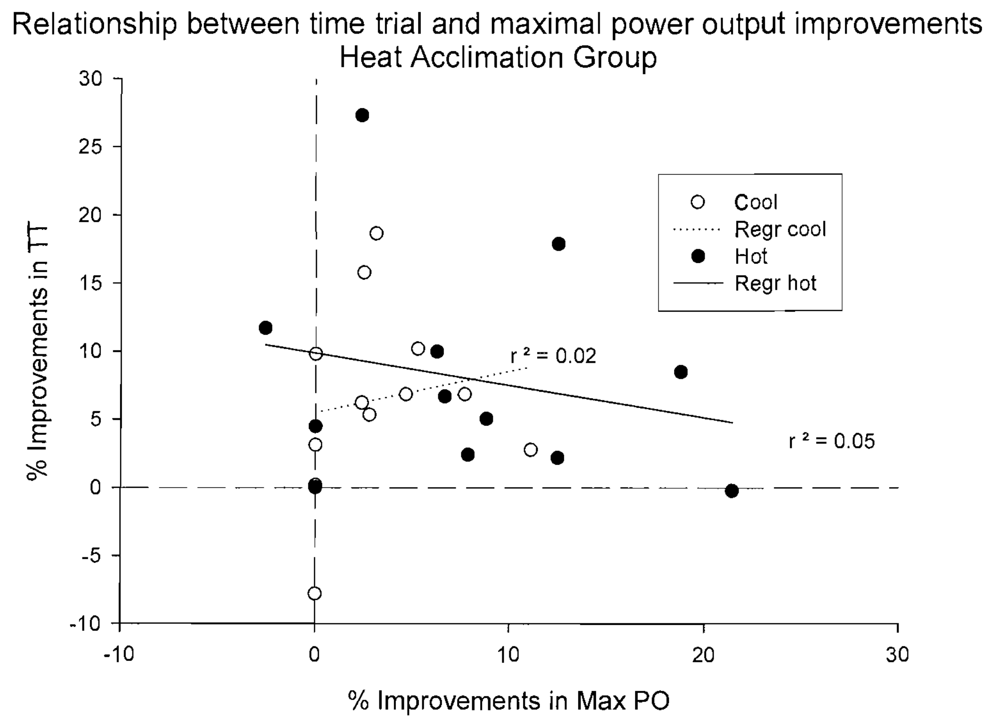
Relationship between VO2max and maximal power output improvements
Heat Acclimation Group
% Improvements in VO2max
% Improvements in Max PO
r² = 0.205
r² = 0.05
Cool
Regr cool
Hot
Regr hot



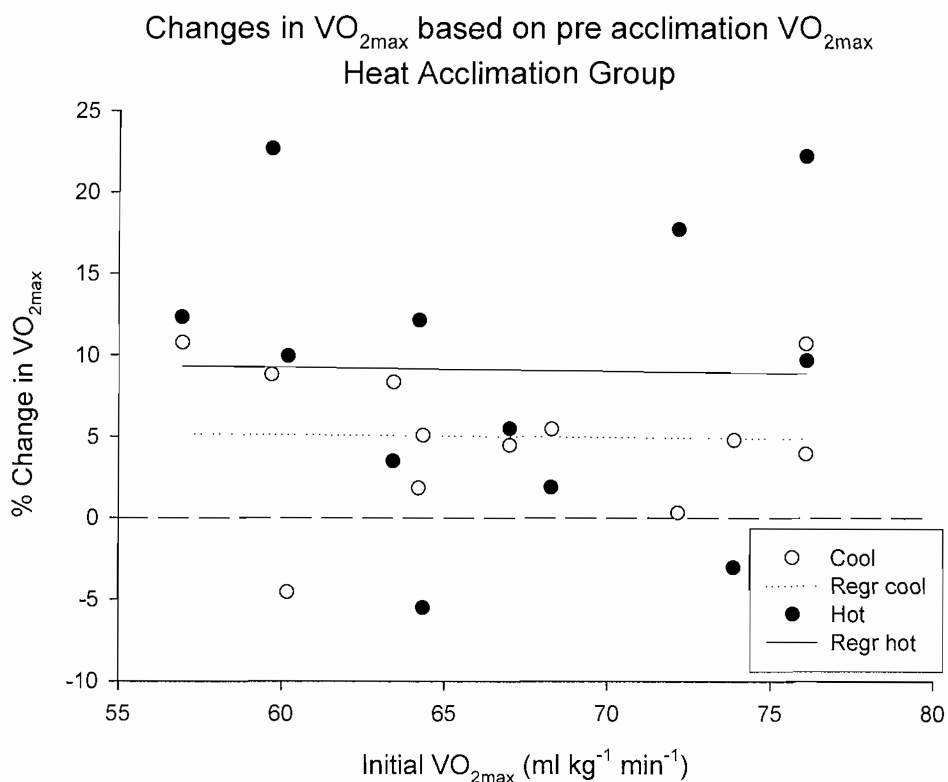
Relationship between VO22max and Qcmax improvements
Heat Acclimation Group
% Improvements in Qcmax
% Improvements in VO2max
Cool
Regr cool
Hot
Regr hot

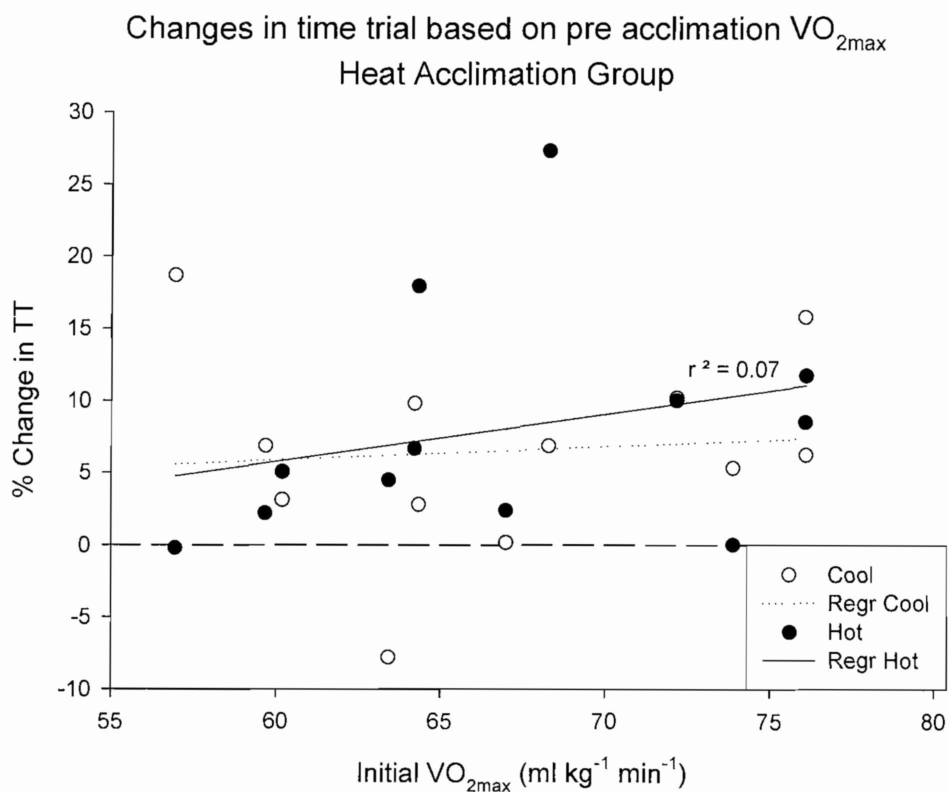


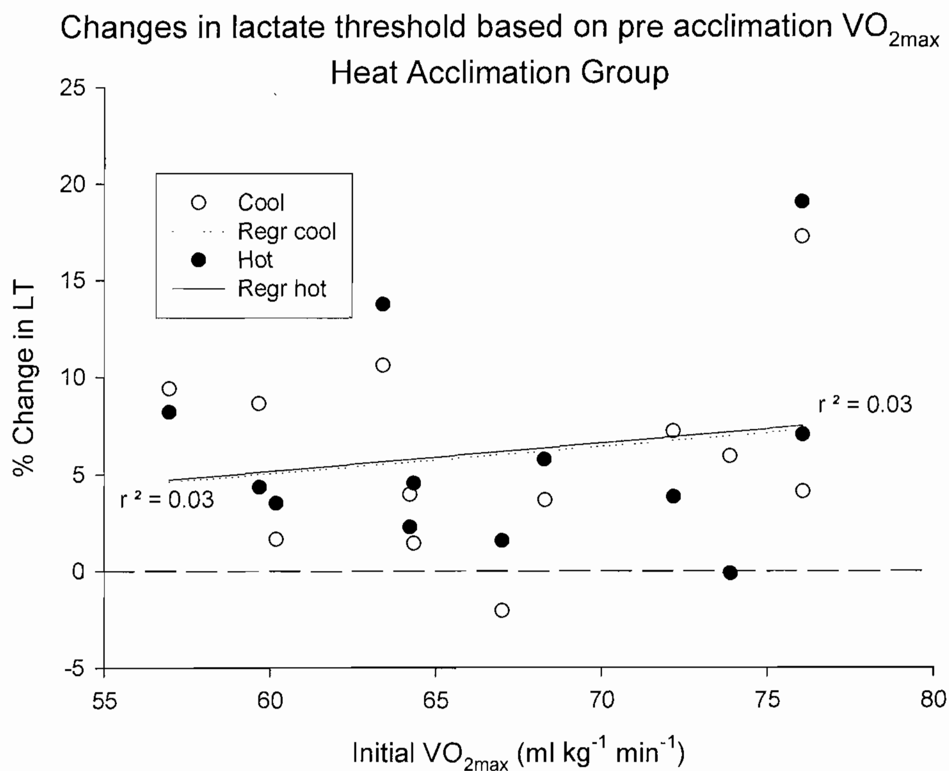
Relationship between time trial and Q_{cmax} improvements
Heat Acclimation Group
% Improvements in TT
% Improvements in Qcmax
Cool
Regr cool
Hot
Regr hot
40
30
20
10
0
-10
-20
-20
-10
0
10
20
30
40

