Fluid Balance Before and During Exercise and the Effects of Exercise-Induced dehydration on Physiological Responses, Substrate Oxidation, Muscle Metabolism, and Performance

by

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ABSTRACT

FLUID BALANCE BEFORE AND DURING EXERCISE AND THE EFFECTS OF EXERCISE-INDUCED DEHYDRATION ON PHYSIOLOGICAL RESPONSES, SUBSTRATE OXIDATION, MUSCLE METABOLISM, AND PERFORMANCE.

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This thesis is an investigation to answer 4 major questions: 1) Do elite hockey players arrive for a game hydrated and do they consume enough fluid to prevent dehydration over the course of a game? 2) Is hydration status repeatable between days and can an athlete who arrives dehydrated prior to training or competition become hydrated in the time before the start of activity? 3) What is the extent of dehydration (%body mass (BM) loss) necessary to change substrate oxidation and skeletal muscle metabolism during exercise in male and female subjects? 4) Will progressive dehydration have a negative effect on endurance performance?

The first study evaluated the pre-game hydration status, sweat loss and fluid intake patterns of elite male junior ice hockey players during a game. Sweat loss was 3.2 ± 0.2L and exceeded net fluid intake (2.1 ± 0.1L). Mean BM loss was 1.3 ± 0.3%, but 8 out of 24 players lost between 1.8 - 4.3% BM. Despite abundant opportunities to hydrate during a hockey game, 33% of players did not drink enough to prevent sweat losses of ≥2% BM.

The second study investigated 1) the day-to-day variability of morning urine specific gravity (USG) and consuming 600mL of water on the hydration status of hydrated
and dehydrated (USG>1.020) subjects, and 2) the effects of consuming water or carbohydrate electrolyte solutions (CES) on hydration status of dehydrated subjects. Morning USG and hydration responses to the ingestion of 600mL of water were repeatable and mildly dehydrated subjects could reach euhydration within 45min after ingesting any type of fluid with no added effect of a CES.

The next two studies (3 & 4) investigated the effects of mild progressive dehydration during 120min of exercise at ~65% VO_{2peak} on whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated females and males. In both studies, muscle glycogenolysis was increased in the initial 60min of exercise in the dehydrated state when BM loss were ≤1%. Increased glycogenolysis appeared due to increases in core temperature during progressive dehydration as there were no differences in plasma epinephrine or the energy status of the cell (free ADP or AMP) between trials. Normal changes in physiological parameters accompanying exercise in a hydrated state were exacerbated with progressive mild dehydration.

The final study determined the impact of dehydration on cycling performance. Active males cycled at ~65% VO_{2peak} for 90min followed by a time trial (TT: 6 kJ/kg BM) with fluid to replace sweat losses (HYD) or without fluid (DEH). DEH subjects began the 90 min trial 0.6% dehydrated and progressively became more dehydrated with a BM loss of 1.4% at 45min, 2.3% at 90min, and 3.1% post-TT. TT performance was significantly compromised with ~2-3% BM loss (HYD 32 ± 4 vs. DEH 36 ± 3 min).
SUBMITTED MANUSCRIPTS & PUBLICATIONS

This thesis is based on the following publications:


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To our great God be all the glory (Proverbs 3:5-6).
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LIST OF TERMS

ADP, adenosine diphosphate
AMP, adenosine monophosphate
ATP, adenosine triphosphate
BM, body mass
CES-L, carbohydrate electrolyte solution light
CES, carbohydrate electrolyte solution
CHO, carbohydrate
CVP, central venous pressure
DEH, dehydrated trial
EPI, epinephrine
FFA, free fatty acids
HR, heart rate
Hsp, heat shock protein
HYD, hydrated trial
La, lactate
NE, norepinephrine
PHOS, glycogen phosphorylase
Q, cardiac output
RER, respiratory exchange ratio
RPE, rating of perceived exertion
SV, stroke volume
Tc, core temperature
Tm, skeletal muscle temperature
Tsk, skin temperature
TT, time trial
USG, urine specific gravity
Uvol, urine volume
VCO₂, volume of carbon dioxide
VO₂, volume of oxygen
W-S, water salt solution
W, water
CHAPTER ONE
INTRODUCTION & LITERATURE REVIEW
1.1 INTRODUCTION

Water is the most important nutrient and the most abundant substance in the human body, comprising between 70-75% of total body mass (BM), and provides the aqueous environment for the functioning of every cell. Water helps to maintain body temperature, metabolize body fat, aid in digestion, lubricate and cushion organs, transport nutrients, and flush toxins from the body (Hall & Guyton, 2011). The complex fluid regulatory system that controls the movement of fluid and solutes throughout the body is similarly vital, replenishing and maintaining levels which maximize performance, such as its function in thermoregulation (Hall & Guyton, 2011). Sweating is the essential means by which excess heat is dissipated from the body during physical activity, consequently resulting in a major source of water and solute loss. The heat loss from sweat can be quite significant, 0.6 kcal for every mL of water evaporated, while the maximum rate of sweating can be up to 50 mL/min or 3 L/hr in trained athletes (Maughan et al. 2007). This high rate of fluid loss cannot be sustained by the body and puts extreme stress on the thermoregulatory system which maintains homeostatic temperature. Under conditions of extreme stress, water losses of up to 25% of total body water are possible, which in most cases are fatal (Hall & Guyton, 2011).

Humans have abundant sweat glands distributed over the body surface and have a high capacity for sweat secretion. The number of sweat glands in humans can vary greatly, ranging from 1.6 to 4 million, with the highest density (>250 glands/cm²) being on the soles of the feet, palms of the hand, and the scalp (Shibasaki et al. 2006). There are two types of sweat glands, the eccrine and the apocrine glands. The largest of the two are the apocrine glands, which are primarily found in the axillae (armpits), the areola of the nipples, and the genitoanal region. The ducts of apocrine glands open into the canals of hair follicles with the principle stimulus for the secretion being epinephrine (Hall & Guyton, 2011). The apocrine sweat is more viscous, is produced in much smaller amounts compared to the eccrine gland, and may contain pheromones (Edgar Folk & Semken, 1991). In contrast, the eccrine glands are the principle glands responsible for thermoregulatory sweating in humans. Eccrine glands are specialized skin appendages present in over 99% of the skin surface, and are innervated by sympathetic cholinergic neurons (Shibasaki et al. 2006). The action of sweating is controlled from a center in the preoptic and anterior regions of the hypothalamus where thermosensitive neurons are located, an action that is also affected by inputs from temperature receptors in the skin (Shibasaki et al. 2006). High skin temperature reduces the hypothalamic set point for sweating and increases the gain of the hypothalamic feedback system in response to variations in core temperature (Tc) (Nadel et al. 1971). The response of sweating to a rise in hypothalamic
temperature is much greater than the response to the same increase in average skin temperature (Nadel et al. 1971). Following sweat secretion from the eccrine glands, the primary mechanism of heat dissipation, particularly when ambient temperature is higher than skin temperature, is evaporative heat loss (Maughan et al. 2007).

Exercise imposes an added challenge to the thermoregulatory system within the body as metabolic heat production from exercise is proportional to the amount of work performed and thus, the magnitude of sweat production is proportional to the rate of energy expenditure or exercise intensity (Costill 1991). The degree of elevation in Tc is directly related to the exercise intensity and ambient temperature such that high intensity exercise in a high ambient temperature will elicit a substantial increase in Tc (2-3°C). Many studies have documented the increase in body temperature (core, esophageal, or rectal temperature measurements) during various exercise intensities and environmental conditions and demonstrated a proportional relationship between exercise intensity and rises in Tc (Parkin et al. 1999, Nielsen et al. 1993, Roberts et al. 1977).

Ultimately, the maintenance of fluid balance is a major concern for athletes competing in all types of exercise. The combination of a high rate of metabolic heat production, high sweat rates, and inadequate fluid intake, results in substantial BM loss, which has been shown to impose significant strain to physiological function. According to the American College of Sport Medicine's (ACSM) Position Stand on Exercise and Fluid Replacement, athletes should consume enough fluid to avoid a 2% BM loss through sweat loss to avert potential detriments to performance (Sawka et al. 2007). It appears that we are designed to handle a certain amount of dehydration (mild BM loss of ~1-2%) during exercise without consequence, however generally we are not designed to sustain a high work rate for long periods of time without consequence when BM losses amount to >2%. The result being a reduction in plasma volume, leading to a lower stroke volume, heightened heart rate, decreased skin blood flow, increased Tc and perceived exertion, all contributing to a compromised performance compared to when exercise is performed in a fluid balance.

1.2 SWEAT LOSS AND FLUID INTAKE DURING EXERCISE

There is substantial individual variation in sweat rate even when environmental conditions, exercise intensity, fitness level, fluid intake, and heat acclimation, are similar (Maughan et al. 2007). As well, there is large variability within an individual in the rate of sweating from day to day owing to changes in environmental factors, exercise intensity, the type of clothing and/or equipment worn, fluid intake, and exercise duration. The increase in sweat rate seen during exercise is
controlled peripherally by increases in the number of activated sweat glands and/or the sweat output per gland (Ichinose-Kuwahara et al. 2010). Due to the variations in sweat loss and fluid intake, some individuals will incur significant deficits in body fluid witnessed by significant reductions in BM such that they become dehydrated (>2%) while others will voluntarily drink enough fluid to replace sweat loss and finish exercise in fluid balance or be minimally dehydrated. From this we see that there appears to be two primary reasons for dehydration during exercise; exercise elicits large sweat losses and the desire to drink fluid during exercise lags behind the rate at which sweat is lost (Greenleaf 1992). To illustrate, during light exercise in a cool environment sweat rates have been shown to be as low as 0.1 L/hr while during high intensity exercise in a hot environment sweat rates may reach 3 L/hr (Rehrer & Burke, 2006). As a result, a high sweat rate during exercise coupled with inadequate voluntary fluid intake results in a significant fluid deficit, which may impair physiological functioning.

### 1.2.1 Effects of Exercise Training & Acclimation on Sweat Loss

Exercise training has been shown to improve thermoregulation in the heat by earlier onset of sweat secretion and by increasing the total amount of sweat that can be produced (Roberts et al. 1977). Ultimately, training heightens the sensitivity of the relationship between sweat rate and Tc and decreases the internal temperature threshold for sweating. Evidence suggests that training appears to elicit hypertrophy (enlarging) of existing sweat glands without increasing the total number (Sato & Sato 1983). Unfortunately it appears that the body does not adapt to dehydration, so exercising in the heat or at a high intensity without fluid intake does not result in additional adaptation in thermoregulation. Instead, progressive dehydration has been shown to reduce the sensitivity of the relationship between sweat rate and Tc leading to relative hyperthermia, an earlier onset of fatigue, and an increased risk of heat illness (Nadel et al. 1980b; Sawka et al. 1985b).

### 1.2.2 Sex Differences & Sweat Loss

There are several studies investigating the impact of gender and training status on sweat loss. Recently, Ichinose-Kuwahara (2010) demonstrated that trained male and female subjects had significantly greater local sweating rates at the forehead, chest, back, forearm, and thigh, compared to untrained subjects during the same exercise session, while the degree of increase in sweat rate with physical training was greater in the males than in females at higher levels of exercise intensity. They concluded that trained and untrained males and trained females increase their sweat rate during exercise by increasing the sweat per gland output, while untrained females increase their sweat per
gland output and also the number of activated sweat glands. As well, untrained females require a higher Tc or work intensity for maximal activated sweat gland response compared to the other groups. It appears that exercise training enhances sweat gland sensitivity to neural input such that an earlier onset of sweating proves greater thermal efficiency. In regards to differences between genders, Kawahata (1960) concluded that testosterone enhanced the sweat response whereas estradiol inhibited it. In support of this, Araki et al. (1979) documented that exercise-induced sweat response increased dramatically after puberty in boys, and that there were no differences in sweat rates in prepubertal males and females. Moreover, the literature is consistent in demonstrating differences in sweat rate between genders and training status at the same relative exercise intensity owing primarily to differences in physical characteristics (body mass and surface area), sweat gland sensitivity (role of testosterone), and sweat gland activation and output (Ichinose-Kuwahara et al. 2010; Gagnon et al. 2009).

1.3 PREVALENCE OF DEHYDRATION IN SPORT

Team sports require athletes to perform repeated work bouts at near maximal effort coupled with periods of low intensity exercise or rest for the duration of a game, often with breaks between sections of the games. As a result, such exercise is associated with large metabolic heat production accompanied by large sweat loss. Games are characterized by large inter- and intra-subject variability in work rates between players from the same sport along with large variability in sweat loss and fluid intake between players. In light of the frequent rest periods during team sports there are ample opportunities to consume adequate volumes of fluid to replace sweat loss and prevent exercise-induced dehydration. Despite this, it has been commonly reported that athletes replace only ~50% of sweat loss incurred during training and competitions (Burke 1997). Sweat loss during exercise can be estimated in field settings by using the calculation below;

\[
\text{Sweat loss (L)} = (\text{Pre body mass (kg)} - \text{Post body mass (kg)}) + \text{Fluid intake (L)} - \text{Urine output (L)}
\]

Moreover, the goal of drinking is to prevent a loss of ~2% BM in order to prevent exacerbated physiological responses during exercise contributing to premature fatigue compared to exercise when hydrated. Broad et al. (1996) suggests that multiple factors influence fluid replacement during exercise, the main factors being the provision of an individual water bottle, proximity of water bottles during sessions, encouragement to drink, duration and number of breaks or substitutions, and awareness of personal sweat rates. Care must be taken when monitoring the fluid habits of athletes during training or competition as fluid intake on a testing day may overestimate an athlete’s habitual...
fluid consumption due to the athlete knowing they are being evaluated. Therefore, since most of the current literature reports that athletes replace ~50% of sweat losses this may be the "high water" mark and the extent of dehydration may be more pronounced when athletes are not being observed.

Palmer & Spriet (2007) evaluated the sweat rate and fluid intake of elite Canadian male junior hockey players during four-1 hour practices and reported that sweat rate during practice was 1.8 ± 0.1 L·h⁻¹ and players replaced 58% (1.0 ± 0.1 L·h⁻¹) of the sweat lost. BM loss averaged 0.8% ± 0.1%, but 33% of players lost more than 1%. Similarly, Palmer et al. (2010) evaluated the on-ice sweat rate, voluntary fluid intake, and sodium balance during practice in male junior ice hockey players drinking water or a carbohydrate-electrolyte solution (CES) and demonstrated that sweat rates were similar between trials (1.5 ± 0.1 L·h⁻¹ vs. 1.5 ± 0.1 L·h⁻¹) while fluid intake during practice was 0.8 ± 0.1 L·h⁻¹ for the water trial versus 0.7 ± 0.1 L·h⁻¹ for the CES trial resulting in a BM loss of 0.9 ± 0.2% versus 1.1 ± 0.2%. Kurdak et al. (2010) monitored fluid intake and sweat loss of football players during a competition and reported that athletes lost 3.1 L over the game and replaced ~55% of sweat losses which resulted in a 2.2% reduction in BM. Similarly, sweat loss in National Basketball Association (NBA) players during a competition ranged from 1.0 – 4.6 L, with a mean loss of 2.2 ± 0.8 L, fluid intake ranged from 0.1 to 2.9 L (mean 1.0 ± 0.6 L), and BM reduction throughout competition was 1.4 ± 0.6% with a large range between players (0.5–3.2%) (Osterberg et al. 2009). Moreover, there are several studies demonstrating the large individual variability in hydration status, sweat losses, and drinking behaviors during exercise and sport, which highlights the need for individualized assessment of hydration status to optimize fluid-replacement strategies.

1.4 PREVALENCE OF PRE-EXERCISE DEHYDRATION

In addition to exercise-induced dehydration many athletes begin practice or competition dehydrated. Pre-exercise dehydration can be determined by measuring urine specific gravity (USG), urine osmolality (Uosm), and plasma osmolality. USG, an indication of the density of urine relative to water, has been shown to be a simple, accurate and valid indicator of whether an athlete is dehydrated prior to exercise (Armstrong et al. 1994, 1995, 1997; Shirreffs & Maughan 1998). Armstrong et al. (1994) was the first to report that USG and Uosm were valid and reliable indicators of hydration status and could be used interchangeably to determine hydration state during field-testing. Oppliger et al. (2005) also reported that both USG and Uosm mirrored acute changes in plasma osmolality and concluded that USG and Uosm were accurate determinants of hydration status in field-testing. Likewise, Stover et al. (2006a) demonstrated that USG measured with refractometry strongly
correlated with Uosm (r = 0.995) and a USG of 1.020 correlated with a Uosm of ~800 mOsm/kg. Using refractometry, the National Athletic Trainer’s Association Position Statement on fluid replacement in athletes classifies hydration state into four conditions; well hydrated (USG < 1.010), minimal dehydration (USG 1.010-1.020), significant dehydration (USG ≥ 1.020), and serious dehydration (USG > 1.030) (Casa et al. 2000). The published Position Stand on exercise and fluid replacement by ACSM also classifies dehydration as a USG exceeding 1.020 and a Uosm greater than 700 mOsmol (Sawka et al. 2007).

Several reports indicate that a high percentage of athletes arrive for training sessions and competitions mildly dehydrated. Maughan et al. (2004) showed that 11 of 31 football players provided pre-competition urine samples with an osmolality of >900mOsm/kg before an important game, which suggests some amount of dehydration. Likewise, Osterberg et al. (2009) reported that 50% of elite NBA basketball players had a pre-game USG in excess of 1.020. Also, Volpe et al. (2009) demonstrated that 66% of 263 male and female collegiate athletes from multiple sports were dehydrated based on USG prior to competition. Bergeron et al. (2006) reported a mean USG of 1.025 ± 0.002 in junior tennis players, indicating significant dehydration prior to a National doubles competition, with 2/5 players with USGs of 1.030 and 1.032. Likewise, our laboratory reported that 79% of junior male hockey players in a water trial and 71% of players in a CES trial arrived at practice dehydrated, with a USG ≥ 1.020 (Palmer et al. 2010). Overall, the majority of athletes arrive to training and competition in a dehydrated state. This however does not account for the time between arrival and the start of exercise that could be used to rehydrate athletes before commencing exercise. Unfortunately what is not known is how much time and how much fluid is necessary for an athlete to rehydrate if they arrive to training or competition dehydrated to ensure they begin exercise in a hydrated state.

1.5 PHYSIOLOGICAL CONSEQUENCES TO DEHYDRATION

1.5.1 Cardiovascular & Thermoregulation

It has been well established that dehydration causes a higher HR, Tc, plasma volume (Pvol) loss, and a lower stoke volume during exercise compared to being euhydrated (Sawka et al. 1985; Nadel et al. 1980a, b). Sawka et al. (1985) were the first to study the effects of graded dehydration levels (3, 5, and 7% BM loss) on thermoregulatory and blood responses during exercise in the heat. Eight heat-acclimated male subjects attempted four heat stress tests (HST). One HST was attempted during euhydration, while three other HSTs were completed while the subjects were
Dehydrated by 3, 5, and 7% BM. Dehydration was achieved by an exercise-heat regimen on the day prior to each HST. After 30 min of rest in a thermoneutral environment, subjects completed the HST consisting of 4 x 25 min bouts of treadmill walking in the heat (49°C) with 10 min rest between bouts. Results demonstrated that 1) a low-to-moderate dehydration level primarily reduced Pvol with little effect on plasma osmolality, whereas more severe dehydration resulted in no further Pvol reduction but a large increase in plasma osmolality; 2) Tc and HR responses increased with severity of dehydration; 3) sweating rate responses for a given rectal temperature decreased with severity of dehydration. Based on these results, Sawka et al. (1985) concluded that an individual’s thermal strain increases linearly with the severity of dehydration during exercise in the heat.

At the onset of exercise there is movement of fluid out of the vascular system to the working muscle and there is a reduction in Pvol by 3-4%, however as shown by Sawka et al. (1985) and others (Coyle & González-Alonso 2001), dehydration exacerbates the Pvol loss at the onset of exercise.

The changes in observed cardiovascular variables over time during prolonged exercise without a change in power output is a phenomena referred to as cardiovascular drift (Hall & Guyton, 2011). Dehydration has been shown to cause cardiovascular drift to occur earlier into exercise compared to a hydrated state as witnessed by a significantly higher HR and lower stroke volume at the same relative exercise intensity (Sawka et al. 1985; Armstrong et al. 1997). Due to the decreased Pvol with dehydration, stroke volume has been shown to decrease, while HR increases in an attempt to maintain cardiac output to meet the demand for oxygen and nutrient supply to the muscle during exercise (Coyle & González-Alonso 2001; Montain & Coyle 1992). For example, HR rises an additional 3 to 5 beats per minute for every 1% BM loss (Montain & Coyle, 1992). As well, the reduction in Pvol causes plasma osmolality to be significantly higher, and the accompanying change in osmotic pressure is sensed by osmoreceptors located primarily in the hypothalamus, leading to the release of hormones such as vasopressin (ADH) to retain water by preventing diuresis at the kidneys and the release of aldosterone acting to retain sodium at the kidneys in an attempt to regain fluid homeostasis (Hall & Guyton, 2011). Just as Pvol expansion of ∼10% can improve VO\textsubscript{2peak} by ∼5% (Coyle et al. 1990), Nybo et al. (2001) demonstrated that 4% dehydration reduced blood volume by ∼5% (Pvol ∼10%) and lowered VO\textsubscript{2peak} by 6% at a skin temperature of 31°C and by 16% when skin temperature was raised to 36°C. As with heat stress alone, a reduced VO\textsubscript{2peak} when dehydrated would make incremental or constant-rate exercise more difficult to sustain or require a slowing of self-paced exercise to achieve a similar sensation of effort (Cheuvront et al. 2011).
Many studies conducted during exercise in the heat have demonstrated that cardiovascular drift strongly correlates with increases in Tc and that a reduction in blood volume is responsible for the dehydration induced changes to cardiovascular drift (Saltin 1964; Montain & Coyle 1992). However, Heaps et al. (1994) demonstrated that cardiovascular drift during exercise is graded in proportion to dehydration and not due to reductions in blood volume; thus, other physiological factors contribute to the dehydration induced cardiovascular strain experienced during moderate intensity exercise like skin and core temperature. As a result, the means by which HR is elevated by dehydration is not fully resolved. It may be a consequence of the reduction in stroke volume in response to hypovolemia, reduced venous return, and central blood volume, which would limit cutaneous perfusion and result in an increased Tc (Hamilton et al. 1991; Sawka & Coyle 1999). Elevations in Tc have been proposed to limit the time for ventricular filling and stroke volume by tachycardia when sympathetic activity is high or by direct temperature effects on intrinsic HR, which has been attributed to the Q_{10} effect or increased sympathetic activation (Gonzalez-Alonso et al. 1997; Mora-Rodriguez et al. 2007; Merry et al. 2010).

In addition to the documented cardiovascular drift during exercise when dehydrated, many studies have demonstrated significant thermal strain with dehydration during exercise at the same relative exercise intensity compared to a hydrated state (Armstrong et al. 1997; Montain & Coyle 1992; Nadel et al. 1980). Heaps et al. (1994) had subjects exercise in the heat (32°C) without fluid replacement until BM was reduced to 2.8%. Following the exercise-induced dehydration protocol subjects had a 2 h rest period where they were given fluid to replace 100% of sweat losses (euhydration) or were given no fluid (dehydrated). In the euhydrated trial, 65% of the ingested water was retained and thus, subjects were in a fluid deficit of 0.9% before starting the experimental trial compared to being dehydrated by 2.8% when no fluid was ingested. After the rest period subjects cycled for 20 min in a temperate environment (21°C) at 65% VO_{2peak} to compare the cardiovascular responses of exercising in a fluid deficit of 0.9% and 2.8% BM loss. HR was elevated 10 ± 2 and 18 ± 2 bpm, and stroke volume was reduced 9 ± 3 and 18 ± 2 mL/beat, when dehydrated by 0.9 and 2.8%, respectively. These observations demonstrated that cardiovascular drift during moderate intensity exercise in a temperate environment is graded in proportion to hydration (Heaps et al. 1994). As mentioned earlier, physical activity is accompanied by an increase in metabolic heat production that will cause Tc to rise if heat loss mechanisms are not evoked. During exercise, some degree of Tc elevation is normal with the increase proportional to the absolute and relative work intensity, along with influence from environmental conditions. To maintain Tc within a homeostatic range during
exercise, skin blood flow is increased to transport heat to the periphery and allow for heat dissipation mainly via evaporative sweat loss. If we do not maintain our Tc within the desired physiological range (37 – 39°C) during exercise via heat dissipation, protein denaturation can occur at high Tc (>40°C) leading to undesirable protein and cell damage (Febbraio et al. 2002).

Moreover, when Tc and skin temperature become elevated, there is a reflex increase in skin blood flow and cutaneous venous volume while HR increases, and cardiac filling and stroke volume decrease (Cheuvront et al. 2010; Brengelmann et al. 1977; Johnson & Park 1984; Rowell et al. 1969; Rowell 1986). During circumstances such as high intensity exercise, when considerable blood is diverted to the skin, blood flow and oxygen delivery to the muscles, as well as to the brain, may become compromised despite maintenance of leg vascular conductance. It has been suggested that a high skin temperature during exercise reduces VO_{2peak} as a consequence of a high skin blood flow, which displaces more blood to the periphery and reduces cardiac filling leading to a reduction in maximal cardiac output, which may impair endurance performance at max VO_{2}. Furthermore, the combination of a reduction in VO_{2peak} and maximal cardiac output results in what Cheuvront terms, a “shrinking cardiovascular reserve,” which is the primary limiting factor to endurance exercise performance (Cheuvront et al. 2011). As well it has been suggested that when a person exercises in the heat, or in this case when dehydrated, the drive to exercise is diminished by an increased Tc (Febbraio et al. 1996a, b; Bruck & Olschewski 1987). Moreover, greater thermal strain accompanying dehydration can lead to earlier fatigue and greater ratings of perceived exertion when compared to exercising hydrated.