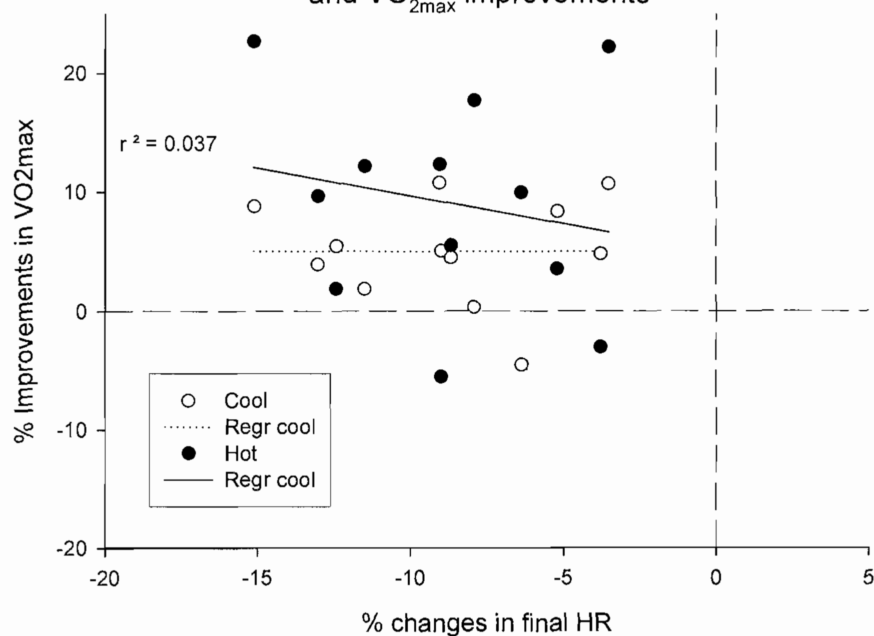


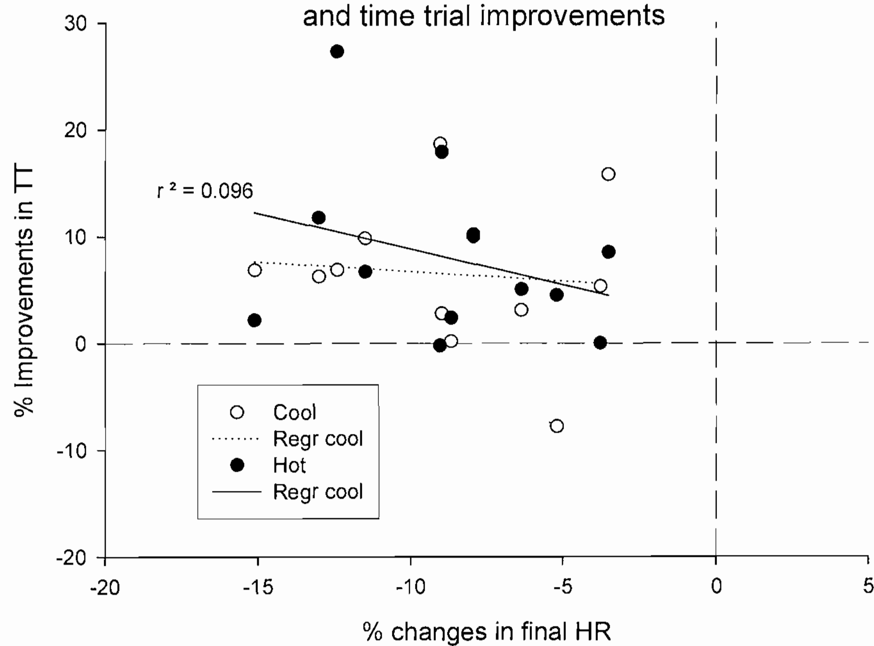
Relationship between time trial and maximal power output improvements
Heat Acclimation Group
% Improvements in TT
% Improvements in Max PO
Cool
Regr cool
Hot
Regr hot
r ² = 0.02
r ² = 0.05
30
25
20
15
10
5
0
-5
-10
-10
0
10
20
30

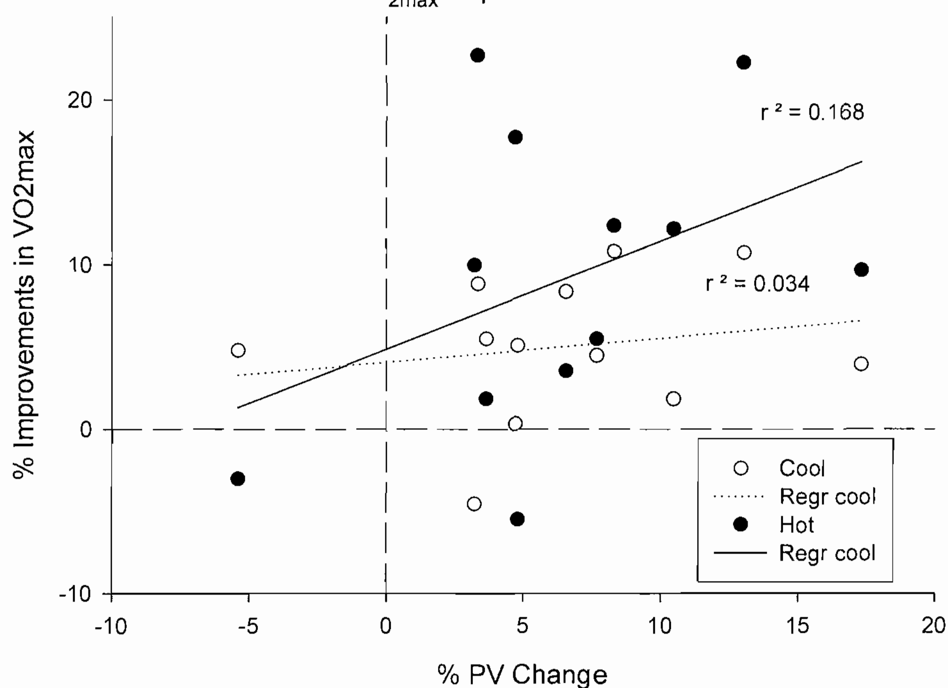

Changes in VO_{2max} based on pre acclimation VO_{2max}
Heat Acclimation Group
% Change in VO_{2max}
Initial VO_{2max} (ml kg^{-1} min^{-1})
Cool
Regr cool
Hot
Regr hot


Changes in time trial based on pre acclimation VO_{2max}
Heat Acclimation Group
% Change in TT
Initial VO_{2max} (ml kg^{-1} min^{-1})
$r^2 = 0.07$
Cool
Regr Cool
Hot
Regr Hot

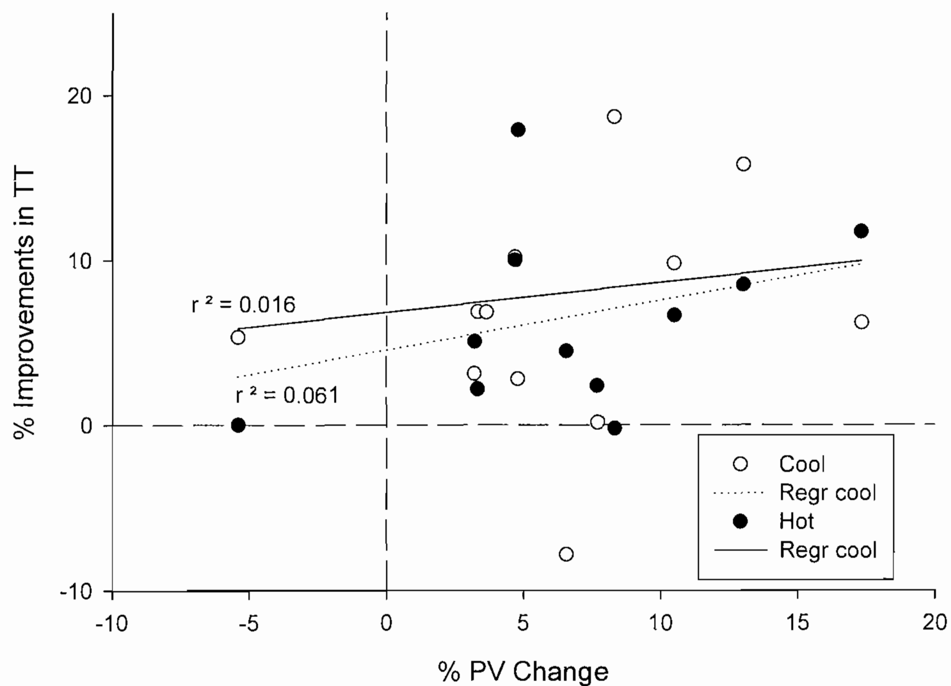

Changes in lactate threshold based on pre acclimation VO_{2max}
Heat Acclimation Group
Cool
Regr cool
Hot
Regr hot
r ² = 0.03
r ² = 0.03
% Change in LT
Initial VO_{2max} (ml kg^{-1} min^{-1})
25
20
15
10
5
0
-5
55
60
65
70
75
80