Furthermore, there is a plethora of literature documenting the cardiovascular and thermal strain associated with exercising dehydrated in a hot environment with much less research conducted in neutral and cold environments. When sweat loss is high and fluid intake is inadequate, cardiovascular and thermal drift is inevitable with the severity of drift proportional to the degree of dehydration (Armstrong et al. 1997; Montain & Coyle 1992; Nadel et al. 1980). The time course by which we begin to see the effects of dehydration on cardiovascular and thermoregulatory function needs to be investigated.

1.5.2 Mental Responses

It has been reported that rating of perceived exertion (RPE) is higher as progressive dehydration increases and that fluid ingestion during exercise can decrease the subjective sensation of fatigue and enhance performance when exercise lasts more than ~40 minutes. (McGregor et al. 1999;
Ishijima et al. 2009; Maughan et al. 2007). Ishijima et al. (2009) had healthy untrained male subjects complete 90 min of cycling exercise at 55% VO$_{2\text{peak}}$ in a hot environment (28°C) with mineral water or a CES to replace sweat loss, or with no fluid replacement. Although the study does not indicate how much BM was lost in the dehydrated trial, mean Pvol loss was ~6% in the dehydrated trial and significantly lower than both of the fluid trials indicating some amount of dehydration. Overall RPE, RPE-cardiovascular and RPE-legs during cycling, as well as HR and Tc, were significantly lower when subjects were given fluid compared to being dehydrated.

As well, it is speculated that hypovolemia associated with exercise dehydration leading to a reduction in brain blood flow may exacerbate the displeasure associated with exercising without fluid leading to greater perceived exertion (Maughan et al. 2007; Kempton et al. 2011). Sherriffs et al. (2004) reported that as subjects became progressively more dehydrated they reported feelings of headache, reductions in their ability to concentrate, and their alertness was reduced, which are all contributing factors to an elevated RPE and premature fatigue during exercise.

Negative effects of dehydration on cognitive performance have been shown in some but not all studies, and the effects of dehydration on brain function are unknown. Kempton et al. (2011) investigated this question using functional magnetic resonance imaging (fMRI) in 10 healthy adolescents (5 males and 5 females). Each subject completed a thermal exercise protocol and non-thermal exercise control condition where they cycled at a low intensity for 90 min. Subjects lost ~1.6% BM via sweating in the thermal exercise versus ~0.5% BM in the control condition, and it was observed that a significant correlation existed between % BM loss and percentage change in ventricular volume indicating that greater reductions in BM were associated with greater increases in the volume of the lateral ventricles. Magnetic resonance imaging (MRI) following the thermal exercise protocol revealed that dehydration led to a significantly stronger increase in fronto-parietal blood-oxygen-level-dependent (BOLD) response during an executive function task (Tower of London) than the control condition, suggesting greater tissue oxygenation. However, the greater tissue oxygenation with dehydration was not paralleled by a change in cognitive performance, which the authors believe to suggest an inefficient use of brain metabolic activity following dehydration. This pattern indicates that participants exerted a higher level of neuronal activity in order to achieve the same performance level. Given the limited availability of brain metabolic resources, these findings suggest that mild dehydration (1-2%) may adversely impact executive functions such as planning and visuo-spatial processing (Kempton et al. 2011). More simply, it may be that various physiological responses that are higher during dehydration (HR, Tc, Tsk, Pvol loss, Posm) could explain the altered
cognitive effects without changes in brain blood flow. The feedback from these factors to the brain may contribute to greater perceived exertion and earlier fatigue during exercise when dehydrated.

It is speculated that central and peripheral receptors sensing changes in blood pressure, osmolality, and temperature at the skin and hypothalamus provide feedback to the brain about the exacerbated physiological responses when exercising dehydrated (decreased Pvol, increased Posm, Tc, HR, Tsk). The brain interprets this feedback as a potential threat to the body anticipating a catastrophic event (i.e. cellular heat injury, protein denaturation) if exercise continues at the given intensity without fluid replacement. This anticipatory effect may be responsible for the increased RPE, decreased motivation, and a slowing of self-selected pace during endurance tests when dehydrated by ~2% BM loss. Figure 1.1 outlines the proposed mechanism for the decreased endurance performance when dehydrated.

**Figure 1.1** Proposed mechanism accounting for the aerobic performance detriments with mild dehydration. Pvol, plasma volume; Tc, core temperature; Posm, plasma osmolality; HR, heart rate; Tsk, skin temperature; RPE, rating of perceived exertion.
1.6 IMPACT OF DEHYDRATION ON PERFORMANCE

1.6.1 Endurance performance

Performance in the Heat

Most of what we have learned about dehydration and endurance performance has come from studies conducted in the heat. These studies induce a certain degree of dehydration through pre-exercise heat exposure or via progressive dehydration during exercise in the heat. Regardless of the means by which dehydration was invoked the physiological responses and effects on performance have shown to be the same. Ebert et al. (2007) investigated the influence of hydration status on thermoregulation and cycle hill climbing. The study evaluated 8 well-trained male cyclists and determined if a reduction in BM due to sweat loss would benefit uphill cycling by increasing the power-to-mass ratio. Subjects cycled for 2 hrs at 30°C and became 2.5% dehydrated, immediately followed by a cycle hill-climb time trial to exhaustion (TTE) on their own bicycle on an inclined treadmill (8%). They reported that TTE was 29% reduced in the dehydrated trial compared to the hydrated trial when fluid was consumed to match sweat loss. Exercise-induced dehydration was detrimental to hill-climbing performance despite reducing the power output required for a given speed. As well, Cheuvront et al. (2003) conducted an extensive review of published studies examining the effects of dehydration on exercise performance and reported that in a warm environment (>30°C) dehydration to the extent of 2-7% BM loss consistently impaired endurance exercise capacity as witnessed by reductions in time to exhaustion and time trial performance during running and cycling. Similarly, Walsh et al. (1994) demonstrated a 31% decrease in time to exhaustion and increased rating of perceived exertion when dehydration amounted to 1.8% BM loss after 60 min of cycling at 70% \( VO_{2\text{peak}} \) followed by a TTE at 90% \( VO_{2\text{peak}} \) (32°C and 60% relative humidity), with was no difference in mean \( VO_2 \) or respiratory exchange ratio. Table 1.1 summarizes the studies discussed in this thesis investigating the effects of dehydration on endurance performance in different environment temperatures.
Table 1.1 Effects of Dehydration on Exercise Performance.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>Subjects</th>
<th>% BM loss</th>
<th>Test Environment</th>
<th>Muscle Characteristics</th>
<th>Effect on Endurance Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebert et al. 2007</td>
<td>2 h at 53%, TTE at 88%</td>
<td>M 8ET</td>
<td>-2.5 %</td>
<td>30°C</td>
<td>-----------</td>
<td>↓ TTE 29%</td>
</tr>
<tr>
<td>Walsh et al. 1994</td>
<td>1 h at 70%, TTE at 90%</td>
<td>M 6ET</td>
<td>-1.8 %</td>
<td>32°C</td>
<td>-----------</td>
<td>↓ TTE 31% ↑ RPE</td>
</tr>
<tr>
<td>Kenefick et al. 2010</td>
<td>30 min at 50%, 15 min TT</td>
<td>M 32UT</td>
<td>-4 %</td>
<td>10°C</td>
<td>20°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Merry et al. 2010</td>
<td>40 min at 70%, 40 min TT</td>
<td>M 6UT 6ET</td>
<td>-1.5 to 2 %</td>
<td>24°C</td>
<td>-----------</td>
<td>NS difference</td>
</tr>
<tr>
<td>McConell et al. 1999</td>
<td>45 min at 85%, 15 min TT</td>
<td>M 8ET</td>
<td>-1 %</td>
<td>21°C</td>
<td>-----------</td>
<td>NS difference</td>
</tr>
<tr>
<td>Hargreaves et al. 1999</td>
<td>2 h at 65%</td>
<td>M 5ET</td>
<td>-2.9 %</td>
<td>20-22°C</td>
<td>↑ glycogen use 16%</td>
<td>NM</td>
</tr>
<tr>
<td>Kenefick et al. 2004</td>
<td>1 h at 50%</td>
<td>M 8UT</td>
<td>-4 %</td>
<td>4°C</td>
<td>-----------</td>
<td>↓ 3% (NS)</td>
</tr>
</tbody>
</table>

TTE, time to exhaustion; TT, time trial; M, males; ET, endurance trained; UT, untrained; RPE, rating of perceived exertion; NS, non-significant (p > 0.05).

Many other studies have documented significant detriments in aerobic performance due to mild dehydration in a hot environment, however the impact on performance was highly variable between individuals, with a 7-60% range in the reduction in aerobic performance (Cheuvront et al. 2011). Kenefick et al. (2010) used incremental heat-stress conditions to systemically evaluate the impact of dehydration on aerobic exercise performance. Thirty-two recreationally active men were divided into four matched groups (n = 8) and tested at one of four euhydrated and dehydrated (4% BM loss) conditions. Subjects completed 30 min of preload cycle exercise (50% VO₂peak) followed by a 15 min self-paced time trial. Time-trial performance in the dehydrated trial was -3%, -5%, -12%, and -23% in 10°C, 20°C, 30°C, and 40°C compared to the hydrated trial, while rectal temperature values were similar within both trial conditions across all ambient temperatures. The results demonstrated that dehydration of 4% BM loss degrades aerobic performance at all temperatures and to a greater extent with increasing heat stress (Kenefick et al. 2010).
**Performance in a temperate environment**

There are several studies that have looked at the impact of dehydration on endurance performance in a temperate environment, however the results are less consistent. Merry et al. (2010) had 6 trained and 6 untrained males cycle in a temperate environment (24°C) for 40 min at 70% VO\textsubscript{2peak} while euhydrated or dehydrated by 1.5-2.0% BM, followed by a 40-min time trial euhydrated or allowed ad libitum drinking in the dehydration trial. During constant workload cycling, HR was increased in the dehydration vs. euhydrated trial for untrained (33 vs. 24 bpm) but not trained (14 vs. 13 bpm) subjects. Similarly, rectal temperature drift happened sooner with dehydration for untrained only, concomitant with their reduced sweat rate. Performance power tended to be reduced with dehydration in both the untrained and trained subjects but the difference was not significant. Ultimately, mild dehydration exacerbated the cardiovascular and thermoregulatory strain and tended to impair endurance performance while aerobic fitness attenuated the physiological effects. However, the results need to be interpreted with caution as the study was underpowered with an n=6. McConell et al. (1999) examined the effect of fluid intake on HR, rectal temperature, and performance during intense endurance exercise at 21°C. Eight well-trained men cycled for 45 min at 80% VO\textsubscript{2peak} while receiving either no fluid replacement, a volume of water that prevented BM loss (FR-100), or 50% of this volume (FR-50). The 45-min exercise bout was followed by a 15-min performance ride. Fluid restricted subjects lost 1.9% BM, while FR-50 and FR-100 resulted in losses of 1.0% and no change in BM. Although values tended to be higher with no fluid, fluid ingestion had no significant effect on HR and rectal temperature during exercise. Work completed during the 15 min performance ride was similar in the three trials (no fluid: 273 ± 8, FR-50: 267 ± 8, FR-100: 269 ± 9 kJ). The authors concluded that there appeared to be little benefit from ingesting water during intense 1-h cycling exercise in mild environmental conditions since fluid ingestion had no significant effect on HR, rectal temperature, or TT performance.

**Performance in the cold**

Kenefick et al. (2004) determined whether dehydration alters thermoregulation and cardiovascular responses to exercise in cold air. On four occasions, eight males walked, in t-shirt, shorts, and shoes, at 50% VO\textsubscript{2peak}, for 60 min at 4°C in both a dehydrated (4% BM loss) and euhydrated state. Rectal temperature was not different between dehydrated-Cold (~37.5°C) and euhydrated-Cold (~37.7°C). Cardiac output and stroke volume were not different within hydration
states and HR was not different between HYPO-Cold and EU-Cold. The results demonstrated that moderate intensity exercise in the cold while dehydrated does not increase thermoregulatory and cardiovascular strain. As well, it was demonstrated that when subjects were dehydrated by 4% BM loss, there was a 3% impairment in a self-paced time trial at an ambient temperature of 4°C, however the results were not significant.

Furthermore, more research is necessary to elucidate the impact of exercise-dehydration in a cool and temperate environment on endurance performance as the variability in subject responses makes the current literature inconsistent in demonstrating whether dehydration is more tolerable in a cooler environment. Instead it seems that the attainment of a critical Tc is the more important factor influencing exercise tolerance and fatigue rather than environmental temperature. However, increases in ambient temperature accelerate the rate at which Tc increases during exercise, and as a result, performance detriments may be seen at a smaller %BM loss in the heat due to greater increases in Tc. Kenefick et al. (2010) determined the effects of 4% dehydration on aerobic performance at 10, 20, 30, and 40°C. Subjects completed 30 min of preload exercise (50% VO$_{2peak}$) followed by a 15 min self-paced time trial. Total work during the time-trial performance was -3%, -5%, -12%, and -23%, in 10, 20, 30, and 40°C. During preload exercise, skin temperature increased by ~4°C per 10°C ambient temperature, while Tc values were similar within euhydrated and dehydrated conditions across all environmental temperatures. A significant relationship was found between skin temperature and the percent decrement in time-trial performance. The authors concluded that dehydration degrades aerobic performance to a greater extent with increasing heat stress and that when skin temperature is >29°C, 4% dehydration degrades aerobic performance by approximately 1.6% for each additional 1°C increase in skin temperature. As well, it was concluded that cardiovascular strain from high skin blood flow requirements combined with blood volume reductions induced by dehydration is an important contributor to impaired performance.

Moreover, the severity of impact on aerobic performance is greater with increasing dehydration and increasing ambient temperature. In light of the many studies evaluating the impact of mild dehydration on endurance performance it can be concluded that a 2% BM loss in both a temperate (20-21°C) and a hot environment (30-32°C) when duration is longer than 90 min impairs performance (Shirreffs 2005). There is very little evidence investigating the impact of dehydration on aerobic performance in cool ambient conditions (<10°C).
1.6.2 Stop & Go Sports

Successful performance in many individual and team sports including basketball, hockey, tennis, football, etc., involves fatigue resistance in combination with optimal cognitive function for decision making and proper execution of complex skills and tasks. In light of this, evaluating sports performance can be difficult. The majority of studies investigating the impact of dehydration on sport performance have assessed how the performance of a skill and/or decision making is affected when subjects are either hydrated or dehydrated. Recent literature in team sports such as soccer, football, and basketball, has shown that a body mass loss of ~2% decreased skill performance (Maughan et al. 2007; Edwards et al. 2007; Maughan et al. 2004; Dougherty et al. 2006; McGregor et al. 1999). For example, McGregor et al. (1999) investigated the effects of 2.4% BM loss on soccer skill performance after 90 min of prolonged intermittent shuttle running in moderate environmental conditions (13-20°C, 57% RH) and reported that when no fluid was consumed during the shuttle test, a 5% decrease in soccer skill performance occurred. Likewise, Nicolas et al. (2000) developed a shuttle running test that mimics the intense stop and go demands of soccer and demonstrated that performance was not affected by ingesting flavored water to prevent a BM loss of <1%, however performance detriments were significant when BM loss was 2% or greater. In addition, Devlin et al. (2001) documented that bowling speed was not affected with 2.8% BM loss, but bowling accuracy, as determined by line and distance, was significantly worsened when conducted in the dehydrated state. Dougherty et al. (2006) reported that decrements in basketball skill performance were apparent with a 2% BM loss in skilled 12-15 yr old basketball players. More recently, MacLeod and Sunderland (2010) investigated the effects of previous-day dehydration to 2% BM loss on skill performance in elite female field hockey players following intermittent exercise in the heat (33°C). The day prior to the exercise trial subjects underwent passive hyperthermia while fluid was consumed to match sweat loss (euhydrated) or fluid was restricted to ensure subjects lost 2% BM (dehydrated) which was maintained for the exercise trial the following day. The following day, subjects performed 50 min of a field hockey specific intermittent treadmill running protocol in euhydrated and dehydrated (2% BM loss) states. Field hockey skill tests were performed before and after the running protocol and results demonstrated that skill performance time and decision making time significantly increased with 2% dehydration. The authors concluded that players who commence match-play dehydrated by 2% BM may be susceptible to detriments in skill and decision-making.

Ultimately, significant levels of dehydration amounting to ~2%, which seem relatively minor and can be induced in healthy individuals or athletes by short periods of voluntary fluid
restriction, can be enough to cause feelings of headache, tiredness, greater difficulty concentrating, and reduced levels of alertness which have been shown to negatively impact performance (Shirreffs et al. 2004).

### 1.7 WHOLE BODY SUBSTRATE OXIDATION

It is well established that exercise in a hot environment increases Tc and skeletal muscle temperature (Tm), whole body carbohydrate (CHO) oxidation, and lactate accumulation compared to exercise in a cooler environment (Mündel 2008; Parkin et al. 1999; Febbrario et al. 1996a & b; Fink et al. 1975; Hargreaves et al. 1996). Since dehydration has been shown to increase Tc and physiological strain during exercise compared to a hydrated state with and without heat stress, it is hypothesized that dehydration alone during exercise would have a similar effect on increasing CHO oxidation and lactate accumulation due to an elevated Tc. Unfortunately, there are few studies that have controlled for dehydration in the heat. Most of the literature investigating heat exposure does not control for hydration state and the effects of heat stress, per se, and makes the findings difficult to interpret. However, it is speculated that the elevated Tc accompanying dehydration would affect substrate oxidation in a similar way as heat stress.

To study the effects of dehydration without heat stress on whole body substrate use, Hargreaves et al. (1996) reported that a 2.9% BM loss over a 2 hr exercise trial in a temperate environment resulted in a significantly higher RER in the fluid restricted trial after 60 (HYD 0.91 ± 0.01 vs. DEH 0.93 ± 0.02) and 120 min (0.86 ± 0.01 vs. 0.89 ± 0.01) of exercise with the difference between trials being greater in the second hour of cycling. Roy et al. (2000) investigated the influence of a diuretic-induced reduction in Pvol on substrate oxidation in ten healthy males. The diuretic significantly reduced Pvol by ~15%. Subjects cycled for 60 min at 61% VO_{2peak} (~23°C) and RER was not significantly different between the diuretic (RER 0.96) and the control (RER 0.93) trial. However, again, there was a trend for greater CHO oxidation in the diuretic trial from 30 – 60 min. Similarly, Armstrong et al. (2006) evaluated the impact of 5% dehydration on 10 min of treadmill running at 70% and 85% VO_{2peak} in trained male collegiate distance runners in a temperate environment (23°C). Dehydration by 5% BM lead to a greater HR and rectal temperature and reductions in cardiac output and stroke volume at both exercise intensities. Unexpectantly, the RER was significantly lower with dehydration during exercise at 70% VO_{2peak}.

Overall, the literature examining just the effects of dehydration on substrate oxidation during exercise is inconsistent, as some studies report that RER increases with dehydration, while
others report a decrease with dehydration, and still others that there was no effect. As well, most of the research investigating the effects of dehydration on substrate oxidation, have been conducted on males and there is no known published research on the effects of dehydration on substrate oxidation in females. What is unclear is the extent of dehydration (%BM loss) necessary to elicit a change in fuel selection. Moreover, our interest is in studying the effects of dehydration among hockey players who compete in a temperate environment. Therefore, it is necessary to determine; (1) the effects of progressive dehydration on substrate oxidation in a temperate environment, (2) the time course of changes to substrate oxidation with increasing levels of dehydration to establish the extent (%BM loss) necessary to increase CHO oxidation during exercise, and (3) to investigate the impact of progressive dehydration on substrate use in women.

1.8 MUSCLE METABOLISM

While few studies have evaluated the impact of progressive dehydration on muscle metabolism in a temperate environment, several studies have examined the combined effect of heat stress and dehydration on muscle metabolism. Gonzalez-Alonso et al. (1999) had male subjects cycle to exhaustion at 61% VO$_{2peak}$ in the heat (35°C) and demonstrated that dehydration to 3.9% BM resulted in increased muscle glycogen use, muscle lactate production, and an 8% increase in CHO oxidation compared with control. The authors suggested that hyperthermia rather than changes in metabolic factors was the main factor underlying the early fatigue in the heat with greater dehydration. Fatigue was not related to elevated muscle lactate, reduced blood glucose or low muscle glycogen, but a critically high Tc (39.8 ± 0.2°C). Another study investigating the effects of dehydration on muscle glycogen use found no effect of heat-induced dehydration on muscle glycogen use or muscle lactate content (Costill et al. 1974).

There are three main hypotheses which have been proposed to explain the substrate shift towards greater carbohydrate metabolism and muscle glycogenolysis during exercise and heat stress; 1) an augmented sympathto-adrenal response leading to greater glycogen phosphorylase (PHOS) activation and flux, 2) increased allosteric activation of glycogen PHOS via increased free ADP and AMP (energy status of the cell) levels and, 3) a higher intramuscular temperature (Tm) during exercise when dehydrated (Febbraio et al. 1996; Febbraio 2000). Figure 1.1 identifies the proposed hypotheses regulating glycogen phosphorylase activity and the rate of glycogenolysis during exercise.
Figure 1.2 Regulation of glycogen phosphorylase (PHOS) activity during exercise. Glycogen phosphorylase kinase (GPKa) converts the inactive form of glycogen phosphorylase (PHOSb) to its active form (PHOSa). The three proposed hypotheses regulating the conversion to and the activity of PHOSa are sympahto-adrenal (epinephrine (EPI)) via cyclic adenosine monophosphate (cAMP), increased allosteric activation via adenosine monophosphate (AMP) and adenosine diphosphate (ADP), and a higher muscle temperature (Tm). As well, calcium (Ca2+) increases the activity of GPKa converting more inactive PHOSb to its active form (PHOSa).

There appears to be only one study that examined solely the effect of dehydration on muscle metabolism in a temperate environment. Hargreaves et al. (1996) investigated the effects of progressive dehydration on skeletal muscle metabolism in males in a thermoneutral environment (20-22°C) and demonstrated that a 2.9% BM loss resulted in 16% greater muscle glycogen use, higher muscle lactate accumulation and higher rectal and muscle temperatures after 2 hr of exercise at 65% VO_{2peak} in trained males. This study was limited by a small sample size (n=5) and only pre and post muscle biopsy sampling times, with no intermediate biopsies taken to elucidate the impact of progressive dehydration on muscle metabolism throughout exercise. Previously it has been suggested that the increases in plasma epinephrine (EPI), norepinephrine (NE), and Tm, secondary to elevations in Tc are contributors to the increased muscle glycogenolysis during exercise in the heat (Febbraio...
2000, Febbraio et al. 1994, 1996). The authors suggested that the same mechanisms were responsible for the dehydration-induced increased glycogenolysis in the temperate environment (Hargreaves et al. 1996).

Physical stress, brought on by exercise and possible potentiation by dehydration, stimulates increased secretion of catecholamines via the hypothalamic-adrenal neural pathways (Febbraio et al. 1998, 2000; Melin et al 1988; Hoffman et al. 1994). This increase is attributed to stimulation of the hypothalamus by osmoreceptors, atrial stretch receptors, and the temperature-regulating center of the brain (Peskind et al. 1993; Hori et al. 1998). During an acute bout of exercise, EPI (not NE) enhances the rate of muscle glycogenolysis via increasing glycogen phosphorylase (PHOS) activation resulting in an increased flux through glycolysis (Turner et al. 1995; Weltman et al. 1994). Elevated rates of muscle glycogen utilization can lead to glycogen depletion and contribute to fatigue. Thus, one possible mechanism whereby dehydration could alter muscle metabolism during exercise is via an exaggerated catecholamine response (Hargreaves et al. 1999). However, Hargreaves et al. (1996) reported no difference in plasma EPI at 60 or 120 min of cycling with ~3% BM loss but significantly greater plasma NE only at 120. The authors suggested that fluid ingestion during exercise attenuates the normal exercise induced increase in EPI, and the blunting of the sympathoadrenal activity may be due to hydration status and Tc, but it is difficult to assess these factors independently. As well, their data to support this are not convincing, since NE was only higher in the fluid restricted trial after 2 hrs of exercise and it does not stimulate muscle glycogenolysis.

The energy status of the cell (via free ADP and AMP) provides powerful allosteric regulators of glycogen phosphorylase and therefore plays a vital role in determining the rate of glycogenolysis. Hargreaves et al. (1996) did not make these calculations in their paper, so it is unclear whether free ADP and AMP further activate glycogen phosphorylase when dehydrated, and this warrants further investigation.

Previous laboratory work suggested that higher Tm was responsible for the increased glycogenolysis and increased reliance on CHO oxidation for muscle ATP production (Febbraio et al. 1994, 1996; Febbraio 2000; Hargreaves et al. 1996; Starkie et al. 1999). Mohr et al. (2010) examined fatigue development in elite soccer players during match play in a hot environment (31°C). Results demonstrated that Tm rose ~3°C (to 40.5 ± 0.4°C) from pre- to post-match while players were dehydrated by >2% BM by the end of the match, despite drinking 1.8 ± 0.1 L. The study provided evidence of a compromised post-match repeated sprint and jump performance and pronounced reduction in high-intensity running ability compared to pre-game testing. The authors attributed these
changes to hyperthermia and/or dehydration that may have accelerated muscle glycogenolysis and fatigue (Mohr et al. 2010). Hargreaves et al. (1996) directly measured a significantly higher Tm when trained male subjects were dehydrated by 2.9% BM after cycling at 65% VO_{2peak} for 2 hrs without fluid in a thermoneutral environment. The authors also attributed the accelerated glycogen use when dehydrated to the higher Tm. The Hargreaves study appears to be the only study that evaluated the impact of dehydration on muscle metabolism in a temperate environment. As well, there appears to be no research examining the effects of dehydration on muscle metabolism in female subjects. Moreover, it remains unclear as to the exact mechanism(s) for the accelerated glycogenolysis during exercise when dehydrated; greater sympathetic activation, a decreased energy status of the cell, and/or increased Tm.