Relationship between degree of acclimation (as changes in final heart rate
during heat acclimation exposures on day 1 and day 10)
and VO_{2max} improvements
r ² = 0.037
Cool
Regr cool
Hot
Regr cool
% Improvements in VO2max
% changes in final HR
20
10
0
-10
-20
-20
-15
-10
-5
0
5

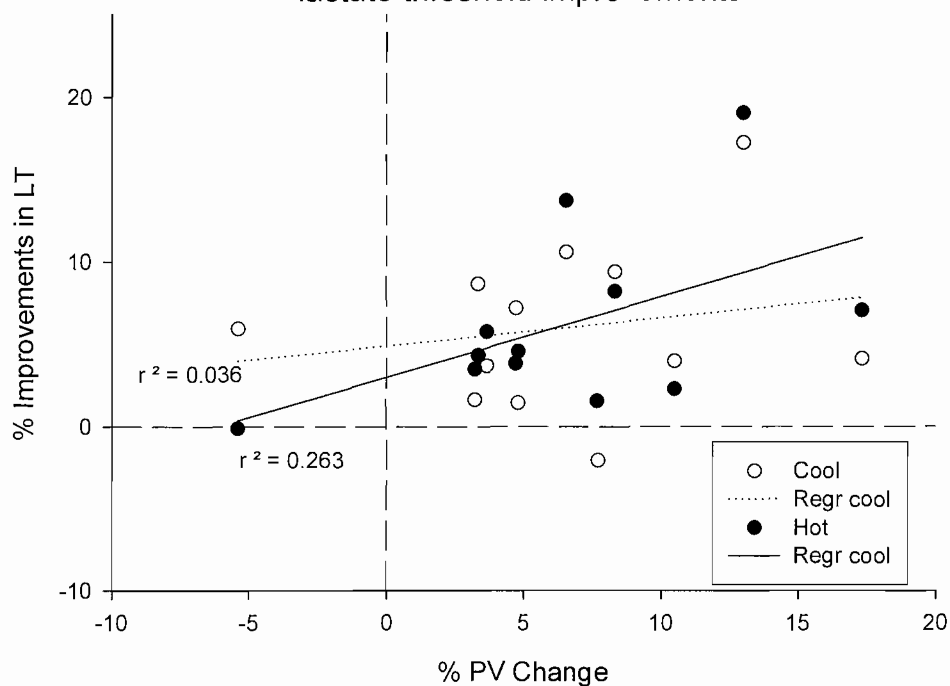

Relationship between degree of acclimation (as changes in final heart rate
during heat acclimation exposures on day 1 and day 10)
and time trial improvements
% Improvements in TT
% changes in final HR
r ² = 0.096
Cool
Regr cool
Hot
Regr cool

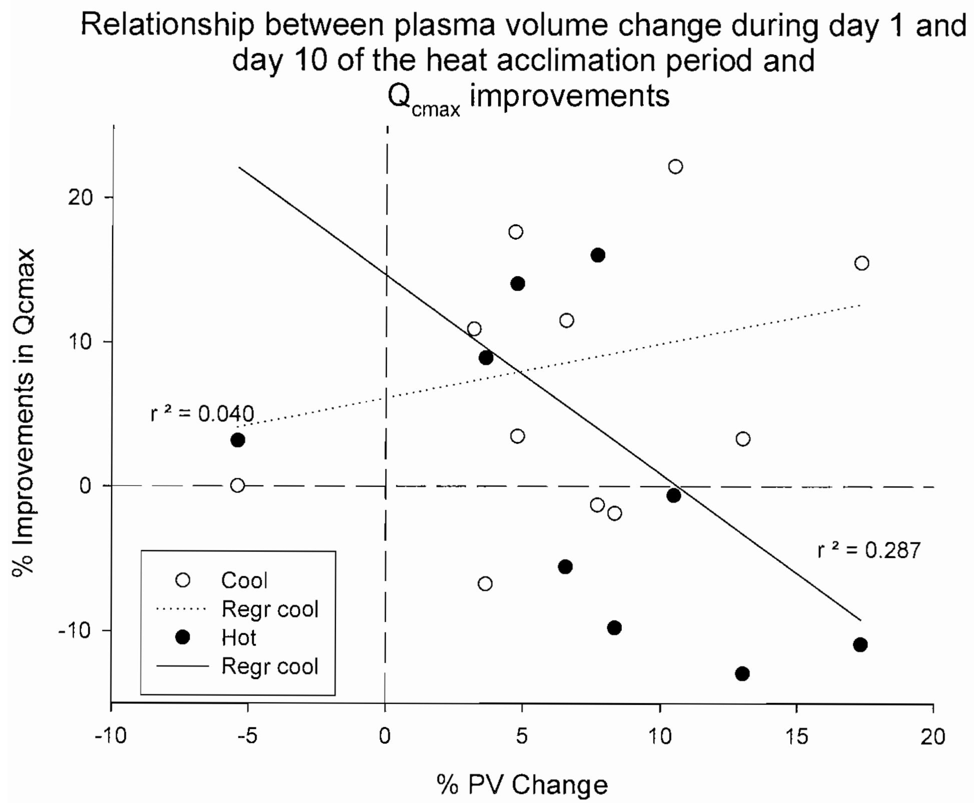

Relationship between plasma volume change during day 1 and
day 10 of the heat acclimation period and
VO2max improvements
% Improvements in VO2max
% PV Change
r ² = 0.168
r ² = 0.034
Cool
Regr cool
Hot
Regr cool

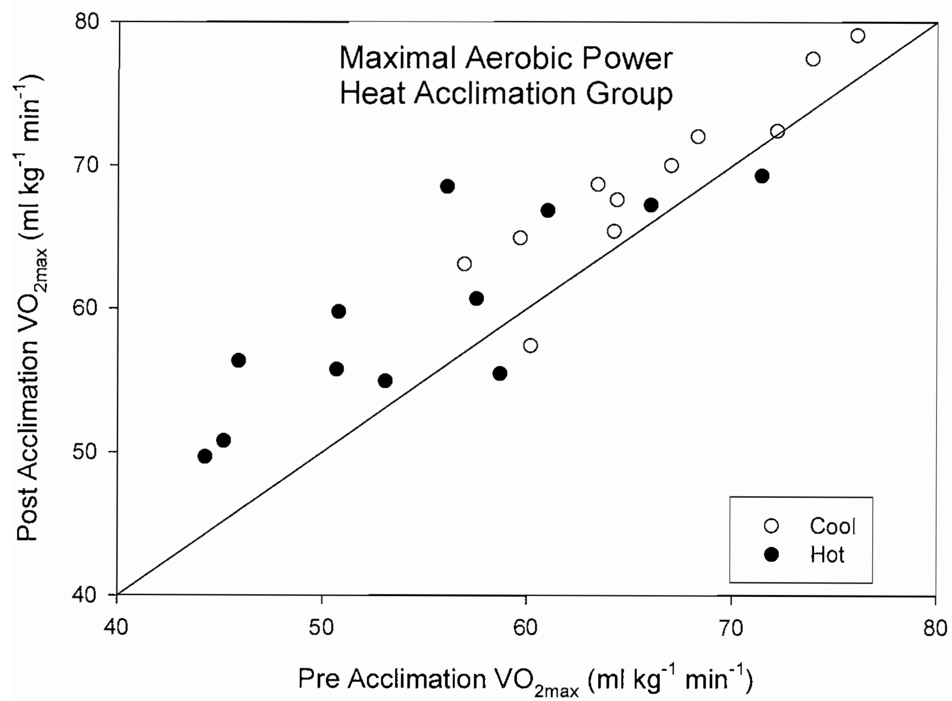
Relationship between plasma volume change during day 1 and
day 10 of the heat acclimation period and
time trial improvements