1.9 CONCLUSIONS

Exercise can result in a significant body fluid deficit when sweat loss is not replaced with adequate fluid intake. When reviewing the prevalence of dehydration in sport, the majority of athletes are arriving to training or competition dehydrated and/or progressively becoming dehydrated throughout exercise as voluntary fluid intake is not matching sweat loss. The literature is consistent in reporting significant performance decrements in endurance and skill performance, when dehydration amounts to ~2% BM loss and beyond, but not during actual ice hockey playing. There are many physiological consequences to exercising dehydrated such as cardiovascular, thermal, and mental strain, and a consistent trend for greater whole body and muscle CHO use. However, the degree of dehydration (%BM loss) necessary to induce physiological strain and changes in whole body and skeletal muscle CHO use has been largely untested. With regards to muscle metabolism, there is only one known study investigating the effects of dehydration on muscle metabolism, which was conducted with a small sample size and only pre and post muscle measurements. Of importance is also the fact that there are no published research investigating the impact of dehydration on substrate oxidation and muscle metabolism in females.
CHAPTER TWO

AIMS OF THESIS
2.1 AIMS OF THESIS

This thesis set out to answer 4 major questions: 1) Do elite hockey players arrive for a game dehydrated and do they consume enough fluid to prevent dehydration over the course of a game? 2) Is hydration status repeatable between days and whether an athlete who arrives dehydrated prior to training or competition can become hydrated in the time before the start of activity? 3) What is the extent of dehydration (\%BM loss) necessary to change substrate oxidation and skeletal muscle metabolism during exercise in male and female subjects? 4) Will progressive dehydration have a negative effect on endurance performance?

Upon undertaking this thesis, it had been established that many athletes are not ingesting enough fluid throughout training and competitions and are ending exercise in a significant fluid deficit. As well, evidence suggested that \~2\% BM loss due to sweating may be associated with decreases in athletic performance. There have been multiple studies investigating fluid intake and sodium balance in athletes during training in sports such as soccer, football, basketball, cricket, swimming, running and cycling, with most of the research being conducted in hot environment (~30°C). As well, there were very few studies looking at the prevalence of dehydration during training in temperate environments (i.e. ice hockey) and no known investigations during a game situation. Therefore, the aim of the first study (Chapter three) was to examine the fluid and sodium balance of elite male junior hockey players during a game.

From this first study we determined that 33\% of the Jr. male hockey players were not doing an adequate job of drinking fluid throughout the game and ended significantly dehydrated (1.8–3.7\% BM loss). As well, we observed that 41\% of the hockey players arrived to the game dehydrated (USG > 1.020), which sparked an interest for a subsequent study (Chapter four) to investigate how fluid intake impacted hydration status over a 60 min time period. What was known from our first study was that hockey players arrived to the arena \~90-120 min prior to the start of the game and then would drink \~600 mL of fluid in the time before the game started. This prompted the research question for our second study; would 600 mL of fluid and 60 min of time improve the hydration status of athletes who arrive to training or competition dehydrated to the point where they begin the game hydrated? If so, what type of fluid (water vs. CES) would be most effective at restoring hydration in dehydrated subjects? In addition, we wanted to monitor the day to day repeatability in morning hydration status (USG) over five days. Based on previous research, we hypothesized that (1) morning USG would be repeatable for subjects between days, (2) fluid retained and hydration state (USG) would be significantly improved within 60 min of consuming 600 mL of water, and (3) drinking 600
mL of a CES solution would increase fluid retention and hydration status of dehydrated subjects to a greater extent than water.

From the first two studies we established the prevalence of dehydration in elite male hockey players and determined that fluid of any type in a volume of 600 mL 60 min prior to competition significantly improves hydration status. In light of the fact that athletes are letting themselves dehydrate during competition, our next aim was to elucidate the impact of progressive dehydration on whole body substrate oxidation and skeletal muscle metabolism. At this point there had been only one known study conducted on male subjects evaluating progressive dehydration over a 2 h cycling bout on muscle metabolism. The study used a very small sample size (n=5), had no intermediate muscle biopsies, and only examined male subjects. The next two studies of this thesis sampled blood and muscle at intermediate time points to evaluate the effects of progressive exercise-induced dehydration in females (Chapter five) and males (Chapter six) in order to establish a time course of changes in physiological and metabolic responses. We hypothesized that as female and male subjects progressively dehydrated during prolonged exercise and increased Tc above the increase in the hydrated state, there would be a greater reliance on whole body CHO oxidation and muscle glycogenolysis. As well, we expected that these differences would be augmented in the second hour of exercise in the dehydrated trial.

From the results of Chapters five and six, we determined that dehydration augmented exercise related physiological strain and promoted greater CHO and muscle glycogen use when BM loss was ~1-2%. However the impact on endurance performance was not determined. The final study of this thesis increased the level of dehydration by combining overnight fluid restriction with progressive dehydration during exercise. This was to determine the effects mild dehydration prior to exercised (~0.6% BM loss) combined with progressive dehydration during cycling on substrate oxidation, skeletal muscle metabolism, and time trial performance. From the results of our previous studies we hypothesized that CHO oxidation and muscle glycogen use would increase as the magnitude of dehydration increased, as compared to the hydrated condition. As well, we predicted that time trial performance would be significantly slower in the dehydrated subjects.

Taken together, this thesis examined the fluid balance of elite male hockey players and the impact of hydration on physiological responses and whole body and skeletal muscle metabolism.
CHAPTER THREE

ESTIMATED FLUID AND SODIUM BALANCE AND DRINK PREFERENCES IN ELITE MALE JUNIOR PLAYERS DURING AN ICE HOCKEY GAME

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3.1 ABSTRACT

Research in many sports suggests that losing ~2% of body mass (BM) through sweating impairs athletic performance, although this has not been tested in ice hockey players. This study investigated pre-game hydration, and on-ice sweat loss, fluid intake, and sodium (Na⁺) balance of elite male junior players during an ice hockey game. Twenty-four players (2 goalies, 7 defensemen, 15 forwards) volunteered to participate in the study (18.3 ± 0.3 y, 86.5 ± 1.6 kg, 184.1 ± 1.3 cm). Players were weighed pre and post game, fluid and sodium intake were monitored throughout the game, and fluid and Na⁺ balance were determined within the time between BM measurements. Sweat Na⁺ loss was calculated based on sweat loss and sweat [Na⁺] determined from sweat patch analysis on the same players during an intense practice. Players arrived to the rink in a euhydrated state and drank 0.6 ± 0.1 L of fluid before the game. Mean playing time for the forwards was 18:85 ± 1:15 min:s and 24:00 ± 2:46 min:s for the defense. Sweat loss was 3.2 ± 0.2 L and exceeded net fluid intake (2.1 ± 0.1 L). Mean BM loss was 1.3 ± 0.3%, with 8/24 players losing between 1.8 - 4.3% BM. Players preferred to drink water and a carbohydrate electrolyte solution before the game and during intermissions, while only water was consumed during each period. Practice mean forehead sweat [Na⁺] was 74 mmol·L⁻¹. Estimated sweat Na⁺ losses of 3.1 ± 0.4 g (~8 g NaCl) coupled with low Na⁺ intake of 0.8 ± 0.2 g (~2 g NaCl) resulted in a significant Na⁺ deficit by the end of the game. This study demonstrated that despite abundant opportunities to hydrate during a hockey game, 1/3rd of the players did not drink enough fluid to prevent sweat losses of 2% BM or higher. Losing 2% BM has been associated with decreases in athletic performance.
3.2 INTRODUCTION

Hypohydration is a major concern to athletes competing in team sports. During exercise, sweating provides a means to dissipate metabolic heat production, but at the expense of losing body fluid. If fluid intake does not closely match sweat loss, a significant body fluid deficit may progressively develop, which has been shown to significantly increase core temperature, heart rate, and ratings of perceived exertion at the same absolute and relative exercise intensities compared to a euhydrated state (Armstrong et al. 1997; Barr et al. 1999; Burke 1999; Gopinathan et al. 1988; Heaps et al. 1994; Maughan 2003; Montain et al. 1998; Nybo & Nielsen 2001; Sawka et al. 2007; Shirreffs 2005). Despite this concern, many athletes only replace ~50% of sweat loss and voluntarily hypohydrate during exercise (Burke 1997, Noakes 1993). Recent literatures in team sports such as soccer, football, and basketball, have shown that a body mass loss of ~2% decreased skill performance (Dougherty et al. 2006, Edwards et al. 2007, Maughan 2003, Maughan et al. 2007, McGregor et al. 1999). A performance study with 2% BM loss has not been done in hockey players.

Hockey is a high-intensity stop-and-go sport, characterized by three 20 min periods with several shifts lasting 30-60 s interspersed with static recovery periods lasting 1-4 min. Between each period is a 15-20 min intermission where players return to their respective dressing rooms to allow for ice re-surfacings. Previous time motion analyzes have shown that the majority of time during a hockey game is spent inactive; essentially, recovering on the bench between shifts or in the dressing room during intermissions (Bracko 2001, Green et al. 1976). In light of this, there are abundant opportunities for players to drink, and therefore, inadequate fluid intake should not occur. One would speculate that the relatively cool micro-environment of a hockey arena (~10 °C) would reduce sweating. However, the equipment worn allowing only the face to be exposed for sweat evaporation coupled with the very high intensity of the intermittent exercise elicits high sweat rates and the potential for hypohydration (Godek et al. 2006, Palmer & Spriet 2008). The rate of sweat loss will also depend on genetic differences, the aerobic fitness status of the player, and the player's hydration status (Burke 1997).
A previous study from our laboratory (Palmer et al. 2010) examined elite male Junior hockey players during a practice and reported that two-thirds of the players arrived at practice in a hypohydrated state (urine specific gravity, USG ≥ 1.020). Average sweat loss over the 90 min practice was high (2.2 ± 0.1 L) and mean fluid intake was less than sweat loss (1.7 ± 0.1 L). While this resulted in players losing an average of only 0.8% body mass due to sweat loss, several players lost ~2% body mass or more. Sweat patch analysis showed a high mean sweat sodium concentration ([Na⁺]) of 74 mmol·L⁻¹ with large variability between players (43 – 115 mmol·L⁻¹). Most athletes chose to drink a carbohydrate electrolyte solution (CES) prior to practice and consumed only water during the practice. High sweat losses coupled with high sweat Na⁺ concentrations resulted in players ending the practice in a significant sodium deficit (-3.5 ± 0.3 g). Following this study, feedback was given to each player about their pre-practice hydration state, sweat loss, fluid intake, and sodium balance, with recommendations on how they can improve their hydration habits before and during training to avoid voluntary hypohydration. Although the mean data appeared positive, it was clear that several players had sub-optimal fluid and sodium intake habits in the practice environment. We hypothesized that players may pay less attention to hydration during an actual hockey game than during practice, due to the intense and emotional nature of competition.

The purpose of the present study was to evaluate the player's (i) pre-game hydration status and habits, (ii) on-ice sweat loss, (iii) on-ice fluid intake, and (iv) sodium balance, (v) drink preference during the game, and (v) provide a gross estimate of sodium losses through sweat. We hypothesized that the pre-game hydration state of the players would be improved before a game vs. practice, and that players would have higher total sweat losses, a higher incidence of dehydration (>2% body mass loss), and higher sodium losses as compared to similar players during intense practices (Palmer et al. 2010). We also had the opportunity to test 6 players on two occasions and predicted that players would improve their hydration status prior to and during a second game after receiving individual feedback following the first game.

3.3 METHODS

3.3.1 Subject Characteristics

Twenty-four elite male hockey players (2 goalies, 7 defensemen, 15 forwards) volunteered to participate in the study. Subjects were members of the Guelph Storm Hockey Club of the Ontario Hockey League and each player had played in the league for at least three months prior to this study. All subjects were informed of the experimental protocol, both orally and in writing, before written informed consent was obtained. Parental consent was obtained
for players under the age of 18 y. The study was approved by the Research Ethics Board at the University of Guelph. Players with the most playing time were selected by the coaching staff and were notified of their participation in the study the day before each game. Six to eight players were monitored in each of four games over two seasons and 6/24 players were tested on two occasions. Subjects mean (± SE) age, mass, and height, were 18.3 ± 0.3 y, 86.5 ± 1.6 kg, 184.1 ± 1.3 cm.

3.3.2 Study Design

Game Protocol and Data Collection

Subjects arrived to the arena at their usual pre-game time (2 - 2.5 hr prior to game time ~ 5:15 PM) (Fig. 3.1).

Figure 3.1 Study timeline and protocol during game testing. *Dressing room water and sports drink bottles measured. #Hockey bench water bottles measured. ^Urine sample for USG measurement. BM, body mass. Int, intermission. P, period.

Participants were asked to defecate if necessary, provide a urine sample for pre-game USG, and then completely empty their bladder. Body mass (BM) was measured ~60-90 min before the game on a portable digital scale (Zenith LG Electronics Canada, Mississauga, Ont.) accurate to ±
0.1 kg with subjects wearing only dry shorts.

After the pre-game BM measurement, any urine passed during the pre-game, game periods and intermissions was collected in order to account for fluid loss from micturation. Players then selected their preferred pre-game, game periods, and intermission beverages (water and/or CES (Gatorade®)) and all fluid intake was carefully monitored. Each water and CES bottle was explicitly labeled with the subject’s name for clear identification and collection, and bottles were weighed prior to and following use to determine mass. Subjects were also given the option to use GatorLYTES® (one pouch = 770 mg Na⁺) mixed in a 591 ml CES bottle (~1040 mg Na⁺, ~95 mmol·L⁻¹). After each game period and intermission, bottles were collected, weighed, and replaced with new bottles. Players were instructed to drink as they normally would during a game. BM was also measured at the end of the game with an empty bladder, again wearing only dry shorts. Forehead sweat patches were not applied during the game as sweat sodium concentrations had been previously determined during practices (Palmer et al. 2010). The availability of drink bottles at all points during the game without the presence of experimenters made it less likely that our presence influenced the hydration habits of the players compared to practice testing. Testing was also repeated on 6/24 players (3 defensemen and 3 forwards) over games separated by one year. Arena temperature and relative humidity (RH) were measured at chest level at centre ice before and after each game using a Digital Thermometer (Fisher Scientific, Ottawa, Ont.). The pre and post-game temperature and RH within a game and between games were not different, and the mean arena temperature and relative humidity for all games was 10.8 ± 0.2 °C and 30 ± 2%.

3.3.3 Analyses

USG was determined from the initial urine sample using a hand-held pocket refractometer (ATAGO USA Inc., Bellevue, Wash.) to determine pre-game hydration status. The refractometer was calibrated with distilled water prior to its use and checked periodically between urine samples. USG values were classified as; USG < 1.020 signifying a hydrated state and a USG ≥ 1.020 indicating hypohydration (Casa et al. 2000, Sawka et al. 2007). Time-motion analysis was assessed with game videotapes.

In a previous study from our laboratory, sweat [Na⁺] was determined via sweat patch analysis on the same junior hockey players and revealed a mean forehead sweat [Na⁺] of 74 mmol·L⁻¹, with a range of 43 – 115 mmol·L⁻¹.
3.3.4 Calculations

Percent BM loss during the game was estimated as the net BM loss (kg) during the game divided by the pre-game BM:

\[
\text{Percent BM loss} = \frac{\text{Pre BM} - \text{Post BM}}{\text{Pre BM}} \times 100
\]

Sweat loss (L) was estimated as the net BM loss (kg) during the game (assuming 1 kg = 1 L) plus total fluid intake (L), minus any urine produced (L) during the game:

\[
\text{Sweat loss} = (\text{Pre BM} - \text{Post BM}) + \text{Fluid intake} - \text{Urine output}
\]

Respiratory water loss, substrate mass loss, and the generation of water from oxidation have been shown to exert little influence on the sweat rate calculated from changes in BM and were therefore ignored in this study (Baker et al. 2009a, Maughan et al. 2007).

Sodium intake was calculated by determining total CES consumption and GatorLYTES® use. One bottle (591 ml) of CES has 270 mg of sodium and one pouch of GatorLYTES® has 770 mg of sodium. As instructed, one pouch of GatorLYTES® is to be mixed into one 591 ml CES bottle for a total of 1040 mg of sodium. Using the subject’s sweat Na⁺ concentration (mmol·L⁻¹), we predicted the total sodium loss over the game for each subject. Recently, Baker et al. (2009b) demonstrated that forehead sweat sodium concentration overestimated whole body sweat Na⁺ loss during cycling by 57%. Accordingly, the Na⁺ loss calculated from forehead sweat patch analysis in this study has been corrected to represent a more accurate whole body Na⁺ loss based on the data reported by Baker et al. (2009b). It is important to note that no study has compared forehead sweat Na⁺ based estimates of total salt loss and whole body wash down procedures in a hockey situation.

3.3.5 Statistical Analysis

All data were tested for normality of distribution and presented as the mean ± SE. Correlations between variables were assessed using a Pearson’s correlation analysis. Differences
between forward and defense data within a game and within repeated players were analyzed with Student’s paired $t$ tests. Goalies were not included in position comparisons as $n = 2$. Statistical significance was accepted when a $p$-value of less than 0.05 was found.
3.4 RESULTS

3.4.1 Pre-Game Urine Specific Gravity and Fluid Intake

A pre-game urine sample revealed that on average players arrived to the rink in a hydrated state (USG 1.016 ± 0.002, Fig. 3.2).

![Graph showing pre-game hydration](image)

**Figure 3.2** Pre-game hydration (USG, urine specific gravity) status. Individual data are presented (n = 24).

Mean USG for forwards was 1.016 ± 0.004 and defense was 1.016 ± 0.004. However, of the 22 skaters, 41% (3/7 defense, 6/15 forwards) arrived hypohydrated (USG ≥ 1.020). Both goalies arrived to the game hydrated with a USG of 1.015 ± 0.002. On average, players consumed 0.6 ± 0.1 L of fluid (0.3 ± 0.1 L water, 0.3 ± 0.1 L CES) before the game. The 9 skaters who arrived to the arena hypohydrated consumed a mean of 0.7 ± 0.1 L of fluid prior to the game.

3.4.2 Game Playing Time

There was large variability in playing time between and within positions. Forwards ranged from 11:45 – 28:27 min:s (18:85 ± 1:15) and defense 12:30 – 33:18 min:s (24:00 ± 2:46). On average, forwards played 31% and the defense played 40% of the game.
3.4.3 Sweat Loss and Fluid Intake

Mean sweat loss during the games was 3.2 ± 0.2 L (range 1.7–6.1 L) (Fig. 3.3).

![Figure 3.3](image)

**Figure 3.3** Net fluid intake (L) and sweat loss (L) during an OHL hockey game. Data are presented as mean ± SE, n = 24. * p < 0.05 significantly greater sweat loss than net fluid intake.

Total fluid intake averaged 2.4 ± 0.2 L and ranged from 0.5 – 3.8 L, with a significantly greater consumption of water (1.6 ± 0.1 L) compared to CES (0.8 ± 0.1 L) for all positions (Fig. 3.4, p < 0.05).

(a)
Figure 3.4 (a) Mean water, carbohydrate electrolyte solution (CES), and total fluid intake during the game and for each position. (b) Distribution of fluid intake across the game. Data are presented as means ± SE, n = 24. * p < 0.01 water vs. CES.

Players consumed only water during each period and averaged 0.3 ± 0.1 L per period (range 0 – 0.9 L). Both water (0.2 ± 0.01 L) and CES (0.2 ± 0.03 L) were consumed during the two intermissions (Fig. 3.5).

Three players chose to drink only water pre-game and throughout the periods and intermissions. Mean total urine output was 0.3 ± 0.1 L (range 0 - 0.95 L). Mean net fluid intake was therefore 2.1 ± 0.2 L with a range of 0.5 - 3.2 L. Players voluntarily replaced ~66 ± 5% of sweat losses before and during the game.

The two goalies lost 2.0 ± 0.7 L of sweat during the game and replaced 72 ± 39% of sweat losses incurred. The defense averaged a sweat loss of 3.7 ± 0.1 L and replaced 75 ± 12% of the sweat they lost, while the forwards lost 3.2 ± 0.1 L of sweat and replaced 62 ± 5%, of the losses incurred during the game.
3.4.4 Fluid Balance and Body Mass Changes

Players were in a negative fluid balance (1.2 ± 0.3 L) by the end of the game (range – 4.2 to 0.5 L). When evaluating sweat loss vs. fluid intake by player position, both the defensemen (-1.3 ± 0.6 L) and the forwards (-1.2 ± 0.2 L) experienced negative fluid balance (Figure 3). In contrast, one goalie and one defenseman ended the game in a positive fluid balance (+0.3 and +0.5 L).

Players lost an average of 1.3 ± 0.3% BM by the end of the game (range – 4.2 to +0.5 kg). Mean BM loss was 1.4 ± 0.7% for defensemen and 1.4 ± 0.2% for the forwards. However, the important finding was that 8/24 players (33%) lost between 1.8 - 4.3% BM.

3.4.5 Sodium Balance

Based on sweat sodium concentrations obtained from the players during practice (mean 74 ± 4.0 mmol·L⁻¹), mean total sodium loss during the game was 3.1 ± 0.3 g (~7.8 g NaCl). Conversely, mean sodium intake was 0.8 ± 0.2 g (0 – 3.34 g), resulting in an average sodium deficit of 2.3 ± 0.4 g by the end of the game. The three players who drank only water replaced no sodium, while eleven players who drank some CES replaced 15 ± 3% of the sodium they lost. Ten players supplemented their CES with GatorLYTES® and consumed 1.4 ± 0.2 g of sodium while losing 3.5 ± 0.5 g, which resulted in the replacement of 40 ± 4% of total sodium lost. Sodium replacement was significantly increased when GatorLYTES® were added to the CES although all players were still in a substantial Na⁺ deficit by the end of the game.

3.4.6 Correlations

Pre-game USG did not correlate with pre-game fluid intake (r = 0.28) or net fluid intake throughout the game (r = 0.12), but was negatively correlated with %BM loss (r = 0.53, Fig. 3.5a). Sweat loss was correlated with net fluid intake (r = 0.39, Fig. 3.5b) and %BM loss (r = 0.74). Sodium loss was also correlated with %BM loss (r = 0.53). There were no significant correlations between playing time and sweat loss (r = 0.14) or total fluid intake (r = 0.24).
Figure 3.5 Relationship between (a) pre-game USG and % BM loss, (b) sweat loss and fluid intake. Data are presented as means ± SE, n = 24.
3.4.7 Repeated Players

The six players who were tested on two occasions (3F, 3D) were well hydrated when they arrived for games (Table 3.1). Mean playing time was significantly greater in game 2 compared to game 1, but sweat loss tended to be lower in game 2 (Table 3.1). However, mean fluid intake was similar in both games, with water and CES consumption also the same.

Table 3.1 Comparison of game 1 (G 1) vs. game 2 (G 2) for six repeated players (3 D, 3 F). Data are presented as means ± SE, n = 6. *p <0.05 sig. diff between games 1 (G1) and 2 (G2).

<table>
<thead>
<tr>
<th></th>
<th>G 1</th>
<th>G 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG</td>
<td>1.009 ± 0.004</td>
<td>1.011 ± 0.004</td>
</tr>
<tr>
<td>Playing Time (min)</td>
<td>17 ± 2.4</td>
<td>25 ± 1.5*</td>
</tr>
<tr>
<td>Net Fluid Intake (L)</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>-water (L)</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>-CES (L)</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Sweat Loss (L)</td>
<td>3.9 ± 0.6</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>% BM Loss</td>
<td>2.0 ± 0.6</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Sodium Balance (g)</td>
<td>5.6 ± 1.2</td>
<td>4.7 ± 0.9</td>
</tr>
</tbody>
</table>

Overall, because of a tendency for lower sweat loss and the similar fluid intake in game 2, the players replaced more of their sweat loss and reduced their percent BM loss in game 2 (Table 1). The sodium lost in game 2 was also lower, but not significantly.

3.5 DISCUSSION

The present study investigated the pre-game hydration status and fluid intake, and on-ice sweat rate, fluid intake and sodium balance of elite male junior ice hockey players in preparation for and during a game situation. We found that (i) 41% of players arrived to the game in a hypohydrated state, but consumed ~600 mL of fluid in preparation for the game which most likely resulted in a proper hydration status prior to the game, (ii) average sweat loss during the game was 3.2 L and high for all positions, (iii) players drank water before and
throughout the game and drank a carbohydrate electrolyte solution (CES) only before the game and during intermissions, (iv) the fluid players consumed before and during the game replaced ~66% of their sweat losses, (v) in spite of numerous opportunities to drink, 33% of players (8/24) lost between 1.8 and 4.3% of their BM by the end of the game, (vi) players lost significant amounts of sodium via sweating, which was not replaced in the fluid they consumed, and (vi) a sub-set of 6 players who were tested during two games, stayed better hydrated and replaced more sodium during the second game.