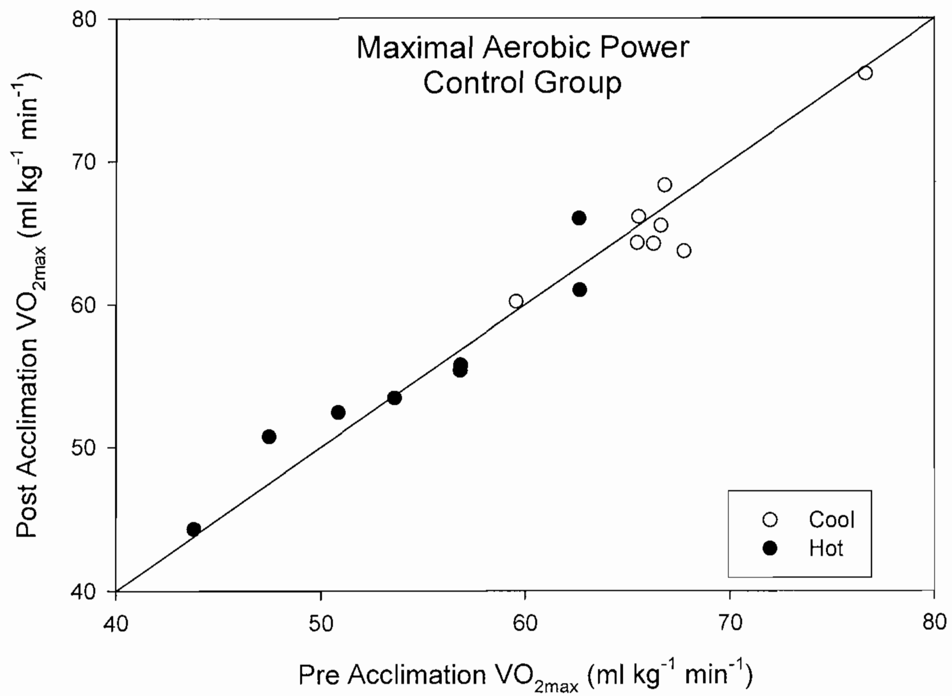


Relationship between plasma volume change during day 1 and
day 10 of the heat acclimation period and
lactate threshold improvements

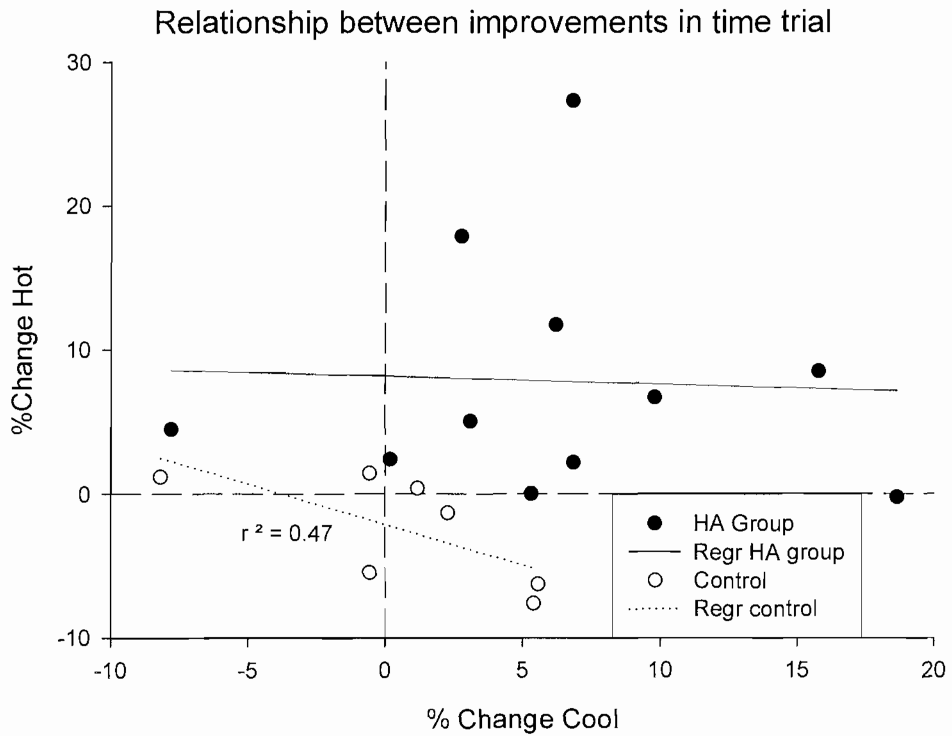



Relationship between plasma volume change during day 1 and day 10 of the heat acclimation period and Q_{cmax} improvements
% Improvements in Qcmax
% PV Change
r ² = 0.040
r ² = 0.287
Cool
Regr cool
Hot
Regr cool
20
10
0
-10
-10
-5
0
5
10
15
20

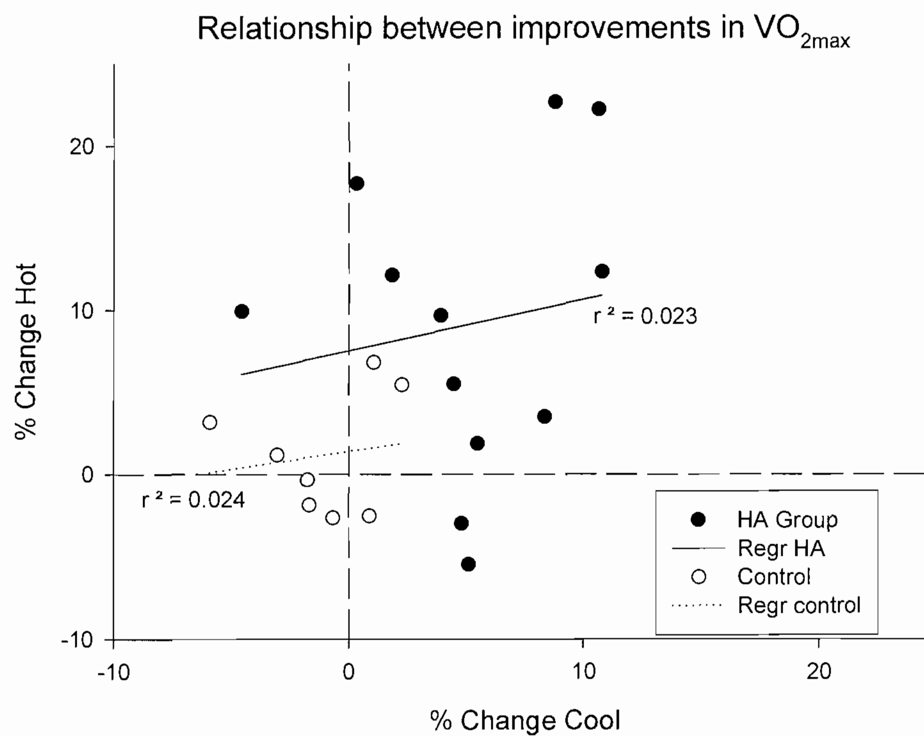

Maximal Aerobic Power
Heat Acclimation Group
Post Acclimation VO_{2max} (ml kg^{-1} min^{-1})
Pre Acclimation VO_{2max} (ml kg^{-1} min^{-1})
Cool
Hot
80
70
60
50
40
40
50
60
70
80