3.5.1 Pre-game Hydration State

The mean pre-game USG in this study (1.016 ± 0.002) was similar to that reported in elite male junior hockey players prior to practices (Palmer et al. 2010, Palmer & Spriet 2008), and similar to elite soccer players (Maughan et al. 2005, Maughan et al. 2007), professional football players (Horswill et al. 2009), and professional basketball players (Osterberg et al., 2005) prior to team training and competition. According to the American College of Sports Medicine (ACSM) and National Athletic Trainer’s Association (NATA) Position Stands on exercise and fluid replacement, urine biomarkers of hydration status allow sufficient sensitivity to detect deviations in fluid balance and a USG value > 1.020 is an indicator of hypohydration (Armstrong et al. 1994, Casa et al. 2000, Oppliger et al. 2005, Sawka et al. 2007, Stover et al. 2006). In the present study, 62% of the players (n = 15) had a pre-game USG < 1.020 and were considered hydrated, while 38% (n = 9) had a USG > 1.020 and were considered to be in a hypohydrated state upon arrival prior to the game. Of interest is the difference in the pre-game USG reported in this study (1.016 ± 0.002) compared to the USG found on the same players prior to a practice (1.023 ± 0.002); more players arrived to the game hydrated as compared to prior to the practice. The nine players who arrived to the arena in a hypohydrated state (USG > 1.020) drank a mean of 0.7 ± 0.1 L of fluid in the 60-90 min prior to the game, which should put these players in a hydrated state before starting the game. This is in contrast to a practice situation where players often arrive to the arena dehydrated and do not have enough time to become hydrated before practice begins.
3.5.2 Sweat Loss

The combination of the arena temperature (~10°C), heavy padded equipment, the high intensity of a hockey game, and the well-trained status of the hockey players in this study resulted in a high average sweat loss of 3.2 ± 0.2 L during the game. This is similar to a study by Godek et al. (2006) who reported that professional male ice hockey players sweat 3.7 ± 0.9 L during a game. They also reported that sweat rate was not different between a practice and game, but sweat losses were higher during the much longer game (3.7 ± 0.9 vs. 2.6 ± 0.6 L). The higher absolute game sweat losses compared to our study may be accounted for by a higher arena temperature (14-18 vs. 10°C) or the larger size of the professional players (96.7 ± 8.5 vs. 86.5 ± 1.6 kg) since the difference in total sweat loss between the games was abolished when sweat loss was expressed per kg body mass (0.037 vs. 0.038 L/kg). Despite the relatively cool environment of the arena, the players in the present study were heavy sweaters with large individual variability (1.3 – 6.1 L) that was independent of playing time. The defensemen sweat the most at 3.7 ± 0.1 L, the forwards sweat 3.2 ± 0.1 L, and the two goalies sweat 2.0 ± 0.7 L. This is in contrast to the work of Palmer & Spriet (2008) who reported that the goalies had the highest sweat loss during a one-hour practice (2.9 ± 0.2 L) and attributed this to their constant involvement in the drills during the practice, while forwards and defense waited their turns before repeating the drills. During a game, goalies only respond to game play in their end of the ice. Forwards and defensemen, however, are constantly active throughout their shift resulting in greater sweat losses incurred over the game compared to practice (Palmer & Spriet 2008). This study affirmed our hypothesis that these players have higher total sweat losses during a game situation vs. practices.

3.5.3 Fluid Balance

Fluid intake during sport is influenced by the opportunities to drink during formal rest periods and informal stoppages in play and shift changes (Broad et al. 1996, Burke 1997). When athletes were monitored in many sports, including football, soccer, rugby, basketball, cricket, and netball, they did not, on average, consume enough fluid to maintain fluid balance, resulting in voluntary hypohydration (Broad et al. 1996, Devlin et al. 2001, Goodman et al. 1985, Kirkendall 1993, Mustafa & Mahmoud 1979, Osterberg et al. 2009, Pohl et al. 1981). Generally,
they replaced only ~50% of sweat losses (Burke 1997), in spite of the fact that some sports offer many opportunities to hydrate. In the present study, the players had abundant opportunities to drink during a game (on the bench between shifts and during intermissions) and replaced ~66% of net fluid loss. This is higher than previously reported in other team sports, possibly due to numerous opportunities to drink and the hydration feedback provided from prior practice testing sessions leading the players tested in this study to be more aware of their hydration habits. According to Shirreffs et al. (2009) hypohydration equivalent to ~2% BM loss during exercise in a hot environment (> 30 °C) significantly impairs endurance performance, but has a lesser and inconsequential effect in temperate environments (20 – 21°C), and may be tolerable in cold environments. In this study, players did an adequate job of replacing lost fluid and averaged a loss of just 1.3% BM. However, 8 players (2 defense and 6 forwards) did a poor job and lost ≥ 1.8 % BM (4.3%, 3.1%, 2.7%, 2.5%, 2.1%, 2.0%, 1.9%, and 1.8%) suggesting they may have voluntarily put themselves in a range where they may have experienced cognitive and physiological consequences as a result of their poor hydration habits. While the atmosphere in the arena was cool at ~10°C, the skin and core temperatures of the players would have mimicked a hot environment. To date, no research investigating the impact of hypohydration (> 2% BM loss) on on-ice hockey performance has been reported to establish if high intensity exercise in a ~10°C environment with high sweat losses negatively affects performance.

3.5.4 Sodium Balance

The mean sweat Na⁺ loss found in this study (-3.1 ± 0.3 g) using a forehead sweat patch over the game was substantially higher than reported by Godek et al. (2006) during a game in elite hockey players (-1.9 g) when sweat Na⁺ loss was assessed via forearm sweat patch analysis. Sodium being the major ion of the extracellular compartment is a pre-requisite for restoration of fluid balance, and hence, Na⁺ replacement during exercise is pivotal to the maintenance of plasma volume for the prevention of cardiovascular and thermoregulatory strain due to inadequate fluid intake (Sanders et al. 2001, Sharp 2006). The important role Na⁺ plays in fluid retention during exercise is supported by the significant relationship found in this study between % BM loss and Na⁺ loss (r = 0.53). The use of Na⁺ containing carbohydrate electrolyte solutions (CES) can offset some of the losses incurred during prolonged and heavy sweating. However, the [Na⁺] in most CES is low and coupled with the fact that the players drank a CES only before the game and during intermissions resulted in a Na⁺ replacement
that was only 15% of estimated losses. Several other players (42%) supplemented the CES with GatorLYTES® and increased the [Na⁺] of the solution from ~20 to 95 mmol·L⁻¹. This improved the replacement of Na⁺ to ~40% of that lost, but low consumption of the CES limited the replacement. The combination of a high sweat [Na⁺] and a high sweat rate poses a problem to Na⁺ balance in elite male hockey players and reinforces the suggestion that these athletes must be educated to replenish sweat Na⁺ losses during intense training sessions and games.

### 3.5.5 Repeat Players

This is the first study to report repeat data on hockey players over two separate games separated by one season. On average, players drank more water and CES, sweat less, and played more in game 2 compared to game 1. Players continued to limit CES intake to pre-game and during intermissions only. Four of six players increased their Na⁺ intake via drinking more CES and supplementing with GatorLYTES®, which increased their Na⁺ replacement from ~7% in game 1 to ~13% in game 2. It may be that the player feedback from game 1 made athletes aware of their hydration habits before and during the game which aided in the improvement of their fluid and Na⁺ balance seen in a follow-up game one year later.

### 3.6 CONCLUSIONS

Several players in this study arrived to the game in a hypohydrated state. However, players consumed an average 0.6 L of fluid prior to the game such that all players would have been well hydrated prior to starting the game. While players replaced an average of ~66% of sweat losses, 33% of the players did a poor job at replacing sweat losses and lost between 1.8 – 4.3% BM despite abundant opportunities to hydrate. Players also lost a significant amount of Na⁺ during the game despite attempting to replenish Na⁺ with a CES alone or in combination with GatorLYTES®. Large individual variability in all the present measurements highlights the importance of individual player feedback to ensure athletes are given the information necessary to continually improve their hydration habits from game to game. The non-obstructive testing approach during the game made it less likely that our presence influenced the hydration habits of the players compared to practice testing. Future research is required to determine whether sweat losses of ~1.5 - 2% body mass and above negatively affects on-ice hockey performance.
CHAPTER FOUR

THE ACUTE EFFECTS OF FLUID INTAKE ON URINE SPECIFIC GRAVITY (USG) AND FLUID RETENTION IN A MILDLY DEHYDRATED STATE

SUBMITTED – J. STRENG. COND. RES.
4.1 ABSTRACT

Many athletes arrive at training sessions and competitions in a mildly dehydrated (DEH) state and are instructed to drink fluids before exercise to reach a euhydrated (HYD) state. Ten recreational athletes (71.9 ± 4.6 kg, 25.2 ± 0.9 y) participated in studies to examine 1) the day-to-day variability of morning urine specific gravity (USG) and consuming 600 mL of water on the hydration status of HYD and DEH (USG > 1.020) subjects, and 2) the effects of consuming water or carbohydrate electrolyte solutions (CES) on the hydration status of DEH subjects. The day-to-day variability in morning USG (CV = 0.2 ± 0.1 %) was low and the hydration responses to the ingestion of 600 mL of water were repeatable. Pre-trial USG was 1.022 ± 0.001 in the DEH trial and decreased below 1.020 by 45 min (1.013 ± 0.003). In the CES study, DEH subjects reached HYD status at 45 min in all conditions (Water (W) 1.013 ± 0.003, Water-Salt (W-S) 1.013 ± 0.003, CES 1.017 ± 0.004, CES-Low (CES-L) 1.011 ± 0.003) as CES ingestion did not affect fluid retention (W 68%, W-S 72%, CES 76%, CES-L 68%). This study demonstrated that morning USG and hydration responses to the ingestion of 600 mL of water were repeatable and that mildly dehydrated subjects could reach euhydration within 45 min following the ingestion of 600 mL of water, with no added effect of carbohydrate-electrolyte solutions.
4.2 INTRODUCTION

Losing as little as 2% body mass through sweating has been shown to compromise physiological functioning during exercise. Cardiovascular and thermoregulatory responses, and ratings of perceived exertion (RPE) were higher when dehydrated, contributing to premature fatigue at the same relative and absolute exercise intensity compared to a hydrated state (Sawka, Young, Francesconi, Muza, & Pandolf, 1985; Montain & Coyle, 1992; Gonzalez-Alonso, Mora-Rodriguez, Below, & Coyle, 1995; Armstrong et al. 1997). Similarly, dehydration prior to exercise exacerbates the rate of increase in heart rate, core temperature, and RPE during exercise compared to starting exercise hydrated (Sawka et al. 1985; Stover et al. 2006b). The severity of these effects is directly related to the degree of hypohydration (Sawka et al. 1985; Montain & Coyle, 1992; Stover et al. 2006b).

The use of urine specific gravity (USG) is a simple, accurate and valid indicator of whether an athlete is dehydrated prior to exercise (Armstrong et al. 1994; Shirreffs & Maughan, 1998a; Armstrong 2005, 2007). Armstrong et al. (1994) was the first to report that USG and urine osmolality (Uosm) were valid and reliable indicators of hydration status and could be used interchangeably to determine hydration state during field-testing. Oppliger et al. (2005) also reported that both USG and Uosm mirrored acute changes in plasma osmolality and concluded that USG and Uosm were accurate determinants of hydration status in field-testing. In support, Stover et al. (2006a) reported that USG measured with refractometry strongly correlated with Uosm ($r = 0.995$) and a USG of 1.020 correlated with a Uosm of ~ 800 mOsm/kg. More recently, Lew et al. (2010) confirmed the reliability of USG and body mass to indicate hydration status between days in adolescents and reported an inverse relationship between morning body mass and USG, revealing that when USG was above 1.020, a 0.003 increase in USG reflected a 1% loss in body mass. Using refractometry, the National Athletic Trainer’s Association (NATA) Position Statement on fluid replacement in athletes classifies hydration state into four conditions; well hydrated (USG < 1.010), minimal hypohydration (USG 1.010-1.020), significant hypohydration (USG ≥ 1.020), and serious hypohydration (USG > 1.030) (Casa et al. 2000). The published Position Stand on exercise and fluid replacement by the American College of Sports Medicine (ACSM) also classifies hypohydration as an USG exceeding 1.020 (Sawka et al. 2007).

Several reports indicate that a high percentage of athletes arrive for training sessions and competitions mildly dehydrated. For instance, Bergeron et al. (2007) reported mean USG to be 1.025 ± 0.002 prior to a junior national tennis competition. Likewise, our laboratory reported a pre-training
USG of 1.024 ± 0.001 in junior male hockey players (Palmer, Logan, & Spriet, 2010) and that 41% of the same players arrived mildly dehydrated prior to a hockey game (Logan-Sprenger, Palmer, & Spriet, 2011). Similarly, Stover et al. (2006b) monitored the pre-practice hydration status of high school football players over a 5 d period during twice a day practices and showed that 60 - 77% of the players were hypohydrated prior to practice each day with a USG >1.020, while the repeatability in morning USG was high within subjects. More recently, Higham et al. (2009) compared daily hydration profiles of competitive adolescents over 4 consecutive days prior to training and reported mean USG to be 1.025 ± 0.001 with high repeatability between days. Overall, the majority of athletes arrive at training and competition sessions in a hypohydrated state and based on morning USG athletes appear to be hypohydrated on a daily basis during training camps.

The goals of this study were to (i) monitor the day to day repeatability in morning USG over 5 consecutive days in adult subjects, (ii) assess the time course of consuming 600 mL of water on the hydration status of euhydration and dehydrated subjects, and (iii) assess whether 600 mL of combinations of carbohydrate and/or electrolyte solutions (CES) are more effective than water at restoring euhydration in dehydrated subjects. Based on previous research, we hypothesized that (i) morning USG would be repeatable for subjects between days, (ii) hydration state (USG) would be significantly improved within 60 min of consuming 600 mL of water, (iii) drinking 600 mL of a CES solution would increase fluid retention and hydration status of dehydrated subjects to a greater extent than water.

4.3 METHODS

4.3.1 Subject Characteristics

Ten recreationally active individuals (6 females, 4 males) volunteered to participate in these experiments. Their mean (± SE) age and body mass were 25.2 ± 0.9 y and 71.9 ± 4.6 kg. In Study 2, one female subject withdrew from the study (n = 9). Each participant engaged in physical activity at least three times per week. Female participants were taking oral contraceptives. All participants were informed of the experimental protocol, both orally and in writing, before written informed consent was obtained. The study was approved by the Research Ethics Board at the University of Guelph.
4.3.2 Experimental Protocol

Study 1

Morning hydration status over 5 consecutive days

Upon waking on five consecutive weekdays participants collected a midstream urine sample. After voiding their bladder, body mass (BM) was determined on a Zenith scale. Each morning urine sample was refrigerated at 4°C and later analyzed for USG. Participants were instructed to weigh themselves at the same time and with the same clothing each day and record their BM and daily fluid intake in a data sheet provided. Participants were asked to drink normally the night before each trial.

Effect of fluid consumption on hydration state

On four weekday afternoons over a two-week period, participants arrived to the laboratory at 3 PM for an experimental trial. Participants restrained from consuming caffeine the day of each trial and dietary intake was replicated for each trial day. On two of the four randomized occasions, participants were allowed to drink *ad libitum* before the trial in order to arrive in a hydrated (HYD) state (USG < 1.020). For the other two experimental trials subjects drank *ad libitum* until 11 AM and then were asked not to drink any fluid up until the time of their trial (3 PM) to ensure participants arrived in a dehydrated (DEH) state (USG > 1.020). Upon arriving to the laboratory participants voided their bladder and provided a small mid-stream urine sample for the determination of pre-trial USG (Fig. 4.1). The participant was then weighed to ascertain pre-trial BM. Each subject drank 300 mL of water at 0 min and another 300 mL of water at 15 min for a total of 600 mL of fluid consumed. Every 15 min (30, 45, 60 min) participants were asked to void their bladder into a measuring cup provided to determine USG and urine volume (Uvol), and were weighed.
Figure 4.1 Study timeline. Subjects consumed 300 mL of fluid at 0 and 15 min. *Subject voided their bladder and provided a small mid-stream urine sample for the determination of urine specific gravity, followed by a body mass measurement. In study 1, there was no urine sample or body mass measurement at 15 min.

Study 2

Effect of carbohydrate and/or electrolyte solutions (CES) on hydration status

On four weekday mornings (once per week), participants arrived at 9 AM to the laboratory for an experimental trial with explicit instructions not to drink any fluid upon waking. Participants replicated the same experimental protocol as Figure 1, however an additional urine sample was collected and a BM measurement was taken at 15 min. Participants completed four randomized trials which varied in the type of fluid ingested; (i) water (W), (ii) a water-sodium solution (40 mmol/L\textsuperscript{-1} Na\textsuperscript{+}, W-S), (iii) a commercially available light carbohydrate-electrolyte solution (G2\textsuperscript{®}, 20 mmol/L\textsuperscript{-1} Na\textsuperscript{+}, 3% carbohydrate, CES-L), (iv) a commercially available carbohydrate-electrolyte solution (Gatorade\textsuperscript{®}, 20 mmol/L\textsuperscript{-1} Na\textsuperscript{+}, 6% carbohydrate, CES).

4.3.3. Analyses

USG and BM were determined using the same methods as outlined in Chapter 2. Each urine sample using a hand-held pocket refractometer (see Chapter TWO methods). Uvol was collected in measuring cups accurate to ± 25 mL.
4.3.4 Statistical Analysis

All data were tested for normality of distribution and presented as the mean ± standard error (SE). Time versus trial data was assessed using a two-way ANOVA. A one-way ANOVA was used to compare differences between fluid types. The significance of the measured differences between two trials was assessed using a paired $t$ test. Statistical significance was accepted as $p < .05$. Associations between variables were investigated using Pearson’s correlation analysis. The CV was calculated using the standard deviation divided by the mean, multiplied by 100.

4.4 RESULTS

4.4.1 Study 1: Morning USG

Morning USG was consistent over consecutive days (M 1.015 ± 0.002, Tu 1.017 ± 0.004, W 1.019 ± 0.002, Th1.018 ± 0.003, F 1.017 ± 0.002). All correlations were significant between any two days (sample correlation in Figure 4.2), and there was no significant difference in USG between days.

![Figure 4.2](image)

**Figure 4.2** Study 1. Correlation between morning urine specific gravity (USG) on Tuesday versus Thursday (n = 10).
The mean coefficient of variation (CV) for individual USGs between days was 0.2 ± 0.1%. Three participants had a mean morning USG of ≥ 1.020, six participants had USGs between 1.012 - 1.019, and one participant had a mean USG < 1.008. Body mass was also consistent between days (M 72.5 ± 5.0, Tu 72.1 ± 4.9, W 71.9 ± 4.9, Th 71.9 ± 4.9, F 71.8 ± 4.8 kg) with a mean individual CV of 0.3 ± 0.4%. Mean daily fluid intake was 3495 ± 511 mL with low variability within participants but large variability between subjects (1538 – 7299 mL). All participants consumed between 1500 – 5000 mL of fluid per day with the exception of one participant who drank between 6000 – 7300 mL per day.

4.4.2 Study 1: Effect of water consumption on USG

**Trial Repeatability**

Test-retest data revealed that USG and Uvol were repeatable between HYD and DEH trials (Table 4.1). The mean data of the two HYD and the two DEH trials were therefore presented below. A strong relationship was found between pre-trial USG and total Uvol (r = 0.54).

**Table 4.1** Repeatability of urine specific gravity (USG) and urine volume (Uvol) in the hydrated (HYD) and dehydrated (DYD) trials (n = 10).

<table>
<thead>
<tr>
<th></th>
<th>USG</th>
<th></th>
<th></th>
<th>Uvol (mL)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HYD</td>
<td>0</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>0</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>Total</td>
</tr>
<tr>
<td>Water 1</td>
<td>1.012 ± 0.002</td>
<td>1.008 ± 0.003</td>
<td>1.004 ± 0.002</td>
<td>1.003 ± 0.001</td>
<td>-----</td>
<td>145 ± 40</td>
<td>131 ± 30</td>
<td>87 ± 22</td>
<td>363 ± 13</td>
</tr>
<tr>
<td>Water 2</td>
<td>1.012 ± 0.002</td>
<td>1.008 ± 0.003</td>
<td>1.003 ± 0.001</td>
<td>1.003 ± 0.001</td>
<td>-----</td>
<td>121 ± 19</td>
<td>108 ± 23</td>
<td>120 ± 17</td>
<td>349 ± 18</td>
</tr>
<tr>
<td>DEH</td>
<td>1.022 ± 0.001</td>
<td>1.021 ± 0.001</td>
<td>1.014 ± 0.003</td>
<td>1.010 ± 0.004</td>
<td>-----</td>
<td>42 ± 9</td>
<td>49 ± 8</td>
<td>74 ± 14</td>
<td>170 ± 14</td>
</tr>
<tr>
<td>Water 1</td>
<td>1.024 ± 0.002</td>
<td>1.021 ± 0.001</td>
<td>1.014 ± 0.003</td>
<td>1.010 ± 0.003</td>
<td>-----</td>
<td>31 ± 10</td>
<td>60 ± 24</td>
<td>74 ± 19</td>
<td>165 ± 50</td>
</tr>
</tbody>
</table>

**Hydration Trial (HYD)**

Pre-trial USG indicated that all participants were well hydrated prior to the trial (1.012 ± 0.002, Table 1) and USG was significantly lower than in the dehydrated trials. The USG was significantly lower at 45 and 60 min than the pre-trial USG (Fig. 4.3a). Mean total Uvol over the trial was 356 ± 7 mL, such that 41% of the fluid consumed was retained. Average pre-trial BM was 73.2 ± 5.1 and was unchanged after the trial at 73.4 ± 5.0 kg.
Figure. 4.3 (a) Effect of time on urine specific gravity (USG) in the dehydrated (DEH) versus hydrated (HYD) trials following the ingestion of 600 mL of fluid. (b) Urine volume (Uvol) in the DEH versus HYD trials. Data are means ± SE (n = 10). *Significantly lower than 0 min. **Significantly greater than the DEH trial.

Dehydrated Trial (DEH)

Pre-trial USG indicated that participants were dehydrated prior to the trial (1.022 ± 0.004, Table 4.1). USG was lower than pre-trial USG and within the euhydrated range at 45 (1.013 ± 0.003) and 60 (1.010 ± 0.002) min following ingestion of 600 mL of water (Fig 4.3a). Mean total Uvol was 167 ± 3 mL and significantly lower than the HYD trial (Fig. 4.3b), such that participants retained 72% of the fluid consumed. BM increased from a pre-trial mass of 73.6 ± 5.1 to 73.9 ± 5.1 kg after 60 min.

4.4.3 Study 2: Impact of CES ingestion on USG

There was no significant difference between pre-trial BM or USG in the four trials. All participants were dehydrated prior to all trials (Table 4.2).

Table 4.2 Pre-trial urine specific gravity (USG) and body mass (BM) in the water (W), water-salt (W-S), carbohydrate-electrolyte solution light (CES-L), and carbohydrate-electrolyte solution (CES) trials. Data are presented as means ± SE (n = 9).

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>W-S</th>
<th>CES-L</th>
<th>CES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>72.0 ± 4.6</td>
<td>72.1 ± 4.5</td>
<td>71.8 ± 4.8</td>
<td>71.8 ± 4.6</td>
</tr>
<tr>
<td>Pre-trial USG</td>
<td>1.024 ± 0.002</td>
<td>1.024 ± 0.001</td>
<td>1.023 ± 0.001</td>
<td>1.023 ± 0.001</td>
</tr>
</tbody>
</table>

Consuming 600 mL of W, W-S, CES, or CES-L decreased USG below the pre-trial USG and 1.020 at 45 and 60 min with no significant differences between trials (Fig. 4.4a).

There were also no significant differences for total Uvol between trials (W 192 ± 49, W-S 166 ± 38, CES-L 193 ± 30, CES 147 ± 30 mL, Fig. 4.4b). Mean fluid retention was 68% in the W and CES-L trials, 72% in the W-S trial, and 76% in the CES trial, but these results were not significantly different.
**USG** was significantly lower than 0 time in the **W**, **W-S**, **CES-L**, and **CES** trials at 45 and 60 min.

### 4.5 DISCUSSION

This study examined the reliability of hydration status over five consecutive days and how **USG** was affected by consuming 600 mL of fluid in the hour preceding training or competition. The main findings of this study were (i) individual morning **USG** was highly repeatable over five consecutive days, (ii) the **USG** and fluid retention responses to ingesting 600 mL of water when euhydrated or dehydrated were also repeatable, (iii) consuming 600 mL of water when dehydrated significantly decreased **USG** to within the hydrated range within 45 min, and (iv) there was no difference in the type of fluid consumed (**W** vs. **W-S** vs. **CES**) in terms of reducing **USG** to within the hydrated range in the hour following consumption.

#### 4.5.1 Morning hydration status over 5 consecutive days

In this study, average morning **USG** over 5 consecutive days (1.017 ± 0.002) demonstrated that the mean **USG** indicated that adult participants were hydrated upon waking. However, 19 out of 50 responses (38%) were below 1.020. Our mean **USG** was lower than previously reported over 4-5 consecutive days with adolescent football players in two separate studies (Stover et al. 2006a, 1.023 ± 0.001; Godek et al. 2005, 1.023 ± 0.002) and adolescent swimmers (Higham et al. 2009, 1.025 ± 0.001). The higher mean **USG** seen in these studies is potentially due to greater sweat losses during training camp without proper rehydration compared to our recreationally active participants who were not involved in a training camp over the 5 days of testing. Another possibility relates to the age of the participants, as the present study employed adult participants vs. the adolescent participants studied in the previous studies. A consistent result between the previous studies and the present study is the strong individual repeatability in morning **USG** between days. This reflects the reliability of using morning **USG** as an indicator of hydration status in recreational and competitive athletes over consecutive days (Shirreffs & Maughan, 1998a). When investigating the relationship between morning **BM** and **USG**, Lew et al. (2010) reported a significant inverse
correlation, such that when USG was ≥ 1.020, a 0.003 increase in USG reflected a 1% loss in BM. The data in the present study along with that of Armstrong et al. (2005) and Lew et al. (2010) suggest that monitoring morning BM is also a useful indicator of hydration status when USG is not available.