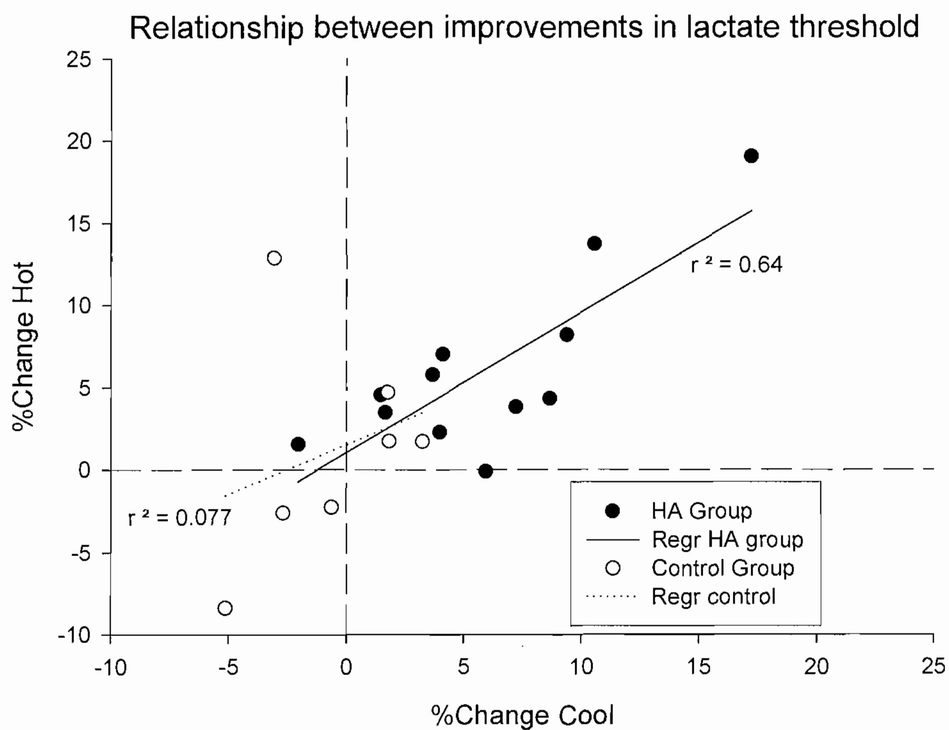


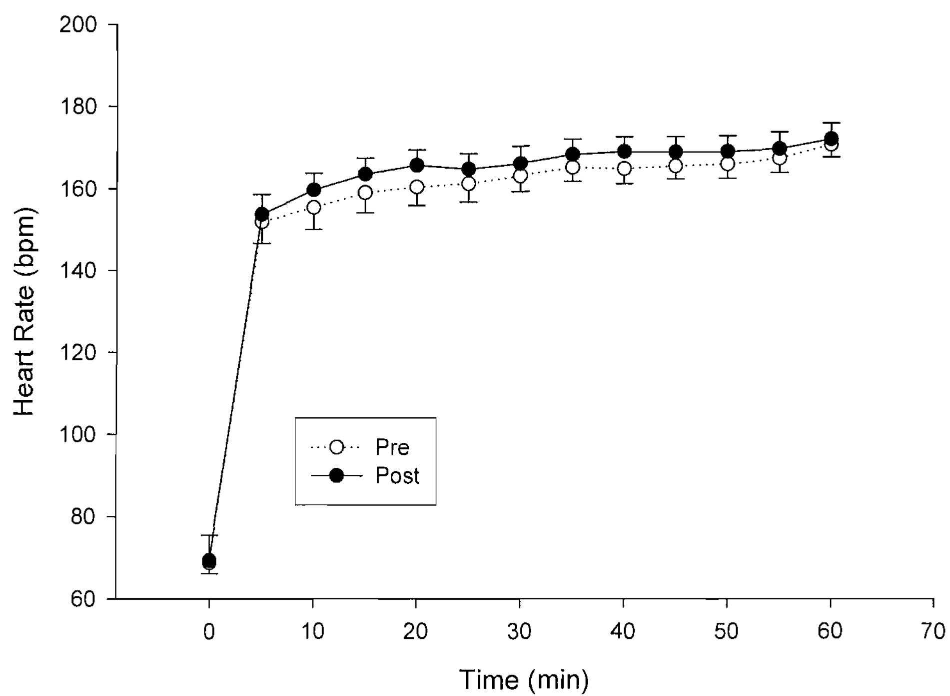
Maximal Aerobic Power
Control Group
Post Acclimation VO_{2max} (ml kg^{-1} min^{-1})
Pre Acclimation VO_{2max} (ml kg^{-1} min^{-1})
Cool
Hot
80
70
60
50
40
40
50
60
70
80

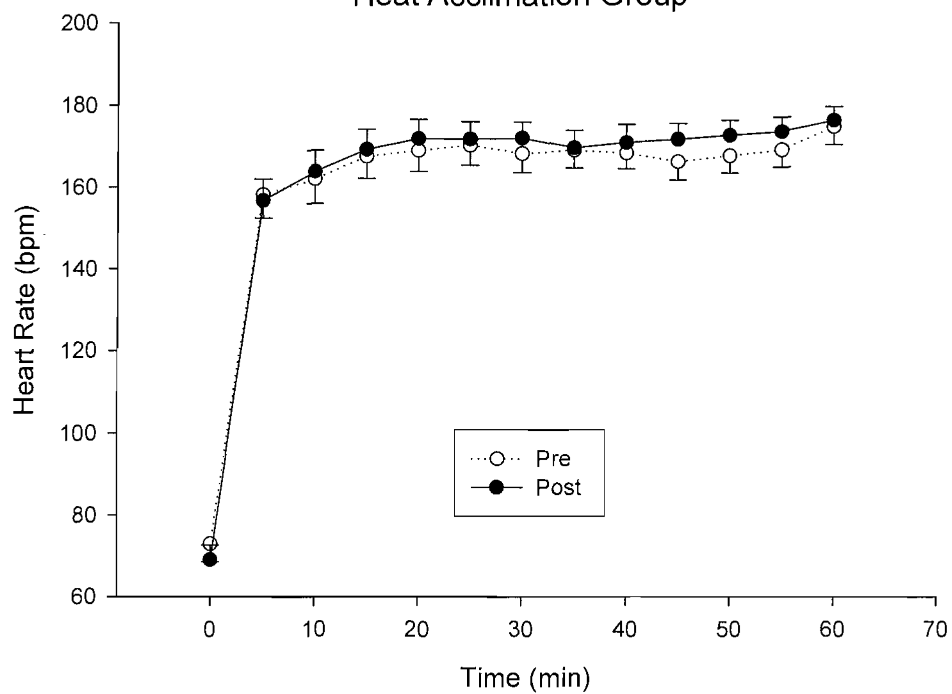


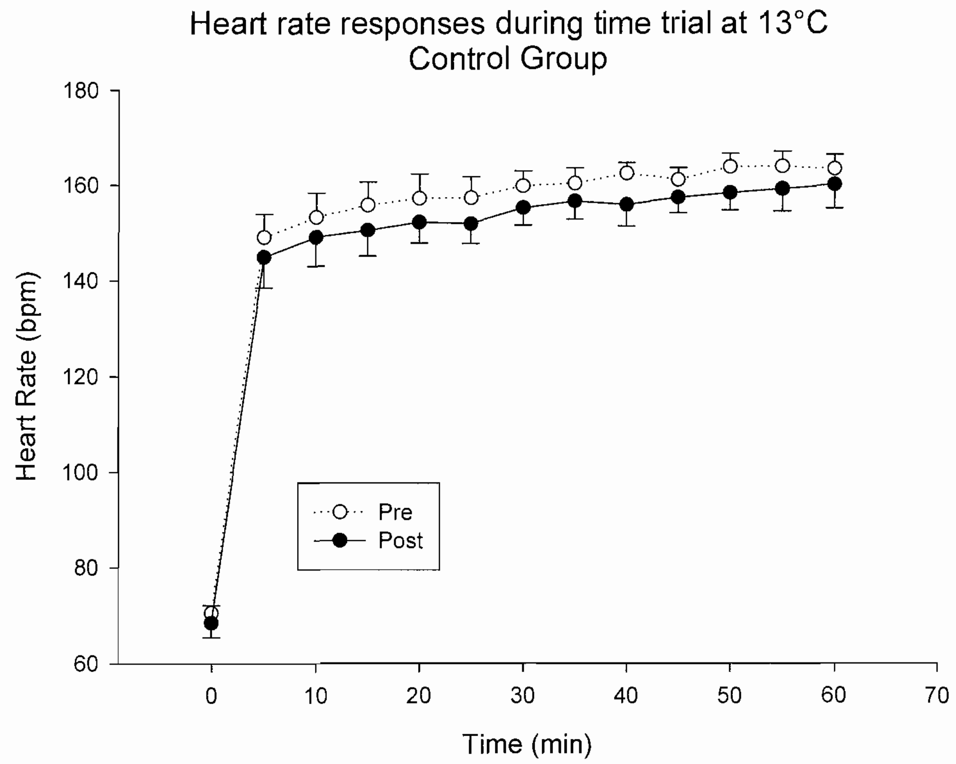
Relationship between improvements in time trial
%Change Hot
% Change Cool
r ² = 0.47
HA Group
Regr HA group
Control
Regr control
30
20
10
0
-10
-10
-5
0
5
10
15
20

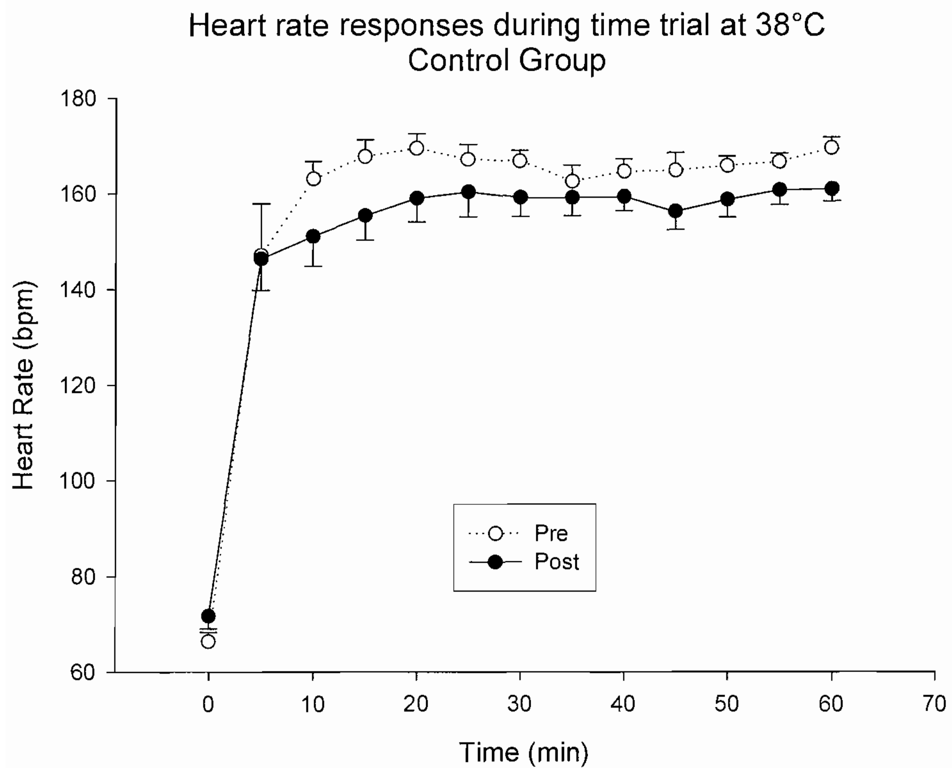

Relationship between improvements in VO_{2max}
% Change Hot
% Change Cool
r ² = 0.023
r ² = 0.024
HA Group
Regr HA
Control
Regr control
20
10
0
-10
-10
0
10
20


Relationship between improvements in lactate threshold
%Change Hot
%Change Cool
r ² = 0.64
r ² = 0.077
HA Group
Regr HA group
Control Group
Regr control
25
20
15
10
5
0
-5
-10
-10
-5
0
5
10
15
20
25


Heart rate responses during time trial at 13°C
Heat Acclimation Group
Heart Rate (bpm)
Time (min)
Pre
Post
200
180
160
140
120
100
80
60
0
10
20
30
40
50
60
70


Heart rate responses during time trial at 38°C
Heat Acclimation Group
Heart Rate (bpm)
Time (min)
Pre
Post
200
180
160
140
120
100
80
60
0
10
20
30
40
50
60
70


Heart rate responses during time trial at 13°C
Control Group
Heart Rate (bpm)
180
160
140
120
100
80
60
0
10
20
30
40
50
60
70
Time (min)
Pre
Post


Heart rate responses during time trial at 38°C
Control Group
Heart Rate (bpm)
180
160
140
120
100
80
60
0
10
20
30
40
50
60
70
Time (min)
Pre
Post

APPENDIX B

INFORMED CONSENT

TITLE: "Mechanisms of Heat Acclimation and Exercise Performance in the Heat"

Protocol 1

INVESTIGATORS: Santiago Lorenzo and Dr. C.T. Minson.

APPROVED BY INSTITUTIONAL REVIEW BOARD: August 13, 2009

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

You are invited to participate in a research study conducted by Santiago Lorenzo M.S. and Dr. Christopher Minson from the University of Oregon, Department of Human Physiology. We hope to learn how specific body systems (cardiovascular and thermoregulatory systems) adapt to exercise in the heat. We will use this data to develop Mr. Lorenzo's dissertation in the Department of Human Physiology. You were selected as a possible participant in this study because you are a healthy young endurance-trained cyclist who meets the specific criteria for investigating the effects of heat acclimation on exercise heat stress.

Why is this study being done?

Performance in the heat has been a greatly researched topic between exercise/environmental physiologists. After a period of heat acclimation, exercise performance in the heat is improved, but the specific mechanisms underlying this effect remain obscure. Advancing the knowledge on this topic can be very beneficial not

only for athletes competing in extreme heat conditions, but also other populations that might be at risk when exposed to hot environments such as the elderly, obese, hypertensive and diabetic populations. This research is thus designed to use the latest minimally invasive techniques for studying the cardiovascular (blood vessels, heart and blood) and thermoregulatory (sweating and skin blood flow) adjustments during dynamic exercise in an effort to shed some light on the human's ability to naturally enhance performance in the heat. Therefore, in order to study these adaptations we will perform a series of studies before heat acclimation (in the heat and cool), and then we will repeat the same set of studies after a period of heat acclimation (also in the heat and cool).

What will happen in the study?

1. You will arrive at Dr. Minson's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately 1.5 hours. You will meet with one of the investigators of the study to complete an initial screening form and health history form, discuss the project, see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. In addition, all women of childbearing potential will need to have a negative urinary pregnancy test before each study day, unless they had a hysterectomy.

2. If you meet all the subject criteria (based on the initial screening form) and are interested in participating in the study we will have you practice kicking at 40 kicks/min on a kicking ergometer.

3. After a discussion of your research participation requirements, we will randomly assign you to one of two research groups for the study. (See discussion below for a description of each group). If you feel uncomfortable participating in the protocol for that particular group for any reason, we will assign you to the other group. Both groups undergo the same studies. One group will go under the heat acclimation process ("heat" group). The other group will serve as the "control" group.

4. You will then return to Dr. Minson's laboratory to participate in the experimental protocol. There will be a total of 12 study days and 10 acclimation days. Each day will take approximately between 2 and 5 hours, depending on the testing day. You will need to wear a t-shirt, shorts, and refrain from eating at least 2 hours prior to

arrival. Females will need to have a negative pregnancy test (meaning that you are not pregnant) prior to starting the study each day. If the test is positive (meaning that you may be pregnant), you will not be allowed to participate in the study.

5. You will be asked to refrain from alcohol and caffeine for 8-12 hours prior to the start of each study day, but not on the acclimation days. In addition you will be asked to refrain from all over-the-counter medications (such as aspirin, ibuprofen, or allergy medication) for the entire 22 testing days. If you are unable to refrain from these substances/activities you will not be able to participate in the study.

6. During the study visits, your heart rate will be monitored by electrocardiogram electrodes placed on your skin (if you are a female subject this will be attached to your body by a female staff member), or by a Polar™ heart rate monitor. Your blood pressure will be measured at periodic intervals by the inflation of a blood pressure cuff around your arm. Periodically, you will breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people at the low concentrations used in the procedure. This is used to study how much work the heart is performing. Two small probes (laser-Doppler probes) will be placed over an area of skin on your forearm. The laser-Doppler probe uses light to measure skin blood flow in these areas, and is taped in place. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your groin-hip intersection. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in these arteries. It's important you know that for any procedure that might cause embarrassment, gender specific research staff will be available.

7. During most of the study days (every day except days 2 and 18) you will be asked to place a rectal probe to measure your body temperature. The probe is made of a thin rubber (flexible) material that is inserted 10 cm (approximately 4 inches) past the anal sphincter. The probe will remain in place throughout the entire study session (up to 5 hours). The probe has a "tail" that will be connected to an external apparatus. The procedure may be a little uncomfortable at first (during insertion) but it should not be painful at anytime. You will be instructed how to self-insert the rectal probe, as well as how to remove it and clean it. If you needed assistance, a lab researcher of the same gender will help you. Once in place, you may not even feel the probe at all. This

technique is widely used and it's considered the "gold standard" procedure for measuring body ("core") temperature. In addition, during some of the study days, you will have a neck collar (days 2 and 18) that will create a light pressure in your neck for approximately 5 seconds. The pressure may feel uncomfortable but should not be painful and it does not prevent you from breathing. Some of the study days (days 1, 3, 5, 17, 19 and 21) will require that you immerse (legs and trunk, but not arms or head) in a water-filled tub to manipulate or control your body temperature. The temperature of the water will be below pain threshold. During these visits, you will need to bring an extra pair of shorts or swimsuit.

8. Bicycle Exercise Session: During some of the study visits and during the heat acclimation period, you will pedal on a bicycle at a moderate rate for a total of 80 minutes (study visits) and 1.5 hours (heat acclimation period).

9. In two study days (Days 2 and 18) you will have 2 small tubes (these are called "microdialysis fibers", and are smaller than the lead of a pencil) placed in the skin of your forearm. First we will numb the area of skin by placing a bag of ice over the area for 5 minutes. Then a small needle will be placed just under the surface of your skin and will exit back out about 1½ inches from where it entered your skin. The small tubes will be placed inside the needle, and the needle will be withdrawn, leaving the small tubes under your skin. There will be two needles inserted in the forearm with one microdialysis fiber threaded through each needle. These will remain in your skin throughout the rest of the study day. We need to wait about 1-2 hours after the small tubes are placed in your skin to let the insertion trauma (redness of your skin around the small tubes) to go away. A small probe (laser-Doppler probe) will be placed over each area of skin where the small tubes are so that we can measure skin blood flow over the small tube. During the protocol we will put some very small doses of drugs through the small tubes in your skin. These drugs will cause the vessels of your skin to either open up or become narrow. You should not feel anything when the drugs are going into your skin. However, it is possible you may feel a slight tingling in the skin where the probe is.

10. We will place your left arm into an arm spray device that will cover your forearm, and heat the area with a fine mist of water from the spray devices. We will heat your arm for a total of 45

minutes. During this time, we will position an ultrasound transducer probe on your upper arm (above your elbow) at the brachial artery, and measure your blood flow velocity for one minute, at minutes 0, 15, 30 and 45. We will also place a small blood pressure cuff on your left wrist, and inflate it to 250 mmHg, stopping blood flow from your hand during this one minute period.