4.5.2 Effect of water consumption on hydration status

This is the first study to examine the impact of consuming 600 mL of water in euhydrated and dehydrated states on hydration status over a 60 min period and confirms that USG is highly repeatable for participants between trials. It is well established that many athletes arrive to training or competition dehydrated (> 1.020), which may exacerbate the cardiovascular and thermoregulatory responses at the beginning of exercise (Godek, Godek, & Bartolozzi, 2005; Bergeron, Waller, & Marinik, 2006; Dougherty, Baker, Chow, & Kenney, 2006; Stover, Zachwieja, Stefan, Murray, & Horswill, 2006a; Stover et al. 2006b; Higham, Naughton, Burt, & Shi, 2009; Osterberg, Horswill, & Baker, 2009). Our results show that if an athlete arrives to training or competition in a dehydrated state and consumes 600 mL of water, the athlete will move into a hydrated state (USG < 1.020) within 45 min. This novel information demonstrates that athletes who arrive to training or competitions in a dehydrated state can quickly rehydrate in order to prevent dehydration related detriments to performance. In two studies from the same laboratory on collegiate wrestlers following acute dehydration of ~3% BM, consuming water to replace 150% of BM losses (3.6 – 3.8 L) in the hour prior to competition was effective in significantly lowering USG into the hydrated range, and lowering urine osmolality (Uosm), and plasma osmolality (Posm) (Valiente, Utter, Quindry, & Nieman, 2009; Utter & Quindry, 2010). Our results are novel in that only 600 mL of fluid consumed was effective at significantly lowering USG within 45 min compared to the USG change seen in the previous studies after rehydrating with 150% of BM losses amounting to 3.6 L (Valiente et al. 2009) and 3.8 L (Utter et al. 2010). Ultimately, our results are relevant for normal exercise situations with mild dehydration as our participants were not dehydrated from previous exercise prior to testing as compared to the latter two studies.

In addition, two studies reported that both USG and Uosm lagged behind Posm in accurately identifying changes in hydration state produced by acute dehydration after rehydrating with 100% BM losses (Popowski et al. 2001, Oppliger et al. 2005). Although Uosm and Posm were not measured in this study we could predict based on the data of the above studies that the significant lowering in USG after consuming 600 mL indicates that Posm was also lowered to a value consistent with effective rehydration. In addition, we showed that when subjects arrived hydrated to the trial,
41% of the 600 mL of water consumed was retained while 72% of the ingested water was retained when participants arrived in a hypohydrated state. As expected, a strong relationship was found between pre-trial USG and total Uvol, such that greater dehydration lead to greater fluid retention to restore fluid homeostasis.

**4.5.3 Impact of carbohydrate and electrolyte fluid ingestion on hydration status**

In this study we expected to see greater fluid retention after consuming 600 mL of salt and/or carbohydrate solutions (W-S, CES, and CES-L). However our results did not demonstrate a significant difference between trials in fluid retention measured by fluid retention/urine output. This is in contrast to other studies where dehydration was induced by exercise and the following rehydration was more severe (~150% BM losses) (Shirreffs and Maughan 1998b). Valiente et al. (2009) and Shirreffs & Maughan (1998b) reported that a greater amount of fluid was retained after exercise when rehydration with a CES compared to water. The authors attributed these results in part to the increased osmolality of the CES to promote greater fluid retention and less acute diuresis. Shirreffs et al. (2007a) also suggested that the amount of fluid retained was directly proportional to the [Na⁺] of the fluid consumed when dehydrated. As well, many previous studies demonstrated that, even if fluid intake is adequate, when the electrolyte concentrations are low there is an increase in urinary excretion and, consequently, the participants are in negative fluid balance (Aragón-Vargas & Madriz-Dávila, 2000; Shirreffs & Maughan 1998b, Shirreffs, Taylor, Leiper, & Maughan, 1993). It was therefore hypothesized that the higher osmolality fluid (CES) would have less of an impact on USG response and greater fluid retention. However, the results of this study reported no difference in the type of fluid consumed on USG response or total Uvol over a 1 hour period after consuming 600 mL of fluid. There was a trend for fluid retention to be slightly greater in the CES trial (76% retention), followed by the W-S (72%), and then the W and CES-L trials (68%), however the differences between conditions were insignificant. Given the effectiveness of plain water in rehydrating the mildly dehydrated participants and retaining 68% of the water, it may not have been realistic to expect a greater effect with the combinations of carbohydrate and electrolyte solutions.

Since many athletes are instructed to drink 6-8 mL/kg BM of a Na⁺ containing fluid in the 1-2 hours before exercise (Shirreffs, Casa, & Carter, 2007b), we adopted this strategy as our subjects did not dehydrate themselves with previous exercise like the studies of Valiente et al. (2009) and Utter et al. (2010) who dehydrated their subjects to ~3% BM losses before rehydrating with 150% BM losses. Instead we focused on a mild dehydration situation (≤ 1% BM loss) that may occur during normal living prior to training and competition as this may be more representative of the normal
situation for the average athlete. If the correlation reported by Lew et al. (2010) between BM and USG is used (a 0.003 increase in USG above 1.020 reflects a 1% loss in BM) then our participants replaced ~41% of fluid loss by consuming 600 mL of fluid. Ultimately, our results demonstrate that if an individual is mildly dehydrated before exercise, consuming 600 mL of any fluid (W vs. W-S vs. CES-L vs. CES) will-reverse the dehydration and put an athlete in a hydrated state (USG > 1.020) within 45 min prior to exercise.

4.6 CONCLUSIONS

In conclusion, this study affirms the reliability of monitoring morning BM and USG over consecutive days in adults as a reliable indication of daily hydration status. Monitoring morning hydration state (USG or BM) is valuable for athletes to ensure they are replenishing daily sweat losses over subsequent training days. Our results were also highly repeatable between days when participants arrived for testing in a hydrated and dehydrated condition and proceeded to consume 600 mL of water. Lastly, this study demonstrated that the consumption of either 600 mL of water, water with electrolytes, or carbohydrate electrolyte solutions, 45 min prior to training or competition is sufficient to ensure that an athlete begins exercise hydrated.
CHAPTER FIVE

THE EFFECTS OF PROGRESSIVE DEHYDRATION DURING PROLONGED CYCLING ON SKELETAL MUSCLE METABOLISM IN HYDRATED FEMALES

SUBMITTED – MED. SCI. SPORTS EXERC.
5.1 ABSTRACT

This study investigated the effects of progressive dehydration on the time course of changes to whole body substrate oxidation and skeletal muscle metabolism during 120 min of moderate intensity cycling in hydrated female subjects. Subjects (n=9) cycled for 120 min at ~65% VO$_{2\text{peak}}$ on two occasions: once with no fluid ingestion (DEH) and once with fluid replacement to match sweat losses (HYD). Venous blood samples were taken at rest and every 20 min and muscle biopsies were taken at 0, 60 and 120 min of exercise. Subjects lost 0.9% body mass (BM) from 0–60 min and 1.1% BM from 60–120 min in the DEH trial (2.0% total). Heart rate (HR) and core temperature (Tc) were significantly higher in the DEH trial from 30–120 min. Plasma volume loss was significantly greater in the DEH trial from 40-120 min. Rating of perceived exertion (RPE) was significantly greater in the DEH trial from 60–120 min. There were no differences in VO$_2$ or sweat loss between trials. RER (HYD 0.85 ± 0.01 vs. DEH 0.87 ± 0.01) and total carbohydrate (CHO) oxidation (175 ± 17 vs. 191 ± 17 g) were significantly higher in the DEH trial. Blood [La] was significantly higher in the DEH trial with no change in plasma FFA and epinephrine. Muscle glycogenolysis was 31% greater in the DEH trial (252 ± 49 vs. 330 ± 33 mmol/kg dm) and muscle [La] was also higher at 60 min. In summary, progressive dehydration in female subjects significantly increased HR, Tc, RPE, plasma volume loss, and whole body CHO oxidation and muscle glycogenolysis, and these changes were already apparent in the first hour of exercise when BM losses were ≤1%. The increased muscle glycogenolysis with dehydration appeared to be due to increased core and muscle temperature, secondary to less efficient movement of heat from the core to the periphery.
5.2 INTRODUCTION

Mild dehydration during exercise can be a major concern for athletes as it may lead to premature fatigue. The cardiovascular and thermoregulatory consequences to exercising dehydrated are well documented with the magnitude of these effects directly proportional to the degree of dehydration (Armstrong et al. 1997; Montain & Coyle, 1992; Nadel et al. 1980; Sawka et al. 1985). For example, as little as a 2% loss in body mass (BM) due to dehydration has been consistently reported to result in elevated heart rate, core temperature, rate of perceived exertion, and plasma osmolality. However, little research has investigated the effects of exercise-induced dehydration on whole body substrate utilization and skeletal muscle metabolism. Hargreaves et al. (1999) investigated the effects of exercise-induced dehydration on muscle metabolism in males and reported that a 3% BM loss resulted in a significantly higher rectal and muscle temperature at 120 min of exercise, with no difference in rectal temperature between trials at any other time point during exercise. The respiratory exchange ratio was significantly higher in the fluid restricted trial after 60 and 120 min of exercise with the difference between trials being greater in the second hour of cycling. The study reported a 16% greater glycogen use during the 120 min of exercise (Hargreaves et al. 1999). Febbraio (2000) reviewed the relevant literature examining exercise in the heat and concluded that when muscle temperature is higher than control during exercise there is increased muscle glycogenolysis. However, many studies did not control for hydration status and have only been conducted in males. Presently there are no studies investigating the time course of progressive exercise-induced dehydration on whole body substrate oxidation and skeletal muscle metabolism in females.

It has been suggested that women thermoregulate less effectively as indicated by higher core temperatures during same load exercise compared to males (Nunneley 1978). Work by Gagnon et al. (2009) also suggested that females experience a quicker rise in core temperature during exercise, which may accelerate muscle glycogen use. The impact of the heightened core temperature during prolonged exercise in females coupled with the thermal stress associated with progressive dehydration on substrate oxidation and muscle metabolism has yet to be elucidated.

There are also limited data on the sweat rate of females during exercise. Hazelhurst and Claassen (2006) investigated the gender differences in sweat response in trained male and female athletes during a 90 min spinning exercise class and demonstrated that sweat loss in males was significantly higher than the female subjects (13.3 vs. 9.4 mL/kg/h⁻¹). Kilding et al. (2009) determined the sweat rate of elite female soccer players during two game-specific training sessions and reported
that mean sweat losses were less than previously reported in male soccer players. Ultimately, these studies suggested that the female sweating response differs from that of males, and sweat rates were significantly lower for the same relative exercise intensity in females even after adjustment for anthropometric measures (Kilding et al. 2009).

In light of the potential thermal and sweat rate differences between genders, the small number of studies evaluating sweat responses in females, and the lack of investigations examining the effects of mild dehydration (1-2%) on muscle metabolism in general, we investigated the effects of progressive exercise-induced dehydration in females to determine the time course of changes in physiological responses and skeletal muscle metabolism. We hypothesized that as female subjects progressively dehydrated during exercise and increased core temperature above the increase in the hydrated state, there would be a greater reliance on whole body carbohydrate oxidation and muscle glycogenolysis during prolonged exercise. As well, we expected that these differences would be augmented in the second hour of exercise in the dehydrated trial.

5.3 METHODS

5.3.1 Subjects Characteristics

Nine recreationally active females, mean age 21.7 ± 0.6 yr, height 155.7 ± 2.8 cm, weight 58.8 ± 2.8 kg, and VO$_{2peak}$ 2.9 ± 0.2 L/min, participated in the study. All subjects engaged in recreational physical activity 2-3 days/wk and were taking oral contraceptives. Subjects were using oral contraceptives or were tested in the follicular phase of the menstrual cycle. Subjects were informed both verbally and in writing of the experimental protocol and potential risks prior to giving their written consent to participate. The Research Ethics Boards of the University of Guelph and McMaster University approved the study.

5.3.2 Pre-experimental Protocol

In preparation for the experiment, subjects visited the laboratory on three separate occasions. On the first visit subjects performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (LODE Excalibur, Quinton Instrument, Groningen, The Netherlands) for the determination of VO$_{2peak}$. Respiratory gases were collected and analyzed using a metabolic cart (MOXUS metabolic system, AEI Technologies, Pittsburgh, USA). Following a 30 min break, subjects cycled for ~20 min at ~65% VO$_{2peak}$ to establish the power output for the subsequent 120 min trials.
On two subsequent occasions, subjects reported to the laboratory for practice trials and cycled at ~65% VO$_{2peak}$ for 120 min without fluid (DEH) or with fluid (HYD) to replace sweat losses. DEH trials occurred first to ascertain sweat losses over the 120 min trial and determine how much fluid subjects needed to drink throughout the HYD trial to maintain fluid balance. All subjects abstained from strenuous exercise and caffeine, and recorded their diet in the 24 h before the trials. Two hours prior to the practice rides, subjects ingested a meal provided for them (790 kcal; 144 g carbohydrate, 35 g fat, 19 g protein) and 250 mL of fluid. Subjects also drank 300 mL of water 90 and 45 min before each trial to ensure they were well hydrated before cycling. Upon arrival to the laboratory, subjects voided their bladder and provided a small mid-stream urine sample to determine urine specific gravity (USG) and completely voided their bladder. A pre-trial BM measurement was made wearing only dry shorts and a sports bra. Following 60 and 120 min of exercise, subjects stopped cycling and dismounted the cycle, removed their shoes and shirt, towed dry, voided if necessary and were weighed wearing only shorts and a sports bra for the determination of sweat loss during the previous hour of exercise. At 60 min, subjects put on a dry t-shirt and recommenced cycling. Any urine produced was included in the calculation of sweat loss (refer to equation Chapter Two). Three-minute respiratory gas measurements were collected every 20 min during exercise to determine the volume of oxygen consumed (VO$_2$), the volume of carbon dioxide produced (VCO$_2$), and to calculate the respiratory exchange ratio (Peronnet & Massicotte, 1991) and whole body carbohydrate (CHO) and fat oxidation with use of the nonprotein RER table and the following equations: $CHO$ oxidation (g) = 4.585 (VCO$_2$) - 3.226 (VO$_2$), and $fat$ oxidation (g) = 1.695 (VO$_2$) - 1.701 (VCO$_2$) (Ferrannini 1988; Peronnet & Massicotte, 1991). Practice trials were separated by 5-7 d.

5.3.3 Experimental Protocol

Subjects arrived to the laboratory on two occasions for the actual experiment. During the experimental trials subjects cycled at ~65% VO$_{2peak}$ for 120 min with fluid to match sweat losses (HYD) or without fluid (DEH). Subjects replicated the same procedure as described above for the practice trials. In addition, heart rate (HR) was collected using a Polar RS400 downloadable HR monitor (Polar Electro., Lachine, QC) and core temperature (Tc) was determined using a calibrated ingestible thermistor (HQ Inc., Palmetto, FL) that was ingested 3-5 h prior to each trial. Prior to exercise, a teflon catheter was inserted into an antecubital vein for blood sampling and was flushed with 0.9% saline to maintain patency. One leg was also prepared for percutaneous needle biopsy
sampling of the vastus lateralis muscle by the Bergström technique (Bergstrom 1962). Three incisions were made in the skin and deep fascia under local anesthesia (2% xylocaine without epinephrine) for three separate biopsies. Immediately before exercise, a venous blood (~5 mL) sample and a muscle biopsy were obtained while the subject rested on a bed. All muscle samples were immediately frozen in the needle in liquid nitrogen and stored in liquid nitrogen for subsequent analyses. Subjects then cycled for 120 min at ~65% VO\textsubscript{2peak} at a constant cadence (80-95 rpm). Venous blood samples were obtained at 20, 40, 60, 80, 100, and 120 min of exercise. HR, Tc, and rating of perceived exertion (RPE) were recorded every 15 min during exercise. RPE was determined using the BORG scale (rating 6-20) (Borg 1970). During the HYD trial subjects were given fluid every 15 min to match sweat loss and drank the fluid after HR, Tc, and RPE measurements were recorded. At 60 and 120 min, the subject stopped cycling and a muscle biopsy was taken with the subject sitting on the cycle ergometer. After the muscle biopsy was taken, subjects removed their shoes and shirt, towed dry, voided if necessary, and were weighed for the determination of BML over the previous 60 min of exercise. The same procedure was replicated for the second trial with muscle biopsies taken from the opposite leg and the trials were randomized and separated by 7 days.

5.3.4 Analyses

**Trial conditions.** Laboratory temperature (°C) and relative humidity (%) were measured using a Digital Thermometer (Fisher Scientific, Ottawa, ON). USG was measured (refer to Chapter Two for details).

**Blood measurements.** Venous blood was collected in sodium heparin tubes. A portion of whole blood (200 µl) was added to 1 mL of 0.6 M perchloric acid and centrifuged. The supernatant was stored at -20°C and later analyzed for blood glucose and lactate with fluorometric techniques (Bergmeyer 1974). A second portion (1.5 mL) was centrifuged and the supernatant was analyzed for plasma free fatty acids (FFA) with an enzymatic colorimetric technique (NEFA C test kit, Wako Chemicals, Richmond, VA). A third portion (1.5 mL) was added to 30 mL of EGTA and reduced glutathione and centrifuged (10,000 g) for 3 min, and the supernatant was analyzed for epinephrine with an enzymatic immunoassay kit (Epinephrine RIA kit, Rocky Mountain Diagnostics Inc., Colorado Springs, CO). The remaining venous blood was used for the determination of whole blood hemoglobin (Hb) and hematocrit (Hct). Hb was measured in duplicate using an automated blood analysis machine (OSM3 Hemoximeter; Radiometer, Copenhagen, Denmark). Hct was measured in triplicate using capillary tubes and a microhematocrit centrifuge and reader (Micro-capillary reader, Damon/IEC Division,
USA). The percent plasma volume change (%Pvol) was calculated using whole blood Hb and Hct measurements (Dill & Costill 1974).

Muscle metabolites. Each muscle biopsy was freeze dried, powdered, and dissected free of visible connective tissue, fat, and blood. One aliquot of freeze-dried powdered muscle (~10 mg) was extracted in 0.5 M HCLO$_4$-1 mM EDTA and neutralized with 2.2 M KHCO$_3$. The supernatant was used to measure phosphocreatine (PCr), creatine (Cr), adenosine triphosphate (ATP), and lactate. Muscle metabolites were normalized to the highest total Cr content measured from all biopsies from each subject. Muscle glycogen content was determined in duplicate using two additional aliquots of freeze dried muscle (2-4 mg). Glycogen was extracted in 0.1 M NaOH and neutralized with 0.1 M HCL - 0.2 M citric acid - 0.2 M Na$_2$PO$_4$, and amyloglucosidase was added to degrade glycogen to glucose, which was measured spectrophotometrically and normalized for total Cr (2).

Muscle calculations. Free ADP (ADP$_f$) and AMP (AMP$_f$) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (Dudley et al. 1987). Specifically, ADP$_f$ was calculated using the measured ATP, Cr, and PCr values, an estimated H$^+$ concentration, and the creatine kinase constant of 1.66 X 10$^9$ (Saltin 1990). AMP$_f$ was calculated from the estimated ADP$_f$ and measured ATP content using the adenylate kinase equilibrium constant of 1.05.

5.3.5 Statistical Analysis

All data were tested for normality of distribution and presented as the mean ± standard error (SE). Time versus trial data was assessed using a two-way ANOVA and specific differences were located using the Student-Newman-Keuls post hoc test. A paired t-test was used to compare single parameter data between trials. Statistical significance was accepted as $p < 0.05$.

5.4 RESULTS

5.4.1 Trial Conditions

No significant pre-trial differences existed between the HYD and DEH trials for laboratory temperature (HYD, 22.7 ± 0.1 vs. DEH, 22.5 ± 0.1°C), relative humidity (34 ± 2.9 vs. 31 ± 3.4 %), pre-trial BM (58.9 ± 2.9 vs. 58.8 ± 2.8 kg), or hydration state (USG; 1.007 ± 0.002 vs. 1.010 ± 0.003).
5.4.2 Body Mass Loss, Sweat Loss, and Fluid Intake

BM was maintained in the HYD trial by drinking a mean of 1.2 ± 0.9 L of fluid over 120 min of cycling (Table 5.1). In the DEH trial, BM was significantly lower at 60 and 120 min of cycling resulting in BM losses of 0.9% and 2.0% (Table 5.1). There were no significant differences in sweat loss between the HYD and DEH trials (Table 5.1). Only two of 9 subjects produced urine after the HYD trial (590 and 250 mL) and only 1 subject after the DEH trial (160 mL).

Table 5.1 Differences in sweat loss, body mass (BM) and percent BM loss between the dehydrated (DEH) and hydrated (HYD) trial at 0, 60, and 120 min of cycling at ~65% VO$_{2peak}$. Values are means ± SE, (n = 9). *Significantly lower than HYD and 0 min in DEH (p < 0.05).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HYD</th>
<th>DEH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Sweat Loss (L)</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>BM (kg)</td>
<td>58.9 ± 2.9</td>
<td>59.0 ± 2.9</td>
</tr>
<tr>
<td>BM loss (%)</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

5.4.3 Oxygen Uptake and Whole Body Substrate Use

VO$_2$ increased in both trials with exercise time and was significantly greater than 20 min at 40, 60, 80, 100, and 120 min in both trials. There was no difference in VO$_2$ between trials (Fig. 5.1a). The RER progressively decreased in both trials over time and was significantly lower than 20 min at all time points in each trial (Fig. 5.1b). The RER was significantly higher in the DEH vs. HYD trial from 40-120 min.
Figure 5.1 (a) VO₂ and (b) respiratory exchange ratio (26) during 120 min of cycling at ~65% VO₂peak in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). VO₂ and RER were significantly greater than 20 min at all time points in both trials (p < 0.05). *Significantly greater than HYD trial (p < 0.05). Arrows (↓) indicate ~1 and 2% BM loss.
CHO oxidation was also significantly greater in the DEH (0-60 min 111 ± 7, 60-120 min 82 ± 7 g) vs. HYD trial (102 ± 7, 73 ± 5 g) and fat oxidation was significantly lower in the DEH (0-60 min 17 ± 4, 60-120 min 30 ± 3 g) vs. HYD trial (21 ± 4, 35 ± 3 g). Total CHO oxidation was significantly greater in the DEH (193 ± 17 g) vs. HYD (175 ± 17 g) trials and total fat oxidation was lower in the DEH (47 ± 1 g) vs. HYD (56 ± 1 g) trials.

5.4.4 Heart Rate, Core Temperature, and Rating of Perceived Exertion

HR significantly increased over time in both trials and became significantly greater than 15 and 30 min at 45 min and beyond in both trials. Subjects had a significantly higher HR from 30 – 120 min of cycling in the DEH vs. HYD trial (Fig. 5.2).

**Figure 5.2** Heart rate response during 120 min of cycling at ~65% VO$_{2\text{peak}}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). HR was significantly greater than 15 and 30 min at 45 min and beyond in both trials ($p < 0.05$). *Significantly greater than HYD trial ($p < 0.05$). bpm, beats per minute. Arrows (↓) indicate ~1 and 2% BM loss.
Tc was significantly greater than 15 min for all time points in both trials. In the DEH trial, Tc was significantly greater than the HYD trial from 30 – 120 min (Fig. 5.3).

Figure 5.3 Core temperature (Tc) during 120 min of cycling at ~65% VO$_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). Tc was significantly greater than 15 min for all time points in both trials ($p < 0.05$). *Significantly higher than HYD trial ($p < 0.05$). Arrows (↓) indicate ~1 and 2% BM loss.

RPE significantly increased over time in both trials and was significantly greater than 15 min from 60 – 120 min. RPE was significantly greater in the DEH vs. HYD trial from 60 – 120 min (Fig. 5.4). The mean RPE over the entire 120 min trial was also significantly greater in the DEH trial (DEH, 13.9 ± 0.6 vs. HYD, 12.3 ± 0.4).
Figure 5.4 Rating of perceived exertion (RPE) during 120 min of cycling at ~65% VO$_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). RPE was significantly greater than 15 min from 60-120 min in both trials ($p < 0.05$). *Significantly higher than HYD trial ($p < 0.05$). Arrows (↓) indicate ~1 and 2% BM loss.

5.4.5 Blood Measurements

Hb and Hct were significantly higher than rest from 20 – 120 min of exercise in both trials (Table 5.2). In the DEH trial, Hb was significantly greater than the HYD trial from 40 – 120 min (Table 5.2). There was a trend for Hct to be higher in the DEH trial throughout exercise, but the difference did not reach significance until 120 min of exercise (Table 5.2). Pvol loss was significantly greater than rest in both trials at all exercise time points and significantly greater in the DEH vs. HYD trial from 40 – 120 min (Table 5.2). Blood glucose was significantly lower than rest between 40 – 120 min in both trials, with no difference between trials (Table 5.2). In the DEH trial, Hb was significantly greater than the HYD trial from 40 – 120 min (Table 5.2).
Table 5.2 Hemoglobin (Hb), hematocrit (Hct), glucose, lactate, and plasma volume loss (Pvol loss), plasma free fatty acid (FFA) and epinephrine (EPI), concentration during 120 min of cycling at ~65% \( \text{VO}_2\text{peak} \) in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE, \((n = 9)\). Blood Hb, Hct, Pvol loss, blood lactate, plasma FFA and EPI, were significantly greater than 0 min at all time points in both trials \((p < 0.05)\). Blood glucose was significantly lower than 0 min from 40 – 120 min in both trials \((p < 0.05)\). *Significantly greater than HYD \((p < 0.05)\).