11. Blood sampling. You will lie down on a table and we will place 1 small flexible needle (these are called "intravenous catheters", and are smaller than the lead of a pencil) placed into a vein in your forearm (between the elbow and hand). The skin will be disinfected before this procedure. This will remain in your vein throughout the study day. We will take between 10 and 60 milliliters of blood from your vein during the course of the study day so that we can measure your catecholamine levels (epinephrine and norepinephrine) and concentrations of other substances that are associated with cardiovascular function. We will not take more than a total of 500 milliliters of blood during the entire length of the 22 study visits. Risks associated with this blood withdrawal will be similar to or less than those associated with standard blood donation programs, where 450-500 ml of blood is routinely withdrawn, and are considered very low. After the session, we will remove the flexible needle in your veins and a bandage will be placed over the area of skin where they were.

    The vials in which we collect the blood will be coded such that only the investigators can determine that the samples came from you and the time each sample was taken. No one else will be able to determine your identity from the sample. Once the study is completed and all samples are analyzed, any remaining or extra sample and the vials will be destroyed. Blood samples are not being collected for diagnostic purposes. The results will not be reviewed by a physician. However, if results fall outside of the normal range, you will be informed that you should consult your primary care physician for an additional medical evaluation.

12. Graded Exercise Test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor) (if you are a female subject this will be attached to your body by a female staff member). After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every

minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study day.

13. You should notify the investigator immediately if you feel any significant discomfort (e.g. chest pain or chest tightness) or concern about your well-being at any time during the study visit. Some examples of discomfort include shortness of breath beyond what is expected from exercise, light-headedness, and nausea.

14. Lactate Threshold Test: This test is very similar to the Graded Exercise Test already explained (see above). The only difference is that the exercise stages will be 3 minutes long and blood samples will be collected by a finger prick (one drop of blood) at the end of each stage. This test will conclude before you reach your maximal effort.

15. Blood Volume Measurement: The amount of blood in your body will be measured at the beginning of the study visit with a carbon monoxide uptake test. For this test, you will breathe on a scuba mouthpiece for about 20 minutes while wearing a nose clip. Through the mouthpiece, you will be breathing mostly oxygen with a small amount of carbon monoxide added to it. Carbon monoxide is a colorless, odorless gas and is used to measure the amount of blood in your body and has a half-life of about 5 hours.

16. Local Heating: Towards the end of the study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 103°F for a period of 40 minutes. You should feel a warm sensation in the skin where the local heaters are placed but it should not be painful.

17. As previously mentioned, you should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during or after the study.

How long will I be in the study?

You will participate in this study over the course of 22 days. Each study day will last approximately 2-5 hours, depending on the study day.

What are the risks of the study?

1. Intravenous catheters: IV procedures will be performed under sterile conditions following standard clinical methods. Due to the repeated IV placement (a total of 9 days will require IV placement: 6 study days and 3 acclimation days), the catherizations will be performed in different veins and also arms will be switched. No same location in a vein will be inserted with a catheter twice within 7 days. Following removal of the catheters at the end of the study, pressure is held for 2 minutes, the area of skin is cleaned with alcohol, and a sterile dressing is applied. There may be some discomfort during the insertion of the small flexible needle into your vein. Once the catheter is in place, the pain should subside. Infusions through the catheter should not be painful, and there should only be minor swelling at the site. At the end of the study, the catheter will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needles are sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein is a small bruise and pain at the site of the needle location which may last several days after removal of the needle.

2. Blood withdrawal: Not more than 60 ml will be withdrawn during each study visit. We will not take more than a total of 500 milliliters of blood during the entire length of the 22 study visits. Risks associated with this blood withdrawal will be similar to or less than those associated with standard blood donation programs (for example, Lane Memorial Bank), where 450-500 ml of blood is routinely withdrawn, and are considered very low. You

will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.

3. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a heart attack, the laboratory is equipped with an Automatic Electronic Defibrillator that is located in the same room where the study is taking place. Specifically, this is located in the cupboard above the telephone in the laboratory of room 166 in Esslinger Hall. Dr. Minson, Mr. Lorenzo and Mrs. Martini have up to date Advanced Cardiac Life Support (ACLS) training. In the event of an emergency, the Department of Public Safety (6-6666) will be called in order to activate the emergency medical system (i.e., 911).

4. Laser-Doppler Probes: These probes send a small light into your skin. You will not feel anything except the probe touching the skin. There are no major risks associated with this procedure.

5. Infusion of Study Drugs: You will have the following drugs infused through the skin by the microdialysis probe. There may be some discomfort during the insertion of the small tubes in your skin. Once the needle is in place, the pain should subside. Infusions through the fibers should not be painful, and there should only be minor swelling at the site. At the end of the study, the fibers will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the small tubes are sterile, there is a slight risk of infection at the sites where the small tubes were placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. If at any time you feel any discomfort, you should notify the research team immediately and the microdialysis infusion will be stopped.

- Acetylcholine: This is a substance that may cause your blood vessels to open. When your "blood vessels open" your blood pressure might fall. However, this is unlikely at the low dose of drug administered.
- Sodium nitroprusside: This is a substance that is used to lower blood pressure in patients and causes your blood vessels to open. When your "blood vessels open" your blood pressure might fall. However, this is unlikely at the low dose of drug administered.

Using sodium nitroprusside in combination with Viagra, Cialis or Levitra can result in severely low blood pressure or even death. A report from the FDA (March-November 1998) showed that from a total of over 6 million Viagra users there were 130 deaths, and 16 of those deaths were reported from individuals who were taking nitrates (such as sodium nitroprusside).

- L-NAME: this stops nitric oxide from being produced and causes the skin vessels to narrow

6. Local Skin Heating: The local skin heaters may cause some minor discomfort. The goal is to warm the area of skin to a temperature that has been determined to be below the threshold for pain. If the local heating becomes painful, you should tell the investigator and the temperature of the local heater will be lowered. There is a slight risk of burning the skin at this site, so it is important that you tell the investigators if you feel any discomfort. The heating device will be promptly removed at any time if you feel any pain associated with the temperature of the local heaters.

7. Arm Spray Device: The arm spray device may cause some minor discomfort. The goal is to warm the forearm area to a temperature that has been determined to be below the threshold for pain (42-44°C). If the arm spray device becomes too painful, you should tell the investigator and the temperature of the water will be lowered. There is a slight risk of burning the skin so it is very important you tell the investigators if you feel any discomfort. You may experience some redness of the forearm area for a brief time after heating.

8. Blood Volume Measurement: This research involves exposure to a small amount of carbon monoxide. Carbon monoxide is a colorless, odorless gas. When humans are exposed to large amounts of carbon monoxide, carbon monoxide can cause symptoms that include headache, fatigue, shortness of breath,

nausea, cherry-red colored lips, dizziness, and death. The amount of carbon monoxide you will be exposed to is less than the amount that normally causes symptoms. During the test we will measure your blood levels of carbon monoxide to make sure your body's carbon monoxide level is below the amount that normally causes symptoms. If your carbon monoxide level is too high or if you have any of the symptoms associated with high carbon monoxide levels, we will treat you with oxygen until the levels return to normal and the symptoms go away. The amount of carbon monoxide you will be exposed to will affect blood levels similarly to being in a tobacco, smoke-filled room, driving in a tunnel or parking structure, or the pollution in a big city such as Los Angeles. The carbon monoxide half-life is approximately 5 hours, but if you breathe supplemental oxygen it's reduced to 80 minutes. The half-life is the period of time required for the concentration or amount of drug in the body to be reduced by one half.

9. Neck Pressure and Neck Suction: During this procedure, a neck collar will be securely fit around your neck. You will feel pressure or stretch on your neck from the collar, but you will have no trouble breathing. The pressure and/or suction will cycle on and off for several trials. If at any time you feel any discomfort, you should notify the research team immediately and the collar will be removed.

10. Emergencies: In the event of an emergency, you will be transported by ambulance to Sacred Heart Medical Center University District or RiverBend.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before each study day. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. In the case that you become pregnant during the study (have a positive pregnancy test), we will ask you to see your physician or a provider in the University of Oregon

Student Health Center (if you are a University of Oregon student). There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not need to pay for any tests or procedures that are done just for this research study. You will get $500 for participating in this study. Once the series of study visits are completed, you will receive a check either in person or vie mail to your address (completed in the informed consent form). If you decide to terminate participation early, you will receive the amount that corresponds to the total study days that you participated (approximately $23 per day). This money is for the inconvenience and time you spent in this study.

Who can answer my questions?