<table>
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<th>Time (min)</th>
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<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/100mL)</td>
<td>HYD</td>
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<td>12.9±0.5</td>
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</tr>
<tr>
<td></td>
<td>DEH</td>
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<td>13.3±0.4*</td>
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<td>13.2±0.3*</td>
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<tr>
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<td>44.3±0.7*</td>
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<tr>
<td>Pvol Loss (%)</td>
<td>HYD</td>
<td>-----</td>
<td>-3.6±0.9</td>
<td>-4.6±0.7</td>
<td>-6.2±1.9</td>
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<td>-5.3±1.2</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>-----</td>
<td>-3.3±1.4</td>
<td>-8.2±0.9*</td>
<td>-9.6±1.1*</td>
<td>-9.5±0.9*</td>
<td>-10.1±1.1*</td>
<td>-11.3±1.2*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>HYD</td>
<td>4.5±0.1</td>
<td>4.1±0.2</td>
<td>4.0±0.1</td>
<td>3.9±0.2</td>
<td>3.8±0.1</td>
<td>3.8±0.1</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>4.5±0.1</td>
<td>4.2±0.1</td>
<td>4.2±0.1</td>
<td>4.1±0.1</td>
<td>4.0±0.1</td>
<td>4.1±0.1</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>HYD</td>
<td>0.8±0.1</td>
<td>1.5±0.4</td>
<td>1.3±0.7</td>
<td>1.3±0.3</td>
<td>1.0±0.2</td>
<td>1.4±0.4</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.8±0.1</td>
<td>2.2±0.6*</td>
<td>1.9±0.5*</td>
<td>2.0±0.6*</td>
<td>2.3±0.7*</td>
<td>2.0±0.6*</td>
<td>2.4±0.7*</td>
</tr>
<tr>
<td>Plasma FFA (mM)</td>
<td>HYD</td>
<td>0.2±0.04</td>
<td>-----</td>
<td>-----</td>
<td>0.4±0.1</td>
<td>0.6±0.1</td>
<td>-----</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.2±0.04</td>
<td>-----</td>
<td>-----</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
<td>-----</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Plasma EPI (nM)</td>
<td>HYD</td>
<td>0.8±0.1</td>
<td>-----</td>
<td>-----</td>
<td>1.3±0.2</td>
<td>1.4±0.2</td>
<td>-----</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.7±0.1</td>
<td>-----</td>
<td>-----</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
<td>-----</td>
<td>1.4±0.2</td>
</tr>
</tbody>
</table>

In the DEH trial, Hb was significantly greater than the HYD trial from 40 – 120 min (Table 5.2). There was a trend for Hct to be higher in the DEH trial throughout exercise, but the difference did not reach significance until 120 min of exercise (Table 5.2). Pvol loss was significantly greater than rest in both trials at all exercise time points and significantly greater in the DEH vs. HYD trial from 40 – 120 min (Table 5.2). Blood glucose was significantly lower than rest between 40 – 120 min in both trials, with no difference between trials (Table 5.2). Blood lactate was significantly increased from rest at 20 min of exercise and beyond in both trials and was also significantly higher in the DEH vs. HYD trial at all exercise time points (Table 5.2). Plasma FFA and epinephrine significantly increased from rest at all time points in both trials, with no significant differences between trials (Table 5.2).
5.4.6 Muscle Fuels and Metabolites

Skeletal muscle PCr significantly decreased in the first 60 min of exercise in both trials and remained significantly lower than rest at 120 min of exercise in both trials, but was not significantly different between trials (Table 5.3).

**Table 5.3** Skeletal muscle fuel and metabolite contents during 120 min of cycling at ~65% VO_{2peak} in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE, (n = 9). PCr, phosphocreatine; Cr, creatine; ATP, adenosine triphosphate; ADPf, free adenosine diphosphate; AMPf, free adenosine monophosphate; dm, dry muscle. PCr and glycogen were significantly lower than 0 at 60 and 120 min. Cr, ADPf, AMPf, and lactate were significantly greater than 0 at 60 and 120 min. *Significantly higher than HYD trial (p < 0.05).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HYD</th>
<th>DEH</th>
<th>DEH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>PCr mmol/kg d.m Cr</td>
<td>80.1 ± 4.3</td>
<td>60.1 ± 6.2</td>
<td>55.7 ± 7.4</td>
</tr>
<tr>
<td>ATP mmol/kg d.m</td>
<td>81.0 ± 3.8</td>
<td>101.0 ± 5.2</td>
<td>105.3 ± 8.9</td>
</tr>
<tr>
<td>ADPf mmol/kg d.m</td>
<td>24.9 ± 1.2</td>
<td>23.3 ± 1.0</td>
<td>24.4 ± 1.3</td>
</tr>
<tr>
<td>Lactate umol/kg dm</td>
<td>133.1 ± 11.8</td>
<td>230.5 ± 27.7</td>
<td>246.7 ± 49.4</td>
</tr>
<tr>
<td>AMPf umol/kg dm</td>
<td>0.7 ± 0.1</td>
<td>2.5 ± 0.7</td>
<td>3.4 ± 1.3</td>
</tr>
<tr>
<td>Glycogen mmol/kg d.m</td>
<td>425 ± 36</td>
<td>223 ± 41</td>
<td>173 ± 34</td>
</tr>
</tbody>
</table>

Cr increases were reciprocal with the PCr decreases. Muscle ATP content was not significantly changed with exercise or between trials (Table 5.3). Muscle free ADP and AMP was significantly increased at 60 and 120 min of exercise in both trials, but was not significantly different between trials (Table 5.3). Muscle lactate content increased with exercise and peaked at 60 min in both trials and was significantly greater at 60 min in the DEH vs. HYD trial (Table 5.3). Muscle glycogen content was similar in the two trials before exercise and significantly lower at 60 and 120 min in both trials compared to rest (Table 5.3). There was a significant 17% greater use of glycogen in the first 60 min of exercise between trials (DEH 238 ± 38 vs. HYD 203 ± 52 mmol/kg dm) and more glycogen use from 60-120 min of exercise in the DEH (92 ± 13 mmol/kg dm) vs. HYD (50 ± 14
mmol/kg dm) trials. Lastly, the glycogen use was also significantly 31% greater during the entire DEH (330 ± 33 mmol/kg dm) vs., HYD (252 ± 49 mmol/kg dm) trials (Fig. 5.5).

**Figure 5.5** Muscle glycogen use during 120 min of cycling at ~65% VO$_{2\text{peak}}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). *Significantly greater than HYD trial (p < 0.05).

### 5.5 DISCUSSION

This study investigated the effects of mild progressive dehydration during exercise at ~65% VO$_{2\text{peak}}$ on whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated females. In the control trial (HYD) of this study subjects drank enough fluid to precisely replace their sweat losses over the 120 min cycling trial. In the DEH trial, all physiological responses to exercise were exacerbated as HR increased from 154 ± 5 at 20 min to 163 ± 5, and 176 ± 5 bpm at 60 and 120 min, representing increases of 4-9 bpm at these time points. Subjects also had elevated Tc values (37.3 ± 0.2°C at rest and 38.1 ± 0.2, 38.7 ± 0.2, and 39.1 ± 0.2°C at 20, 60 and 120 min) and were 0.2, 0.5 and 0.6°C higher at 20, 60 and 120 min in the DEH vs. HYD trial. Even in the first hour of exercise in DEH (~1% BM loss), RPE, Pvol loss and blood [La] were all higher and there was a significantly greater reliance on whole body carbohydrate, higher muscle lactate content and a trend for higher
muscle glycogen use ($p = 0.15$). In the second hour, BM loss progressed from 1-2% and the additional physiological parameters remained higher and whole body carbohydrate oxidation and muscle glycogen use were also significantly greater in the DEH trial. The 2% BM loss over two hours of exercise increased whole body carbohydrate oxidation by 9% and muscle glycogen use by 31% in female subjects who were hydrated prior to exercise.

5.5.1 The effects of dehydration on substrate oxidation & muscle metabolism

Hargreaves et al. (1999) demonstrated in trained males that a 3% BM loss over a 2 h trial resulted in a significantly higher whole body RER after 60 and 120 min of exercise compared to the euhydrated trial. The study also observed a 16% greater muscle glycogen use over the entire trial with fluid restriction. In contrast, Walsh et al. (1994) demonstrated that mild dehydration of 1.3% BM loss in trained males did not change RER after 60 min of cycling at 70% VO$_{2\text{peak}}$ in the heat (32°C). It seems likely that the low level of dehydration may be the reason for no effect on RER. In comparison, the present study, which was conducted on recreational trained females, demonstrated that RER was significantly higher in the DEH trial as early as 40 min of cycling when dehydration was <1% BM loss and remained significantly higher than the HYD trial for the duration of the trial. Our RER and glycogenolysis data matches Hargreaves et al. (1999) despite this study being conducted on recreationally trained females and not trained males. As well, our results suggest that some of the increased pyruvate production in the DEH trial was oxidized and some was converted to lactate.

The major question is what accounts for the increased glycogenolysis in the DEH trial? There are three main hypotheses which have been proposed to explain the substrate shift towards greater carbohydrate metabolism and muscle glycogenolysis during exercise and heat stress; 1) an augmented sympatho-adrenal response leading to greater glycogen phosphorylase (PHOS) activation and flux, 2) increased allosteric activation of glycogen PHOS via increased free ADP and AMP (energy status of the cell) levels and, 3) higher intramuscular temperature during exercise when dehydrated (Febbraio 2000). Hargreaves et al. (1999) reported no difference in plasma EPI at 60 or 120 min of cycling with 3% BM loss but significantly greater plasma norepinephrine content only at 120 min. The authors suggested that fluid ingestion during exercise attenuates the normal exercise-induced increase in EPI, and the blunting of the sympatho-adrenal activity may be due to hydration status and Tc, but it is difficult to assess these factors independently. The present study also found no difference in EPI response with fluid restriction (2% BM loss) in females, which down plays the role
of EPI as the reason for the significantly greater muscle glycogen use and muscle lactate concentration at 60 min while exercising dehydrated.

The energy status of the cell (free ADP and AMP) exerts powerful allosteric regulation of glycogen PHOS and therefore plays a vital role in determining the rate of glycogenolysis. In light of the augmented glycogen use accompanying progressive DEH we predicted that the energy status of the cell may be decreased (higher free ADP and AMP) to a greater extent when dehydrated and explain the accelerated glycogen use. However, this was not observed, suggesting that the energy status of the cell was not altered by mild dehydration.

Previous work suggests that higher Tc and muscle temperatures are responsible for the increased glycogenolysis and increased reliance on carbohydrate oxidation for muscle ATP production (Febbraio 2000; Febbraio et al. 1994, 1996; Hargreaves et al. 1994; Shirreffs et al. 2004; Weltman et al. 1994). Research investigating local hyperthermia in the working muscle demonstrated an increase in muscle temperature, muscle glycogenolysis, and muscle [La], independent of changes in circulating EPI (Febbraio et al. 1996). Starkie et al. (1999) cooled one leg prior to two-legged cycling and reported increased glycogenolysis in the non-cooled or hotter leg when both legs were exposed to the same [EPI]. Febbraio (2000) concluded in a review that increases in Tc of >0.5°C significantly increased intramuscular carbohydrate utilization during moderate intensity exercise in the heat. In the present study in a neutral environment, Tc was already 0.2-0.5°C higher from 20-60 min of exercise in the DEH trial. While Tm was not measured in this study, the work from Hargreaves' laboratory (Febbraio 2000; Hargreaves et al. 1996; Starkie et al. 1999) suggests that Tm would also have been higher in the first hour of exercise of the present study. Therefore, muscle temperature (Q10 effect) appears to be the primary mechanism inducing the shift in intramuscular glycogenolysis and whole body carbohydrate oxidation during progressive dehydration in females. It is currently unknown why dehydration preferentially increases CHO metabolism and not fat metabolism and future research needs to elucidate the impact of dehydration on the perturbations to intramuscular metabolism. One would predict that the Q10 effect would also increase fat metabolism, however the results of this study demonstrated that fat oxidation was actually reduced with mild dehydration, and carbohydrate oxidation was more sensitive than fat oxidation to increases in Tc. The down-regulation of fat oxidation cannot be explained by FFA delivery as there was no difference between trials in plasma FFA, nor can the impact of muscle pH on CPT1 be considered as a mechanism for the decreased fat oxidation as there was no significant difference in muscle acidity between trials. Moreover, mild dehydration may affect the uptake of FFA into the muscle or the use of intramuscular triacylglycerol,
however this is merely speculation and the mechanisms by which dehydration causes a reduction in fat oxidation remain unclear and call for further investigation. Unfortunately, we were unable to measure intramuscular triacylglycerol or the activity of hormone sensitive lipase and pyruvate dehydrogenase due to tissue limitation.

5.5.2 Effects of dehydration on cardiovascular and thermal responses

It is well established that fluid ingestion attenuates the increases in HR and Tc and the decreases in stroke volume and cardiac output that occur during prolonged exercise without fluid ingestion (Armstrong et al. 1997; Cheuvront et al. 2010; Febbraio et al. 1994; Hamilton et al. 1991; Montain & Coyle 1992; Morimoto 1990; Sawka et al. 2001). An early study demonstrated that when heat-acclimatized male subjects were dehydrated to 3, 5, and 7% BM loss by an exercise-heat regimen and then walked in a hot environment (49°C) at a low intensity for 140 min, HR and Tc increased linearly with the severity of dehydration (Sawka et al. 1985). In a similar way, our results demonstrated that as mild dehydration increased from 0-1% and 1-2% BM during exercise in the DEH trial, HR and Tc became progressively higher than the elevations in the HYD trial.

Hypovolemia and the displacement of blood to the skin for evaporative cooling make it difficult to maintain central venous pressure (CVP) during exercise when fluid is restricted (Sawka et al. 1985). CVP is regulated by the continuous adjustment of blood volume to the changing size of the vascular bed to maintain cardiac output. Heat stress and/or exercise induced dehydration provides a threat to this control as inadequate fluid intake during periods of sweat loss reduces Pvol (Morimoto 1990). In light of the significantly greater loss in Pvol found in the DEH vs. the HYD trial after ~20 min of cycling, a reduction in CVP and stroke volume may account for the significantly elevated HR to maintain cardiac output when stroke volume was compromised. An accompanying baroreflex that would decrease cutaneous blood flow and heat transfer to the periphery leading to heat storage may account for the augmented Tc found in the DEH trial. In support of this, Nadel et al. (1980) reported that diuretic-induced dehydration of 2.7% BM loss lead to restrictions in core to skin heat transfer, which forced esophageal temperature to nearly 39.0°C during 30 min of cycling at 55% VO_{2peak} in the heat, compared to 38.4°C in euhydrated subjects. In the present study, female subjects had higher Tc values in the last 90 min of exercise in the DEH vs. HYD trials, while the sweat rates were similar, suggesting that the lack of heat transfer to the periphery accounted for the elevated Tc in the DEH trial with as little as 1-2% BM loss.
5.5.3 Effects of dehydration on ratings of perceived exertion

In this study, RPE mirrored the rise in HR and Tc with progressive dehydration and became significantly higher in the DEH trial at 60 min of cycling when subjects had lost ~1% BM. Similar results have been reported in other studies investigating the effects of progressive dehydration on RPE (Ishijima et al. 2009; McGregor et al. 1999). It is speculated that hypovolemia associated with exercise dehydration leading to a reduction in brain blood flow may heighten the perceived exertion associated with exercising without fluid leading to greater perceived exertion (Maughan et al. 2007). More simply, it may be that the elevations in Tc, HR, and reduced Pvol in the DEH trial are sensed and the feedback to the brain results in the greater RPE during exercise at the same relative intensity in a mildly dehydrated state. Sherriff et al. (2004) reported that as subjects became progressively more dehydrated to 2.7% BM loss they reported feelings of headache, reductions in their ability to concentrate, and their alertness was reduced, which are all contributing factors to an elevated RPE during exercise.

5.6 CONCLUSIONS

This study is the first to investigate the effects of progressive mild dehydration during exercise at ~65% VO$_{2\text{peak}}$ on the time course of changes in whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated females. Whole body carbohydrate oxidation and skeletal muscle glycogenolysis were significantly increased early into exercise when BM loss was <1-2%, which we attribute to dehydration-induced increases in Tc and skeletal muscle temperature as there were no differences in plasma EPI or the energy status of the cell (free ADP or AMP) between the HYD and DEH trials. In addition, the traditional changes in physiological parameters accompanying exercise in a HYD state were exacerbated with mild dehydration of 1-2% BM loss.
CHAPTER SIX
THE EFFECTS OF PROGRESSIVE DEHYDRATION DURING PROLONGED CYCLING ON SKELETAL MUSCLE METABOLISM IN HYDRATED MALES
SUBMITTED – APPL. PHYSIOL. NUTR. METAB.
6.1 ABSTRACT

This study investigated the effects of progressive mild dehydration during moderate intensity exercise on whole body substrate oxidation and skeletal muscle metabolism in recreationally active males. Subjects cycled for 120 min at ~65% VO$_{2peak}$ in a neutral environment with water to replace sweat losses (HYD) or without fluid (DEH). Blood samples were taken at rest and every 20 min and muscle biopsies taken at rest, 40, 80, and 120 min of exercise. Subjects lost 0.8%, 1.8% and 2.7% BM after 40, 80 and 120 min of cycling in the DEH trial while sweat loss was similar between trials. HR was significantly greater in the DEH trial from 60-120 min and core temperature (Tc) was greater from 75-120 min. Rating of perceived exertion (RPE) was higher in the DEH trial from 30-120 min. There were no differences in VO$_2$, RER, or total CHO oxidation (HYD, 312 ± 9 vs. DEH, 307 ± 10 g) between trials. Blood [La] was significantly greater in the DEH trial from 20-120 min with no difference in plasma [FFA] or [EPI]. There were trends for greater glycogen use in the DEH trial from 0-40, 40-80, and 80-120 min, and glycogenolysis was significantly greater over the entire DEH vs. HYD trial (24%, 0-120 min: 433 ± 44 vs. 349 ± 27 mmol/kg dm). Muscle [La] was significantly higher at all time points in the DEH trial. In summary, dehydration of <2% BM and beyond elevated physiological and mental parameters, as well as muscle glycogenolysis. The dehydration-induced increase in muscle glycogenolysis may have been due to increased muscle temperature, secondary to less efficient movement of heat from the core to the periphery and increases in Tc.
6.2 INTRODUCTION

It has been widely demonstrated that athletes replace less than 50% of sweat losses, resulting in significant fluid deficits during exercise (review Burke 1997). The cardiovascular, thermoregulatory and cognitive penalties associated with exercising dehydrated are well documented and consistent in showing that the exercise-induced increases in HR, Tc and RPE are exacerbated with dehydration and directly proportional to the degree of dehydration (Armstrong et al., 1997; Gonzalez-Alonso et al., 1995; Montain & Coyle 1992; Nadel et al., 1980; Sawka 1985). Despite the large amount of research demonstrating the cardiovascular and thermoregulatory changes with dehydration, only one study has investigated the effects of dehydration on whole body substrate oxidation and skeletal muscle metabolism. Hargreaves et al. (1996) investigated the effects of dehydration on muscle metabolism in males and reported that a 2.9% BM loss over 2 hours of cycle exercise resulted in 16% more glycogen use. The RER was significantly higher in the fluid restricted trial after 60 and 120 min of exercise with the difference between trials being greater in the second hour of cycling. This was accompanied by significantly higher rectal and muscle temperatures at the end of exercise only. Goulet et al. (2008) performed a meta-analysis on the effects of dehydration on endurance exercise and reported that 14 studies observed decrements in performance beginning at ~2% BM loss. Also, it has consistently been shown that a 1-2% BM loss is commonplace among athletes in training and competition (Burke 1997; Godek et al., 2005; 2009; Logan-Sprenger et al., 2011; Maughan et al., 2004; Palmer et al., 2008, 2010). No studies have examined how progressive dehydration affects muscle glycogenolysis during prolonged exercise.

Therefore the aims of this study were to (1) investigate the time course of changes in skeletal muscle metabolism with progressive dehydration during 120 min of cycling at ~65% VO$_{2peak}$ in recreationally active males, and (2) determine how much BM loss is necessary to effect changes in whole body and muscle metabolism. We hypothesized that (1) whole body CHO oxidation and muscle glycogen use would be greater than in the hydrated trial from 40-80 min and 80-120 min of cycling as dehydration increased, and (2) increased CHO oxidation and glycogenolysis would coincide with a higher Tc in the dehydrated trial.

6.3 METHODS

6.3.1 Subject Characteristics

Nine recreationally active males, mean age 21.6 ± 0.5 y, height 178.1 ± 0.9 cm, weight 77.3 ± 2.2 kg, and VO$_{2peak}$ 4.4 ± 0.2 L/min, volunteered to participate in the study. All subjects
engaged in recreational physical activity 3-4 days/wk. Subjects were informed both verbally and in writing of the experimental protocol and potential risks prior to giving their written consent to participate. The Research Ethics Boards of the University of Guelph and McMaster University approved the study.

6.3.2 Pre-experimental Protocol
Same as Chapter 5.3.2

6.3.3 Experimental Protocol
Same as Chapter 5.3.3
At 40, 80, and 120 min the subject stopped cycling and a muscle biopsy was taken with the subject sitting on the cycle ergometer. After the muscle biopsy was taken, subjects removed their shoes and shirt, towed dry, and were weighed for the determination of BM loss over the previous 40 min of exercise. The same procedure was replicated for the second trial with muscle biopsies taken from the opposite leg and the trials were randomized and separated by 7 days.

6.3.4 Analyses
Same as Chapter 5.3.4

6.3.5 Statistical Analysis
Same as Chapter 5.3.5

6.4 RESULTS
6.4.1 Trial Conditions
No significant pre-trial differences existed between the HYD and DEH trials for laboratory temperature (HYD 22.6 ± 0.1 vs. DEH 22.8 ± 0.2 °C), relative humidity (32 ± 2.8 vs. 33 ± 2.7 %), pre-trial BM (77.1 ± 2.2 vs. 77.5 ± 2.2 kg), or hydration state (USG 1.013 ± 0.003 vs. 1.015 ± 0.003).
6.4.2 Body Mass Loss, Sweat Loss, and Fluid Intake

Body mass was maintained in the HYD trial by consuming a mean of 2.3 ± 0.2 L of fluid over the 120 min of cycling (Table 6.1). In the DEH trial BM was significantly lower at 40, 80, and 120 min (Table 6.1). There was no significant difference in sweat loss between the HYD vs. DEH trials (Table 6.1). Only one subject micturated after the HYD trial (350 mL) and the DEH trial (400 mL).

Table 6.1 Differences in sweat loss, body mass (BM) and percent BM loss between the hydrated (HYD) and dehydrated (DEH) trials at 0, 40, 80, and 120 min of cycling at ~65% VO$_{2\text{peak}}$. Values are means ± SE, ($n$ = 9). *Significantly lower than HYD and 0 min in DEH ($p$ < 0.05).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HYD</th>
<th>DEH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweat Loss (L)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>40</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>80</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>120</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>BM (kg)</td>
<td>77.1 ± 2.2</td>
<td>77.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>77.0 ± 2.1</td>
<td>76.1 ± 2.1*</td>
</tr>
<tr>
<td>80</td>
<td>77.1 ± 2.2</td>
<td>77.1 ± 2.2</td>
</tr>
<tr>
<td>120</td>
<td>77.1 ± 2.2</td>
<td>77.1 ± 2.2</td>
</tr>
<tr>
<td>BM loss (%)</td>
<td>-----</td>
<td>0.8 ± 0.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8 ± 0.2%</td>
</tr>
</tbody>
</table>

6.4.3 Oxygen Uptake and Whole Body Substrate Use

Mean VO$_2$ increased in both trials with exercise time and was significantly greater than 0 min at all time points in both trials, but there was no difference between trials (Fig. 6.1a). The RER progressively decreased in both trials over time and was significantly lower than 20 min at 80, 100, and 120 min in both trials, with no differences between trials (Fig. 6.1b).
Figure 6.1 (a) VO₂ and (b) respiratory exchange ratio (RER) during 120 min of cycling at ~65% VO₂peak in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). Arrows (↓) indicate ~1, 2 and 3% BM loss.

There was no significant difference in total CHO (HYD, 312 ± 9 vs. DEH, 307 ± 10 g) or fat oxidation (53 ± 8 vs. 55 ± 17 g) between trials. CHO oxidation decreased over time from 0-40
(HYD, 116 ± 7 vs. DEH, 115 ± 6 g), 40-80 min (105 ± 5 vs. 107 ± 6 g), and 80-120 min (91 ± 4 vs. 85 ± 5 g), but was not significantly different between trials. Fat oxidation increased over time from 0-40 (HYD, 12 ± 2 vs. DEH 13 ± 2 g), 40-80 (18 ± 2 vs. 18 ± 2 g), and 80-120 min (23 ± 2 vs. 24 ± 3 g) but there were no trial differences.

6.4.4 Heart Rate, Core Temperature, and Rating of Perceived Exertion

HR increased significantly over time in both trials and was significantly higher in the DEH vs. HYD trial from 60-120 min of cycling (Fig. 6.2).

Figure 6.2 Heart rate (HR) during 120 min of cycling at ~65% VO\textsubscript{2}peak in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). HR was significantly greater than 15 min at all time points in both trials (p < 0.05). *Significantly greater than the HYD trial (p < 0.05). bpm, beats per minute. Arrows (↓) indicate ~1, 2 and 3% BM loss.