You may talk to Santiago Lorenzo at any time about any question you have on this study. Mr. Lorenzo's phone number is (541) 346-4507 or (541) 484-2646. You may also contact Dr. Minson by calling (541) 346-4105 or (541) 953-2231. In addition, you may also contact Dr. Paul Kaplan by calling the Student Health Center at (541) 346-4597.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The University of Oregon is not able to offer financial compensation nor absorb the costs of medical treatment should you be injured as a result of participation in this research. If such complications arise,

the researchers will assist you in obtaining appropriate medical treatment that will be provided at the usual charge.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel Human Subjects	Office for Protection of
Office of the President	University of Oregon
University of Oregon	Eugene, OR 97403
Eugene, OR 97403	(541) 346-2510
(541) 346-3082	

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be $100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive $500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a “subject identification number”. The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Minson’s office.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Office for Protection of Human Subjects, 5219 University of Oregon, Eugene, OR 97403, 541/346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

____________ ________________________________

(Date) (Signature of Participant)

____________ ________________________________

(Date) (Signature of Individual Obtaining Consent)

APPENDIX C

INFORMED CONSENT CHRONIC ARM HEATING

TITLE: **"Mechanisms of Heat Acclimation and Exercise Performance in the Heat"**

Protocol 2, Chronic Arm Heating

INVESTIGATORS: Santiago Lorenzo and Dr. C.T. Minson.

APPROVED BY INSTITUTIONAL REVIEW BOARD: August 13, 2009

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

You are invited to participate in a research study conducted by Santiago Lorenzo M.S. and Dr. Christopher Minson from the University of Oregon, Department of Human Physiology. We hope to learn how specific body systems (cardiovascular and thermoregulatory systems) adapt to exercise in the heat. You were selected as a possible participant in this study because you are a healthy young male or female, between the ages of 18 and 30, who meets the specific criteria for investigating the effects of heat acclimation on exercise heat stress.

Why is this study being done?

Changes in skin blood flow have been seen after a period of heat acclimation. We wish to see if we can reproduce these same changes in the skin by only exposing the forearm to chronic heating, as opposed to the entire body. This would provide information about how the human body adapts to heat stress.

What will happen in the study?

18. You will arrive at Dr. Minson's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately 15 minutes. You will meet with one of the investigators of the study to complete an initial screening form and health history form, discuss the project, see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. In addition, all women of childbearing potential will need to have a negative urinary pregnancy test before each study day, unless they had a hysterectomy.

19. You will then return to Dr. Minson's laboratory to participate in the experimental protocol. There will be a total of 2 study days and 10 "training" days. The 2 study days may take up to 5 hours each, and the training days will take approximately 1 hour. You will need to wear a t-shirt, shorts, and refrain from eating at least 4 hours prior to arrival. Females will need to have a negative pregnancy test (meaning that you are not pregnant) prior to starting the study each day. If the test is positive (meaning that you may be pregnant), you will not be allowed to participate in the study.

20. You will be asked to refrain from alcohol and caffeine for 8-12 hours prior to the start of each study day, but not on the training days. In addition you will be asked to refrain from all over-the-counter medications (such as aspirin, ibuprofen, or allergy medication) for the entire 12 testing days. If you are unable to refrain from these substances/activities you will not be able to participate in the study.

21. During the two study days (Days 2 and 18) you will have 2 small tubes (these are called "microdialysis fibers", and are smaller than the lead of a pencil) placed in the skin of your forearm. First we will numb the area of skin by placing a bag of ice over the area for 5 minutes. Then a small needle will be placed just under the surface of your skin and will exit back out about 1½ inches from where it entered your skin. The small tubes will be placed inside the needle, and the needle will be withdrawn, leaving the small tubes under your skin. There will be two needles inserted in the forearm with one microdialysis fiber threaded through each needle. These will remain in your skin throughout the rest of the study day. We need to wait about 1-2 hours after the small tubes are placed in your skin to let the insertion trauma (redness of your

skin around the small tubes) to go away a small probe (laser-Doppler probe) will be placed over each area of skin where the small tubes are so that we can measure skin blood flow over the small tube. During the protocol we will put some very small doses of drugs through the small tubes in your skin. These drugs will cause the vessels of your skin to either open up or become narrow. You should not feel anything when the drugs are going into your skin. However, it is possible you may feel a slight tingling in the skin where the probe is. Towards the end of the study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 103°F for a period of 40 minutes. You should feel a warm sensation in the skin where the local heaters are placed but it should not be painful.

While we wait for the redness to go away, we will place your left arm into an arm spray device that will cover your forearm, and be warmed with a fine mist of water from the sprayers in the device. We will heat your arm for a total of 45 minutes. During this time, we will position an ultrasound transducer probe on your upper arm (above your elbow) at the brachial artery, and measure your blood flow velocity for one minute, at minutes 0, 15, 30 and 45. We will also place a small blood pressure cuff on your left wrist, and inflate it to 250 mmHg, stopping blood flow from your hand during this one minute period. We will also measure forearm blood flow by temporarily blocking venous (vein) blood flow for ~8 seconds then releasing it for 8 seconds. This will be repeated 5-6 times every ten minutes. It is not uncomfortable.

22. Chronic Arm Heating: During the 10 "training" days, you will be asked to rest in a chair, and place both arms into an arm spraying device. Your arms will be randomized to either a "control" or warming condition. Your arms will be placed in the arm sprayer for the same 45 minute protocol as mentioned above. One sprayer will be set to a cool temperature and the other to a warm temperature of 42-44°C (107-111°F).
23. As previously mentioned, you should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during or after the study.

How long will I be in the study?

You will participate in this study over the course of 12 days. Each study day will last approximately 1-5 hours, depending on the study day.

What are the risks of the study?

11. Laser-Doppler Probes: These probes send a small light into your skin. You will not feel anything except the probe touching the skin. There are no major risks associated with this procedure.

12. Infusion of Study Drugs: You will have the following drugs infused through the skin by the microdialysis probe. There may be some discomfort during the insertion of the small tubes in your skin. Once the needle is in place, the pain should subside. Infusions through the fibers should not be painful, and there should only be minor swelling at the site. At the end of the study, the fibers will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the small tubes are sterile, there is a slight risk of infection at the sites where the small tubes were placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. If at any time you feel any discomfort, you should notify the research team immediately and the microdialysis infusion will be stopped.

    - Acetylcholine: This is a substance that may cause your blood vessels to open. When your "blood vessels open" your blood pressure might fall. However, this is unlikely at the low dose of drug administered.
    - Sodium nitroprusside: This is a substance that is used to lower blood pressure in patients and causes your blood vessels to open. When your "blood vessels open" your blood pressure might fall. However, this is unlikely at the low dose of drug administered.

    Using sodium nitroprusside in combination with Viagra, Cialis or Levitra can result in severely low blood pressure or even death. A report from the FDA (March-November 1998) showed that from a total of over 6 million Viagra users there were 130 deaths, and 16 of those deaths were reported from individuals who were taking nitrates (such as sodium nitroprusside).

    - L-NAME: this stops nitric oxide from being produced and causes the skin vessels to narrow

13. Local Skin Heating: The local skin heaters may cause some minor discomfort. The goal is to warm the area of skin to a temperature that has been determined to be below the threshold for pain. If the local heating becomes painful, you should tell the investigator and the temperature of the local heater will be lowered. There is a slight risk of burning the skin at this site, so it

is important that you tell the investigators if you feel any discomfort. The heating device will be promptly removed at any time if you feel any pain associated with the temperature of the local heaters.

14. Arm Spray Device: The arm spray device may cause some minor discomfort. The goal is to warm the forearm area to a temperature that has been determined to be below the threshold for pain (42-44°C, 107-111°F). If the arm spray device becomes too painful, you should tell the investigator and the temperature of the water will be lowered. There is a slight risk of burning the skin so it is very important you tell the investigators if you feel any discomfort. You may experience some redness of the forearm area for a brief time after heating.

15. Emergencies: In the event of an emergency, you will be transported by ambulance to Sacred Heart Medical Center University District or RiverBend.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before each study day. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. In the case that you become pregnant during the study (have a positive pregnancy test), we will ask you to see your physician or a provider in the University of Oregon Student Health Center (if you are a University of Oregon student). There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not need to pay for any tests or procedures that are done just for this research study. You will get paid $10/hour for participating in this study. Once the series of study visits are completed, you will receive a check either in person or via mail to your address (completed in the informed consent form). If you decide to terminate participation early, you will receive the amount that corresponds to the total study hours that you participated. This money is for the inconvenience and time you spent in this study.

Who can answer my questions?

You may talk to Santiago Lorenzo, M.S. at any time about any question you have on this study. Mr. Lorenzo's phone number is (541) 346-5527. You may also contact Emily Martini, M.S., Research Coordinator by calling (541)-346-5807 or (541)-829-3120 or Dr. Minson by calling (541) 346-4105 or (541) 953-2231. In addition, you may also contact Dr. Paul Kaplan by calling the Student Health Center at (541) 346-4597.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The University of Oregon is not able to offer financial compensation nor absorb the costs of medical treatment should you be injured as a result of participation in this research. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment that will be provided at the usual charge.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a

UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel Human Subjects	Office for Protection of
Office of the President	University of Oregon
University of Oregon	Eugene, OR 97403
Eugene, OR 97403	(541) 346-2510
(541) 346-3082	

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be $100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive $500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a “subject identification number”. The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Minson’s office.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Office for Protection of Human Subjects, 5219 University of Oregon, Eugene, OR 97403, 541/346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

______________ ____

(Date) (Signature of Participant)

______________ ____

(Date) (Signature of Individual Obtaining Consent)

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