Tc increased significantly over time in both trials and was significantly higher in the DEH vs. HYD trial from 75-120 min (Fig. 6.3).
Figure 6.3 Core temperature (°C) during 120 min of cycling at ~65% \( \text{VO}_{2\text{peak}} \) in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). Tc was significantly greater than 15 min for all time points in both trials \((p < 0.05)\). *Significantly higher than HYD trial \((p < 0.05)\). Arrows (↓) indicate ~1%, 2%, and 3% BM loss.

RPE significantly increased over time in both trials and was significantly higher in the DEH trial from 30-120 min (Fig. 6.4). The mean RPE over the entire trial was also significantly greater in the DEH vs. HYD trial \((12.9 \pm 0.3 \text{ vs. } 14.4 \pm 0.6)\).
Figure 6.4 Rating of perceived exertion (RPE) during 120 min of cycling at ~65% \(\text{VO}_{2\text{peak}}\) in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). RPE was significantly greater than 15 min from 45 – 120 min in both trials \((p < 0.05)\). *Significantly higher than HYD trial \((p < 0.05)\). Arrows (↓) indicate ~1%, 2%, and 3% BM loss.

6.4.5 Blood Measurements

Hb and Hct were significantly higher than rest from 20-120 min of exercise in both trials (Table 6.2). In the DEH trial, Hb was significantly greater from 40-120 min and Hct was significantly greater from 60-120 min (Table 6.2). Pvol loss was significantly greater in the DEH trial from 40-120 min of exercise (Table 6.2). Blood glucose was unaffected by time or trials (Table 6.2). Blood lactate was significantly increased above rest at all time points in both trials and was greater in the DEH trial at all exercise time points (Table 6.2). Plasma FFA and epinephrine significantly increased from rest in both trials with no differences between trials (Table 6.2).
Table 6.2. Whole blood and plasma parameters during 120 min of cycling at ~65% \( \text{VO}_2 \text{peak} \) in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). *Significantly different than 0 min (\( p < 0.05 \)). †Significantly greater than HYD (\( p < 0.05 \)).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/100mL) HYD</td>
<td>14.2 ± 0.4</td>
<td>14.9 ± 0.3†</td>
<td>15.0 ± 0.3†</td>
<td>15.0 ± 0.3†</td>
<td>14.9 ± 0.3†</td>
<td>14.8 ± 0.3†</td>
<td></td>
</tr>
<tr>
<td>DEH</td>
<td>14.0 ± 0.4</td>
<td>15.0 ± 0.2†</td>
<td>15.5 ± 0.3‡*</td>
<td>15.5 ± 0.3‡*</td>
<td>15.4 ± 0.3‡*</td>
<td>14.8 ± 0.2‡*</td>
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</tr>
<tr>
<td>Hct (%) HYD</td>
<td>45.2 ± 1.2</td>
<td>47.3 ± 0.9†</td>
<td>47.4 ± 0.9†</td>
<td>47.7 ± 1.1†</td>
<td>47.1 ± 1.0†</td>
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<tr>
<td>DEH</td>
<td>44.9 ± 1.4</td>
<td>47.7 ± 1.0†</td>
<td>48.3 ± 0.8‡*</td>
<td>48.3 ± 0.7‡*</td>
<td>49.1 ± 0.7‡*</td>
<td></td>
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</tr>
<tr>
<td>Pvol Loss (%) HYD</td>
<td>-----</td>
<td>-6.0 ± 1.3†</td>
<td>-4.9 ± 1.2†</td>
<td>-4.5 ± 1.3†</td>
<td>-5.1 ± 1.4†</td>
<td>-4.9 ± 1.5†</td>
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<tr>
<td>DEH</td>
<td>-----</td>
<td>-7.1 ± 1.2‡</td>
<td>-8.3 ± 1.2‡*</td>
<td>-8.9 ± 1.5‡*</td>
<td>-8.5 ± 1.4‡*</td>
<td>-9.4 ± 1.5‡*</td>
<td></td>
</tr>
<tr>
<td>Glucose (mM) HYD</td>
<td>4.0 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.7 ± 0.2</td>
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<tr>
<td>DEH</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.2</td>
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</tr>
<tr>
<td>Lactate (mM) HYD</td>
<td>0.5 ± 0.1</td>
<td>2.0 ± 0.3†</td>
<td>1.5 ± 0.3†</td>
<td>1.4 ± 0.3†</td>
<td>1.4 ± 0.2†</td>
<td>1.2 ± 0.2†</td>
<td></td>
</tr>
<tr>
<td>DEH</td>
<td>0.7 ± 0.3</td>
<td>2.6 ± 0.6‡*</td>
<td>2.3 ± 0.5‡*</td>
<td>1.9 ± 0.3‡*</td>
<td>1.9 ± 0.3‡*</td>
<td>2.0 ± 0.5‡*</td>
<td></td>
</tr>
<tr>
<td>Plasma FFA (mM) HYD</td>
<td>0.15 ± 0.02</td>
<td>-----</td>
<td>-----</td>
<td>0.21 ± 0.03†</td>
<td>0.38 ± 0.1†</td>
<td>-----</td>
<td>0.89 ± 0.1†</td>
</tr>
<tr>
<td>DEH</td>
<td>0.14 ± 0.02</td>
<td>-----</td>
<td>-----</td>
<td>0.19 ± 0.04‡</td>
<td>0.44 ± 0.1†</td>
<td>-----</td>
<td>0.84 ± 0.1†</td>
</tr>
<tr>
<td>Plasma EPI (nM) HYD</td>
<td>0.41 ± 0.03</td>
<td>-----</td>
<td>-----</td>
<td>0.93 ± 0.1†</td>
<td>1.13 ± 0.1†</td>
<td>-----</td>
<td>1.65 ± 0.2†</td>
</tr>
<tr>
<td>DEH</td>
<td>0.41 ± 0.04</td>
<td>-----</td>
<td>-----</td>
<td>1.06 ± 0.1†</td>
<td>1.11 ± 0.1†</td>
<td>-----</td>
<td>1.66 ± 0.2†</td>
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</table>

6.4.6 Muscle Fuels & Metabolites

Skeletal muscle PCR content significantly decreased in the first 40 min of exercise and remained lower than rest at 80 and 120 min of exercise in both trials, but there were no differences between trials (Table 6.3). Skeletal muscle Cr changes were reciprocal with the PCR changes. Muscle ATP content was significantly lower than rest at 120 min in both trials, with no differences between trials. ADPf and AMPf were significantly higher than rest at all time points during exercise in both trials, with no differences between trials (Table 6.3). Muscle lactate content increased with exercise and peaked at 40 min in both trials and was significantly greater in the DEH trial at 40, 80 and 120 min of exercise (Table 6.3).
Table 6.3. Skeletal muscle fuel and metabolite contents during 120 min of cycling at ~65% VO$_{2peak}$ in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). PCr, phosphocreatine; Cr, creatine; ATP, adenosine triphosphate. ADPf, free adenosine diphosphate; AMPf, free adenosine monophosphate. †Significantly different than 0 min (p < 0.05). *Significantly greater than HYD (p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>HYD</th>
<th></th>
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<th>DEH</th>
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<td>80 min</td>
<td>120 min</td>
<td>0 min</td>
<td>40 min</td>
<td>80 min</td>
</tr>
<tr>
<td>PCr</td>
<td>(mmol/kg dm)</td>
<td>80.3 ± 4.9</td>
<td>63.8 ± 4.7†</td>
<td>59.4 ± 4.6†</td>
<td>58.0 ± 4.7†</td>
<td>84.6 ± 3.3</td>
<td>57.1 ± 4.3†</td>
<td>58.7 ± 5.0†</td>
</tr>
<tr>
<td>Cr</td>
<td>(mmol/kg dm)</td>
<td>74.7 ± 3.2</td>
<td>90.8 ± 7.0†</td>
<td>95.2 ± 6.7†</td>
<td>93.0 ± 5.3†</td>
<td>70.5 ± 2.8</td>
<td>98.1 ± 7.1†</td>
<td>96.8 ± 6.2†</td>
</tr>
<tr>
<td>ATP</td>
<td>(mmol/kg dm)</td>
<td>25.6 ± 1.2</td>
<td>26.4 ± 1.1</td>
<td>25.2 ± 0.9</td>
<td>24.8 ± 0.8</td>
<td>26.9 ± 0.8</td>
<td>26.5 ± 0.6</td>
<td>26.1 ± 0.9</td>
</tr>
<tr>
<td>ADPf</td>
<td>(umol/kg dm)</td>
<td>144 ± 19</td>
<td>246 ± 48†</td>
<td>231 ± 24†</td>
<td>205 ± 31†</td>
<td>128 ± 10</td>
<td>238 ± 32†</td>
<td>247 ± 52†</td>
</tr>
<tr>
<td>AMPf</td>
<td>(umol/kg dm)</td>
<td>1.0 ± 0.3</td>
<td>2.8 ± 0.9†</td>
<td>2.0 ± 0.4†</td>
<td>2.9 ± 0.9†</td>
<td>0.6 ± 0.3</td>
<td>1.8 ± 0.5†</td>
<td>2.9 ± 1.1†</td>
</tr>
<tr>
<td>Lactate</td>
<td>(mmol/kg dm)</td>
<td>1.8 ± 0.4</td>
<td>9.3 ± 2.6†</td>
<td>8.1 ± 1.7†</td>
<td>5.2 ± 1.5†</td>
<td>2.1 ± 0.5</td>
<td>13.1 ± 3.3*†</td>
<td>12.4 ± 2.2*†</td>
</tr>
<tr>
<td>Glycogen</td>
<td>(mmol/kg dm)</td>
<td>522 ± 58</td>
<td>313 ± 63†</td>
<td>228 ± 54†</td>
<td>165 ± 43†</td>
<td>572 ± 61</td>
<td>323 ± 67†</td>
<td>240 ± 53†</td>
</tr>
</tbody>
</table>

Muscle glycogen content was similar in the two trials before exercise and significantly lower at 40, 80, and 120 min in both trials compared to rest. Total glycogen use (0-120 min) was significantly greater (24%) in the DEH trial (HYD, 349 ± 27 vs. DEH, 433 ± 44 mmol/kg dm)(Fig. 6.5). However there was no significant differences in glycogen use from 0-40 (19%, HYD, 209 ± 30 vs. DEH, 249 ± 43 mmol/kg dm, p = 0.13), 40-80 (19%, 77 ± 14 vs. 92 ± 19 mmol/kg dm, p = 0.47), and from 80-120 min (46%, 63 ± 12 vs. 92 ± 24 mmol/kg dm, p = 0.09).
**Figure 6.5** Muscle glycogen use during 120 min of cycling at ~65% VO$_2$peak in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). *Significantly greater than HYD (p < 0.05).

### 6.5 DISCUSSION

This study investigated the effects of mild progressive dehydration during exercise at ~65% VO$_2$peak on whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated males. In the control trial (HYD) of this study we prevented dehydration by having subjects drink enough fluid to precisely replace their sweat losses over the 120 min cycling trial. During this trial, HR increased from 150 ± 4 bpm at 15 min to 160 ± 5 and 165 ± 4 bpm at 60 and 120 min while Tc increased from 37.2 ± 0.1°C at rest to 37.8 ± 0.1°C at 15 min and 38.1 ± 0.1 and 38.2 ± 0.1°C at 60 and 120 min. In the DEH trial, when the subjects progressively dehydrated by sweating, they lost ~1, 2, and 3% BM at 40, 80, and 120 min and added progressive dehydration to the physiological demands of exercising for 120 min at ~65% VO$_2$peak. All physiological responses to exercise were exacerbated in the DEH trial as HR increased from 150 ± 5 at 15 min to 166 ± 3 and 172 ± 4 bpm at 60 and 120 min, representing increases of 6-7 bpm at the 60 and 120 min time points. Subjects also had elevated Tc values (37.1 ± 0.1°C at rest and 37.8 ± 0.1, 38.3 ± 0.1, and 38.7 ± 0.2°C at 15, 60 and 120 min) and were 0.2 and
0.5°C higher at 60 and 120 min in the DEH vs. HYD trial. Even in the first 40 min of exercise in DEH (~1% BM loss), RPE, Pvol loss, blood [La], were all higher and there was a significantly higher muscle lactate content and a trend for increased muscle glycogen use ($p = 0.17$). From 40-80 and 80-120 min, BM loss progressed from 1-2% and 2-3% and the additional physiological parameters remained higher, and HR and Tc were also significantly greater in the DEH trial. The 3% BM loss over two hours of exercise increased muscle glycogen use by 24%.

6.5.1 The effects of progressive dehydration on muscle metabolism

Previously, Hargreaves et al. (1996) reported that net muscle glycogen use was 16% greater over a 120 min trial at 67% VO$_{2\text{peak}}$ when trained males were dehydrated by 2.9% BM. The RER was significantly higher in the fluid restricted trial after 60 and 120 min of exercise with the difference between trials being greater in the second hour of cycling. The present study examined the time course in changes to muscle metabolism at 40, 80, and 120 min of cycling at ~65% VO$_{2\text{peak}}$ with progressive dehydration to ascertain how much dehydration is necessary to see a shift in substrate oxidation and muscle glycogen use. There were no differences in whole body substrate oxidation when subjects were dehydrated by 1, 2, or 3% BM, although there were trends for accelerated muscle glycogen use in each 40 min exercise segment and there was a significant 24% increase over the entire DEH trial compared to HYD. An interesting finding in this study was the greater total muscle glycogen use in the DEH trial with no difference in whole body carbohydrate oxidation. This suggests that the extra glycogen metabolized to pyruvate was not oxidized, but converted to lactate. This speculation is supported by the augmented blood and muscle lactate contents throughout the DEH trial. There are at least three potential mechanisms that may account for the increased muscle glycogenolysis and glycogen phosphorylase (PHOS) activity in the DEH trial without also affecting the activity of pyruvate dehydrogenase. They include an increased sympatho-adrenal response leading to elevated circulating [EPI] and activation of PHOS, a decreased energy status in the cell manifested by elevated ATP/ADP ratio and AMPf levels (as they act as allosteric activators of PHOS), and increased Tm (Febbraio 2000). Hargreaves et al. (1996) reported no difference in plasma [EPI] at 60 or 120 min of cycling with 2.9% BM loss at 120 min and the present study also found no difference in EPI response with fluid restriction up to 3% BM loss in males.

Estimates of free ADP and AMP in the DEH and HYD trials of the present study did not reveal any differences, downplaying the suggestion that these allosteric regulators can explain the increased PHOS activity and glycogenolysis in the DEH trial.
Studies examining the importance of local hyperthermia on muscle glycogenolysis increased the Tm of one leg during exercise and reported increased muscle glycogenolysis and [La] in the hot leg only, independent of changes to Tc or circulating [EPI]. This demonstrated that increasing local Tm can increase glycogenolysis at a given Tc and when both legs were exposed to the same [EPI] (Febbraio et al. 1996; Starkie et al. 1999). In a review article by Febbraio et al. (2000), the authors state that increases in Tc of >0.5°C consistently increased intramuscular CHO utilization during moderate intensity exercise in the heat. In the present study, Tc was 0.3-0.5°C higher in the final 30-45 min of exercise in a neutral environment in the DEH trial. While Tm was not measured in this study, the work from Hargreaves and Febbraio predicts that Tm would have been higher during exercise in the DEH trial of the present study (Febbraio 2000; Hargreaves et al. 1996; Starkie et al. 1999).

Therefore, an increased Tm and the Q₁₀ effect appears to be the most plausible explanation for the increased muscle glycogenolysis reported during progressive dehydration in males in the present study. It is currently unknown why dehydration preferentially increases CHO metabolism and not fat metabolism, but it may be related to the ability to quickly mobilize muscle CHO vs. the relatively slower mobilization of fat fuels not coming from intramuscular triglycerides.

The present data also suggests that activity of PHOS may be more sensitive to increased Tm (increased pyruvate production) as compared to the activity of pyruvate dehydrogenase (PDH), resulting in no increase in pyruvate oxidation and more lactate formation. As this was the case the increased glycogen use with dehydration appeared to be wasted as excess pyruvate produced was converted to lactate and not oxidized. At the present time, there does not appear to be an explanation for this finding and the final study in this thesis was not able to corroborate this finding, as CHO oxidation was elevated with dehydration!

6.5.2 Effects of dehydration on cardiovascular and thermal responses

It is well established that fluid ingestion attenuates the increases in HR and Tc and the decreases in stroke volume and cardiac output that occur during prolonged exercise without fluid ingestion (Armstrong et al. 1997; Cheuvront et al. 2010; Febbraio et al. 1994; Hamilton et al. 1991; Morimoto 1990; Nadel et al. 1980; Sawka et al. 1985). An early study demonstrated that when heat-acclimatized male subjects were dehydrated to 3, 5, and 7% BM loss by an exercise-heat regime and then walked in a hot environment (49°C) at a low intensity for 140 min, HR and Tc increased linearly with the severity of dehydration (Sawka et al. 1985). In a similar way, our results demonstrated that as
dehydration increased from 0-1%, 1-2%, and 2-3% BM during exercise in the DEH trial, HR and Tc became progressively higher than the elevations in the HYD trial.

Hypovolemia and the displacement of blood to the skin for evaporative cooling make it difficult to maintain central venous pressure (CVP) during exercise when fluid is restricted (Sawka et al. 2001). CVP is regulated by the continuous adjustment of blood volume to the changing size of the vascular bed to maintain cardiac output, and heat stress and/or exercise induced dehydration provides a threat to this control as inadequate fluid intake during periods of sweat loss reduces plasma volume (Morimoto 1990). In light of the significantly greater loss in Pvol found in the DEH vs. the HYD trial after ~40 min of cycling, a reduction in CVP and stroke volume may account for the significantly elevated HR to maintain cardiac output when stroke volume was compromised. An accompanying baroreflex that would decrease cutaneous blood flow leading to heat storage may account for the augmented Tc found in the DEH trial. In support of this, Nadel et al. (1980) reported that diuretic-induced dehydration of 2.7% BM lead to restrictions in core to skin heat transfer, which forced esophageal temperature to nearly 39°C during 30 min of cycling at 55% VO2peak in the heat. In our study, male subjects had higher Tc values in the last 45 min of exercise in the DEH (38.7°C) vs. HYD (38.1°C) trials, while the sweat rates were the same, suggesting that the lack of heat transfer to the periphery accounted for the elevated Tc in the DEH trial with as little as ~1-2% BM loss.

6.5.3 Effects of dehydration on ratings of perceived exertion

In this study, RPE became significantly higher in the DEH trial after only 30 min of cycling when subjects had lost <1% BM. Similar results have been reported in other studies investigating the effects of progressive dehydration on RPE (Ishijima et al. 2009; McGregor et al. 1999). It is speculated that hypovolumia associated with exercise dehydration leading to a reduction in brain blood flow may exasperate the sense of effort associated with exercising without fluid leading to greater perceived exertion (Maughan et al. 2007). More simply, it may be that the temperature and cardiovascular centres that sense elevations in Tc, HR, and reduced Pvol feedback to the brain and increase the RPE during exercise at the same relative intensity in a mildly dehydrated state. Sherriffs et al. (2004) reported that as subjects became progressively more dehydrated to 2.7% BM loss they reported feelings of headache, reductions in their ability to concentrate, and their alertness was reduced, which may all contribute to an elevated RPE during exercise.
6.6 CONCLUSIONS

This study investigated the time course of changes in whole body substrate oxidation and skeletal muscle metabolism, as well as cardiovascular, thermal, and mental responses in recreationally active, hydrated males with progressive mild dehydration during exercise at ~65% VO_{2peak}. All traditional changes in physiological parameters accompanying exercise in a HYD state were exasperated with mild dehydration of ~1-2% BM loss. Muscle glycogenolysis was significantly increased in the DEH vs. HYD condition over the entire trial (0-120 min) with no difference in whole body CHO oxidation between trials. The increased glycogenolysis was attributed to increases in Tm and the Q_{10} effect, as there were no differences in plasma EPI or the energy status of the cell (free ADP or AMP) between the HYD and DEH trials. There does not appear to be an obvious explanation for the lack of increased whole body CHO oxidation in the face of the dehydration-induced increase in muscle glycogenolysis.
CHAPTER SEVEN
THE EFFECT OF DEHYDRATION ON MUSCLE METABOLISM & CYCLING PERFORMANCE DURING PROLONGED EXERCISE IN MALES SUBMITTED – MED. SCI. SPORTS EXERC.
7.1 ABSTRACT

Dehydration of ~1-2% body mass (BM) loss has been shown to augment the normal physiological responses to exercise compared to being hydrated and may impair endurance performance. Little is known about the impact of dehydration on substrate oxidation and muscle metabolism. This study combined overnight fluid restriction with lack of fluid intake during exercise to determine the effects of dehydration on substrate oxidation, skeletal muscle metabolism, heat shock protein (Hsp) response, and time trial (TT) performance. Nine males cycled at ~65% \( \text{VO}_{2\text{peak}} \) for 90 min followed by a TT (6 kJ/kg BM) either with fluid to replace sweat losses (HYD) or without fluid (DEH). Blood samples were taken throughout exercise and muscle biopsies were taken at 0, 45, 90 min, and after the TT. Urine specific gravity (USG) and osmolality (Uosm) were significantly higher in the DEH trial before exercise. DEH subjects started the trial with a 0.6% BM loss from overnight fluid restriction and were dehydrated by 1.4% after 45 min, 2.3% after 90 min of exercise, and 3.1% BM after the TT. HR was greater from 50–90 min and core temperature (Tc) was higher from 60 – 90 min in the DEH trial. RPE was higher in the DEH trial from 30 – 90 min. There were no differences in mean \( \text{VO}_2 \), \( \text{VCO}_2 \), or total sweat loss between trials. RER was greater in the DEH trial from 60 min onward and total CHO oxidation was greater in DEH (HYD, 168 ± 10 vs. DEH, 202 ± 12 g). Blood [La] was greater in the DEH trial from 40 – 90 min. Blood glucose, plasma FFA and epinephrine, and serum Hsp72 were not different between trials. Glycogen use was greater in the DEH trial from 0 – 45 min (15%, HYD, 207 ± 47 vs. DEH, 282 ± 44 mmol/kg dm) and 0 – 90 min (17%, HYD 359 ± 35 vs. DEH, 308 ±35) with a trend for greater glycogen use from 45 – 90 min (24%, HYD 62 ± 20 vs. DEH 77 ± 18). Muscle [La] at 90 min and just before the TT (1.9 ± 0.3 vs. 2.3 ± 0.5) was higher in the DEH trial, and muscle glycogen was low in both trials, resulting in little glycogen use during the TT in HYD and DEH. Muscle [La] after the TT (7.0 ± 1.3 vs. 8.2 ± 1.5 mmol/kg dm) was higher in the DEH trial. Muscle Hsp72 protein increased in both trials with time with a trend for greater increase in the DEH trial (HYD 1.8 ± 0.5 vs. DEH 2.4 ± 0.5 fold increase). TT performance was slower in the DEH trial (31.8 ± 4.1 vs. 36.0 ± 3.1 min). Tc, Hb, Het, Pvol loss, Posm and serum Hsp72 were all greater after the DEH TT. In summary, TT performance was 13% slower when dehydrated by 2-3% BM with all physiological measures exacerbated compared to responses when HYD. The accelerated muscle glycogen use during 90 min of moderate intensity exercise with DEH did not affect performance, rather an augmented Tc and RPE is more of a domineering factor slowing performance when DEH.
7.2 INTRODUCTION

As little as 1-2% BM loss from sweating has been shown to compromise physiological functioning during prolonged exercise. Multiple studies have demonstrated that the magnitude of exercise-induced physiological responses such as Tc, HR, plasma osmolality and central venous pressure are exacerbated by dehydration and directly proportional to the degree of dehydration (Armstrong et al. 1997; Montain & Coyle, 1992; Nadel et al. 1980; Sawka et al. 1985). Unfortunately, the reality in sport is that the majority of athletes only replace ~50% of sweat losses during exercise leading to significant fluid deficits, and as a result experience amplified physiological responses compared to when drinking enough fluid to replace sweat losses (Burke, 1997). For example, we reported in a study conducted on elite male junior ice hockey players that 33% did not drink enough fluid during a game to prevent sweat losses of ≤2% BM loss (Logan-Sprenger et al. 2011). This is just one of the many studies documenting the significant fluid deficit many athletes incur as a result of insufficient fluid intake during exercise when sweat loss is high (Burke 1997).

Despite the large number of studies investigating the physiological responses to dehydration, very few have investigated the impact of dehydration on whole body substrate oxidation, skeletal muscle metabolism, and performance. One recent study conducted in recreationally active males who cycled for 2 hrs at 65% VO2peak reported that muscle glycogen use was 24% greater in the dehydrated compared to the hydrated trial (Chapter six). However, whole body CHO oxidation was not increased with dehydration to 2.7% BM loss. The relationship between the changes in substrate oxidation and muscle metabolism with progressive dehydration and performance was not examined in the previous study so it is unknown if performance was affected.

Dehydration has been shown to impair endurance performance, however what is not known is whether the performance detriments are a result of glycogen depletion or other physiological factors. For example, Armstrong et al. (1985) demonstrated that pre-exercise dehydration of 1.5 – 2% BM loss reduced performance of track running at distances of 1.5, 5, and 10k. As well, Walsh et al. (1994) had trained males cycle for 60 min at 70% VO2peak and then complete a time trial to exhaustion at 90% VO2peak either consuming water with 120 mL NaCl every 10 min or no fluid. TT performance was significantly slower when subjects were dehydrated by ~2% BM loss (fluid 9.8 ± 3.9 vs. fluid restricted 6.8 ± 3.0 min). In both of these studies alterations in metabolism were not ascertained, so the contribution of metabolism to the performance detriments is unclear.

Therefore, the purpose of this study was to investigate the effects of progressive dehydration on TT performance and several metabolic, physiologic and mental variables that may be
related to performance in active males. By adopting an overnight fluid restriction protocol we exacerbated the magnitude of the progressive dehydration during moderate intensity exercise and the following TT. We hypothesized that carbohydrate oxidation, muscle glycogen use, heat shock protein 72 response, and all other physiological measures (HR, Tc, Pvol loss), would be greater with dehydration during moderate intensity exercise compared to when hydrated. As a result, we predict that time trial performance will be significantly slower. By measuring intermediate blood (every 20 min) and skeletal muscle measurements (0, 45, 90 min, and post-TT), mental responses (rating of perceived exertion), and other physiological parameters (core temperature, heart rate) we will attempt to determine if metabolism is contributing to performance detriments when dehydrated or point to another physiological factor.

7.3 METHODS

7.3.1 Subjects

Nine trained males, mean age 21.6 ± 0.5 yr, height 178.1 ± 0.9 cm, weight 77.5 ± 3.0 kg, and VO$_{2peak}$ 4.4 ± 0.2 L/min, participated in the study. All subjects engaged in physical activity 4-6 days/wk. On average subjects cycled for ~4-5 h/wk. Subjects were informed both verbally and in writing of the experimental protocol and potential risks prior to giving their written consent to participate. The Research Ethics Boards of the University of Guelph and McMaster University approved the study.

7.3.2 Pre-experimental Protocol

In preparation for the experiment, subjects visited the laboratory on three separate occasions. On the first visit subjects performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (LODE Excalibur, Quinton Instrument, Groningen, The Netherlands) for the determination of VO$_{2peak}$. Respiratory gases were collected and analyzed using a metabolic cart (MOXUS metabolic system, AEI Technologies, Pittsburgh, USA). Following a 30 min break, subjects cycled for ~20 min at ~65% VO$_{2peak}$ to establish the power output for the subsequent 90 min trial.

On two subsequent occasions, subjects reported to the laboratory for practice trials and cycled at ~65% VO$_{2peak}$ for 90 min followed by a TT. On the first practice trial, subjects completed the trial without fluid (DEH) to ascertain sweat loss over the entire trial and determine how much fluid they needed to drink throughout the second practice trial (HYD) to maintain fluid balance.
Subjects woke, urinated, and had their BM taken in shorts for five consecutive days prior to each trial to ascertain mean morning BM. All subjects abstained from strenuous exercise and caffeine, and recorded their diet in the 24 h before the trials. Subjects in the DEH trial abstained from drinking fluid from 6 PM the evening prior to the trial until they arrived at the laboratory. Two hours prior to the practice rides, subjects ingested a meal provided for them (790 kcal; 144g carbohydrate, 35g fat, 19g protein) and 250 mL of fluid. Additionally, subjects in the HYD trial drank as they normally do the night before and drank 300 mL of water 90 and 45 min before the trial to ensure they were well hydrated before cycling. Upon arrival to the laboratory, subjects voided their bladder and provided a small mid-stream urine sample to determine USG and urine osmolality (Uosm) and completely voided their bladder. Subjects were weighed in shorts to determine pre-trial BM. The effect of the overnight fluid dehydration protocol was ascertained by subtracting the pre-trial BM from the mean BM measurements of the five consecutive mornings prior to each trial. Following 45 and 90 min of exercise, subjects stopped cycling and dismounted the cycle ergometer, removed their shoes and shirt, toweled dry, and were weighed wearing only shorts for the determination of sweat loss during the previous 45 min of exercise. At 45 min, subjects put on a dry t-shirt and recommenced cycling. Refer to 5.3.2 for the calculation of sweat loss, RER, carbohydrate and fat oxidation. After each respiratory gas measurement, subjects completed a 2 min simulated hill climb at 85% VO_{2peak} for a total of 4 intervals to incur greater sweat loss over the 90 min trial.

Following the 90 min trial at ~65% VO_{2peak} subjects rested for 5-7 min. Subjects then remounted the cycle ergometer and completed a TT where they were asked to complete 6 kilojoules (kJ)/kg BM of work in the fastest time possible. The total work (kJ) that each subject needed to complete was calculated from the subject’s mean morning BM for that trial. Monetary prizes were established to motivate subjects to work hard and finish each TT in the fastest time. Subjects were reminded of the monetary prizes associated with the fastest cumulative TT over the four trials (practice + experimental) before commencing each trial. Throughout the trial, subjects were blinded to elapsed time and power output but were able to see the amount of work (kJ) completed. After the TT, subjects removed their shoes and shirt, toweled dry, and were weighed for the determination of BM loss over the TT. Practice trials were separated by 5-7 d.

7.3.3 Experimental Protocol

Subjects arrived to the laboratory on two occasions for the actual experiment. During the experimental trials subjects cycled at ~65% VO_{2peak} for 90 min followed by a TT where subjects
completed 6 kJ/kg BM of work in the fastest time possible. In one trial subjects drank fluid to match sweat losses (HYD) or without fluid (DEH). Subjects replicated the same procedure as described above for the practice trials. Prior to exercise, a teflon catheter was inserted into an antecubital vein for blood sampling and one leg was also prepared for percutaneous needle biopsy sampling (same as 5.3.3). Venous blood samples were obtained at 20, 40, 60, 75, and 90 min of exercise. HR, Te, and RPE were recorded every 10 min during exercise. During the HYD trial subjects were given fluid every 15 min to match sweat loss. At 45 and 90 min, the subject stopped cycling and a muscle biopsy was taken with the subject sitting on the cycle ergometer. During the TT, power output, HR, Te, and RPMs were recorded at 15, 30, 45, 60, 75, 90, and 100% of total work (kJ) completed. Also, a venous blood sample was taken at 50% (TT1) and 90% (TT2) of total work completed. Immediately following the completion of the TT a muscle biopsy was taken with the subject sitting on the cycle ergometer. After the muscle biopsy was taken, subjects removed their shoes and shirt, towel-dry, and were weighed for the determination of BM loss over the TT. The same procedure was replicated for the second trial with muscle biopsies taken from the opposite leg. The trials were randomized and separated by 7 days.

7.3.4 Analyses

Same as 5.3.4

Hsp72 Western Blots. Skeletal muscle tissue from the vastus lateralis was homogenized in ice-cold buffer (1:9 wt/vol dilution) suitable for whole cell protein extraction and preserving phosphorylation states of proteins. Homogenates were sonicated for 5 s to ensure the nuclear membrane was completely broken, centrifuged at 1,500 g for 15 min at 4°C, and the supernatant was removed, and protein content was determined using BSA as standards.

Whole cell lysate protein was mixed with equal volumes of sample buffer (0.5 M Tris base, 13% glycerol, 0.5% SDS, 13% β-mercaptoethanol, and bromophenol blue) and separated according to their molecular weight on gels consisting of a 12% acrylamide separating gel overlaid by a 4% acrylamide stacking gel. A molecular weight standard (catalog no. 161-0373 Bio-Rad) was run concurrently on each gel for accurate determination of the proper molecular weight of the protein. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes and blocked in Tris-buffered saline (TBS) for 1 h and then washed twice with 0.01% Tween 20 in TBS (TTBS) for 5 min each wash. Membranes were then incubated at 4°C in primary antibody specific to Hsp72 (anti-Hsp70 polyclonal antibody, 1:5,000, SPA-812, Stressgen) in TTBS (2% BSA, catalog no. A-2153,
Sigma). Following incubation, membranes were washed in TTBS and incubated with secondary antibody according to the manufacturer's instructions. Antibody detection was performed using the enhanced chemiluminescence method (Syngene Chemigenius2; PerkinElmer, Waltham, MA), and quantified with densitometry (Gene Tools software; PerkinElmer). Repeats were done on all samples. Ponceau Staining was performed after quantification to ensure loading was even in each well.

7.3.5 Statistical Analysis

Same as 5.3.5

7.5 RESULTS

7.5.1 Trial Conditions

No pre-trial differences existed between the HYD and DEH trials for laboratory temperature (HYD, 23 ± 0.1 vs. DEH, 23 ± 0.2°C), and relative humidity (32 ± 0.3 vs. 33 ± 0.4%). Pre-trial hydration status was significantly different between trials based on USG (HYD, 1.015 ± 0.002 vs. DEH, 1.024 ± 0.002) and Uosm (700 ± 111 vs. 968 ± 49 mmol/L).

7.5.2 Body Mass Loss, Sweat Loss, & Fluid Intake

In the HYD trial, subjects drank a mean of 1.6 ± 0.2 L of fluid and BM decreased by only 0.5 kg or 0.6% BM (Table 7.1). As a result of overnight fluid restriction, DEH subjects started the trial with a BM loss of 0.6% (0.5 ± 0.1 kg). BM was lower at 45, 90 min, and after the TT in the DEH vs. HYD trial and there was no difference in sweat loss between trials (Table 7.1).
Table 7.1 Differences in sweat loss, body mass (BM) and percent (%) BM loss between the hydrated (HYD) and dehydrated (DEH) trial at 0, 45, 90 min of cycling at ~65% VO$_{2peak}$ and after the time trial. Data are means ± SE (n = 9). *Significantly lower than HYD (p < 0.05).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HYD</th>
<th>DEH</th>
</tr>
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<tbody>
<tr>
<td>Mean Morning BM</td>
<td></td>
<td></td>
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<tr>
<td>0-45</td>
<td>45–90</td>
<td>90 Post TT</td>
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<tr>
<td>Sweat Loss (L)</td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>45</td>
<td>90</td>
</tr>
<tr>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
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<tr>
<td>77.3 ± 3.0</td>
<td>77.4 ± 2.9</td>
<td>77.2 ± 2.8</td>
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<tr>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
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</table>
| 75.3 Oxygen Uptake & Whole Body Substrate Use

There was no difference in mean VO$_2$ with exercise time and between trials (Fig. 7.1a). The RER progressively decreased in both trials and was lower than rest at 40, 60, and 80 min in the HYD trial and at 80 min in the DEH trial (Fig. 1b). RER was higher in the DEH vs. HYD trial from 60-90 min (Fig. 7.1b).
Figure 7.1 (a) VO\textsubscript{2} and (b) respiratory exchange ratio (RER) during 90 min of cycling at ~65% VO\textsubscript{2peak} in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9). RER was significantly lower than rest at 40, 60, and 80 min in the HYD trial and at 80 min in the DEH trial (\(p < 0.05\)). *Significantly greater than HYD trial (\(p < 0.05\)). Arrows (↓) indicate ~1% and ~2% BM loss.

The rate of CHO oxidation significantly decreased over time in both trials and was lower than 20 min at 60 and 80 min in the HYD trial and at 80 min in the DEH trial (Fig. 7.2a). Conversely, the rate of fat oxidation increased over time in both trials and was greater than 20 min at 60 and 80 min in the HYD trial and at 80 min in the DEH trial (Fig. 7.2b). There was a trend for CHO oxidation to be greater and fat oxidation to be lower in the DEH trial at all time points, but the difference was not significant until 60 min of cycling. Total CHO oxidation was greater in the DEH trial (HYD, 168 ± 10 vs. DEH, 202 ± 12 g), and total fat oxidation was lower in the DEH trial (67 ± 1 vs. 54 ± 1 g).
**Figure 7.2** (a) carbohydrate and (b) fat oxidation during 90 min of cycling at 65 % VO$_2$peak in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9). Carbohydrate oxidation was lower than 20 min at 60 and 80 min in the HYD trial and at 80 min in the DEH trial. Fat
oxidation was greater than 20 min at 60 and 80 min in the HYD trial and at 80 min in the DEH trial. *Significantly greater than HYD trial ($p < 0.05$). Arrows ($\downarrow$) indicate ~1% and ~2% BM loss.

7.7.4 Heart Rate, Core Temperature, and Rating of Perceived Exertion

HR significantly increased over time and was greater than 10 min from 30 – 90 min in both trials (Fig. 7.3). Subjects had a higher HR from 50 – 90 min of cycling in the DEH vs. HYD trial (Fig. 7.3).

Figure 7.3 Heart rate response during 90 min of cycling at 65% $\text{VO}_{2\text{peak}}$ in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE ($n = 9$). Heart rate was greater than 10 min at 30 – 90 min in both trials. *Significantly greater than the hydrated trial ($p < 0.05$). bpm, beats per minute. Arrows ($\downarrow$) indicate ~1%, ~2%, and ~3% BM loss.

During the TT, HR was significantly greater than the 15% data point at 75 – 100% of work completed in both trials (Table 7.2). There was no difference in HR between trials (Table 7.2).
Table 7.2 Performance time and heart rate at 15, 30, 45, 60, 75, 90, and 100% of work completed during the time trial in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9).

†Significantly greater than 15% of work completed (p < 0.05). *Significantly greater than HYD (p < 0.05).

<table>
<thead>
<tr>
<th>Work Completed</th>
<th>15%</th>
<th>30%</th>
<th>45%</th>
<th>60%</th>
<th>75%</th>
<th>90%</th>
<th>100%</th>
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<tr>
<td></td>
<td>31.8 ± 4.1</td>
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<tr>
<td>Time (min)</td>
<td>HYD</td>
<td>DEH</td>
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<td>DEH</td>
<td>HYD</td>
<td>DEH</td>
<td>HYD</td>
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<tr>
<td>Heart Rate (bpm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HYD</td>
<td>180 ± 3</td>
<td>182 ± 3</td>
<td>182 ± 3</td>
<td>183 ± 3</td>
<td>184 ± 3†</td>
<td>185 ± 3†</td>
<td>187 ± 3†</td>
</tr>
<tr>
<td>DEH</td>
<td>179 ± 2</td>
<td>181 ± 2</td>
<td>180 ± 3</td>
<td>179 ± 4</td>
<td>183 ± 3†</td>
<td>183 ± 2†</td>
<td>188 ± 3†</td>
</tr>
</tbody>
</table>

Tc was significantly greater than 10 min at all time points in both trials. Tc was significantly greater in the DEH vs. HYD trial from 60 – 90 min (Fig. 7.4a). Throughout the TT, Tc increased only in the DEH trial and was significantly greater than 15% of work competed at 45 – 100% of work completed (Fig. 7.4b). Tc was greater in the DEH vs. HYD trial at all time points (Fig. 7.4b). As well, Tc was greater in the DEH vs. HYD trial before the start of the TT (HYD 37.5 ± 0.2 vs. DEH 38.1 ± 0.2 °C) (Fig. 7.4b).
Figure 7.4 Core temperature (°C) during (a) 90 min of cycling at 65% VO$_{2\text{peak}}$ (b) performance trial in the hydrated (HYD) and dehydrated (DEH) conditions. Values are means ± SE (n = 9). *Significantly greater than HYD trial ($p < 0.05$). Arrows (↓) indicate ~1%, ~2% and ~3% BM loss.

RPE increased over time in both trials and was greater than 10 min at 90 min in the HYD trial and from 40 – 90 min in the DEH trial (Fig 7.5). RPE was greater in the DEH trial from 50 – 90 min of cycling (Fig 7.5).
Figure 7.5 Rating of perceived exertion (RPE) during 90 min of cycling at 65% \( \text{VO}_{2\text{peak}} \) in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9). RPE was greater than 10 min at 90 min in the HYD trial and at 40 – 90 min in the DEH trial. *Significantly greater than HYD trial (\( p < 0.05 \)). Arrows (↓) indicate ~1 and ~2% BM loss.

7.5.5 Blood Measurements

Hb and Hct were increased above rest at all time points in both trials (Table 7.3). In the DEH trial, Hb was higher than the HYD trial from 60 min onward (Table 7.3). There was a trend for Hct to be slightly greater in the DEH trial throughout exercise, but the difference was insignificant (Table 7.3). Pvol loss was greater than rest at all time points in both trials and was greater in the DEH vs. HYD trial from 75 min onward (Table 7.3). Posm was greater than rest at 60-90 min in the HYD trial and from 40-90 min in the DEH trial (Table 7.3). Posm was greater in the DEH vs. HYD trial from 40 min onward (Table 7.3). During the TT, Hb, Pvol loss, and Posm were significantly greater at TT1 and TT2 in the DEH vs. HYD trial. Blood glucose decreased with exercise time and was lower than rest at 40-90 min in the HYD trial and at 90 min in the DEH trial, with no differences between trials (Table 7.3). Blood lactate was increased from rest at 20 min of exercise and beyond in both trials and was higher in the DEH vs. HYD trial at 40 -90 min (Table 7.3). As well, during the TT
DEH subjects had a blood [La] that was the same as the HYD subjects at TT1 and a significantly higher blood [La] at TT2 despite power output being significantly lower (Table 7.3). Plasma FFA was not affected with exercise time and there was no difference between trials. Plasma epinephrine significantly increased from rest at all time points in both trials, however there were no differences between trials at any time point (Table 7.3). During the TT, plasma EPI was greater in both trials at TT2 compared to TT1, with no between trial differences (Table 7.3). During the 90 min trial there was no effect of exercise time or trial differences in serum Hsp72 (Table 7.3). Serum Hsp72 content was significantly greater than rest at TT1 and TT2 in both trials, and was significantly greater in the DEH vs. HYD trial at TT1 and TT2 (Table 7.3).
Table 7.3 Hemoglobin (Hb), hematocrit (Hct), glucose, lactate, and plasma volume loss (Pvol loss), plasma osmolality (Posm), plasma free fatty acid (FFA), plasma epinephrine (EPI) concentration, and serum Hsp72 content, during 90 min of cycling at ~65% VO\textsubscript{2peak} and at 50% (TT1) and 90% (TT2) of work completed in the time trial in the hydrated (HYD) and dehydrated (DEH) trials. Data are means ± SE (n = 9). †Significantly greater than 0 min (p < 0.05). *Significantly greater than HYD (p < 0.05). ^Significantly greater than TT 1 (p < 0.05).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Time (min)</th>
<th>Time Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Hb (g/100mL)</td>
<td>HYD</td>
<td>13.9 ± 0.4</td>
</tr>
<tr>
<td>DEH</td>
<td>13.9 ± 0.5</td>
<td>14.8 ± 0.5†</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>HYD</td>
<td>41.1 ± 0.8</td>
</tr>
<tr>
<td>DEH</td>
<td>41.6 ± 0.8</td>
<td>42.7 ± 0.7†</td>
</tr>
<tr>
<td>Pvol Loss (%)</td>
<td>HYD</td>
<td>-----</td>
</tr>
<tr>
<td>DEH</td>
<td>-----</td>
<td>-3.8 ± 0.9†</td>
</tr>
<tr>
<td>Posm (mOsm/dL)</td>
<td>HYD</td>
<td>288 ± 1.5</td>
</tr>
<tr>
<td>DEH</td>
<td>290 ± 1.1</td>
<td>292 ± 1.0†</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>HYD</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>DEH</td>
<td>3.9 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>HYD</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>DEH</td>
<td>0.9 ± 0.4</td>
<td>2.5 ± 0.4†</td>
</tr>
<tr>
<td>Plasma FFA (mM)</td>
<td>HYD</td>
<td>0.7 ± 0.03</td>
</tr>
<tr>
<td>DEH</td>
<td>0.7 ± 0.04</td>
<td>-----</td>
</tr>
<tr>
<td>Plasma EPI (nM)</td>
<td>HYD</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>DEH</td>
<td>0.7 ± 0.1</td>
<td>-----</td>
</tr>
<tr>
<td>Serum Hsp72 (ng/mL)</td>
<td>HYD</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>DEH</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>
7.5.6 Muscle Fuels & Metabolites

Muscle PCr was lower than rest at all time points in both trials, but not different between trials and $[\text{Cr}]$ was reciprocal with the PCr changes (Table 7.4). Muscle ATP content was not altered with exercise in the HYD trial but was lower than rest after the TT in the DEH trial with no difference between trials (Table 7.4). ADPf and AMPf were higher than rest at all time points during exercise in both trials, with no differences between trials (Table 7.4). Muscle lactate was greater than rest at all time points in both trials and greater in the DEH trial at 90 min and after the TT (Table 7.4). Muscle glycogen content was similar in the two trials before exercise and significantly lower after 45 and 90 min, and after the TT in both trials compared to rest (Table 7.4).

Table 7.4 Skeletal muscle fuel and metabolite contents during 90 min of cycling at $\sim 65\%$ $\text{VO}_{2\text{peak}}$ and immediately after the completion of the time trial in the hydrated (HYD) and dehydrated (DEH) trials. Data are means $\pm$ SE ($n = 9$), mmol/kg dry muscle (dm). PCr, phosphocreatine; Cr, creatine; ATP, adenosine triphosphate; ADPf, free adenosine diphosphate; AMPf, free adenosine monophosphate. †Significantly greater than 0 min ($p < 0.05$). *Significantly higher than HYD trial ($p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>HYD</th>
<th>DEH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td><strong>PCr (mmol/kg dm)</strong></td>
<td>90.9 ± 5.4</td>
<td>72.1 ± 5.6</td>
</tr>
<tr>
<td><strong>Cr (mmol/kg dm)</strong></td>
<td>71.1 ± 5.5</td>
<td>90.0 ± 5.7</td>
</tr>
<tr>
<td><strong>ATP (mmol/kg dm)</strong></td>
<td>28.6 ± 3.6</td>
<td>23.5 ± 1.6</td>
</tr>
<tr>
<td><strong>ADPf (umol/kg dm)</strong></td>
<td>124 ± 14</td>
<td>184 ± 40†</td>
</tr>
<tr>
<td><strong>AMPf (umol/kg dm)</strong></td>
<td>0.6 ± 0.1</td>
<td>1.7 ± 0.8†</td>
</tr>
<tr>
<td><strong>Lactate (mmol/kg dm)</strong></td>
<td>0.7 ± 0.2</td>
<td>2.2 ± 0.4†</td>
</tr>
<tr>
<td><strong>Glycogen (mmol/kg dm)</strong></td>
<td>467 ± 26</td>
<td>221 ± 25†</td>
</tr>
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</table>

In the DEH trial there was more glycogen used in the first 45 min (15%, HYD, 232 ± 36 vs. DEH, 282 ± 44 mmol/kg dm, $p = 0.05$) and over the entire 90 min trial (17%, HYD 308 ± 35 vs. DEH, 359 ± 35 mmol/kg dm, $p = 0.04$), with a trend for greater glycogen use from 45-90 min (24%, HYD, 76 ± 14 vs. DEH, 77 ± 17 mmol/kg dm, $p = 0.29$) (Fig. 7.7). There was no difference in muscle glycogen use between trials during the TT (HYD, 88 ± 26 vs. DEH, 74 ± 21 mmol/kg dm).
Figure 7.6 Muscle glycogen use from 0 – 45 min, 45 – 90 min, 0-90 min and during the time trial (TT) in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9).

7.5.7 Hsp72 Protein Changes

There was no difference between trials in Hsp72 protein content at rest. Skeletal muscle Hsp72 protein was significantly greater than rest at the end of the TT in both the HYD (1.4 fold) and DEH (2.0 fold) trials. There was no significant difference between trials (Fig. 7.8).
Figure 7.7 Heat shock protein (Hsp) 72 protein change at rest and at the end of the time trial (TT) in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9). *Significantly greater than rest ($p<0.05$).

7.5.8 Time Trial Performance

Subjects in the DEH trial were 13% slower in completing the TT than the HYD trial (HYD 31.8 ± 4.1 vs. DEH 36.0 ± 3.1 min) (Fig. 7.9).
Figure 7.9 Time trial (TT) performance between the hydrated (HYD) and dehydrated (DEH) trials (n=8). Performance was 13% slower in the DEH vs. HYD trial ($p < 0.05$).

Power output was significantly lower in the DEH vs. HYD trial throughout the TT (Fig. 7.10).

Figure 7.10 Power output during the time trial (TT) in the hydrated (HYD) and dehydrated (DEH) trials. Values are means ± SE (n = 9). *Significantly greater than HYD ($p < 0.05$).
7.6 DISCUSSION

This study investigated the effects of starting exercise mildly dehydrated combined with exercise-induced dehydration on whole body substrate oxidation, serum Hsp72 response, skeletal muscle metabolism, and TT performance. All physiological and blood parameters, in addition to carbohydrate oxidation and muscle glycogen use were greater during the 90 min trial when subjects were dehydrated by ~1-2% BM. TT performance was significantly compromised (13%) with dehydration of ~2-3% BM loss, as power output was lower throughout the TT. Additionally, DEH subjects had higher Tc, Posm, blood and muscle lactate, and serum Hsp72. Muscle glycogen was already very low at the start of the TT in both trials and may have limited the performance in both the DEH and HYD trials.

7.6.1 Effect of dehydration on substrate oxidation and muscle glycogen use

This study demonstrated that whole body CHO oxidation was greater when subjects were dehydrated by ~1.5% BM. There was a trend for CHO oxidation to be greater in the DEH trial throughout exercise even at the beginning of the trial when subjects were 0.6% dehydrated from the overnight fluid restriction, however the difference between trials was not significant until 60 min of exercise when subjects had lost ~1.5% BM (1% = ~22.5 min; 2% = 75 min and 3% = post TT). There are very few studies that have demonstrated an effect of mild dehydration on RER in a temperate environment. Fallowfield et al. (1996) demonstrated that RER significantly increased with progressive dehydration to 2.7% BM loss. In their study male subjects ran on a treadmill at 70% VO$_{2\text{peak}}$ until exhaustion with no fluid (NF) or with fluid replacement (FR) consuming 2 mL/kg BM every 15 min throughout exercise. CHO oxidation was significantly increased (NF 73% vs. FR 64% of total energy expenditure) and fat metabolism was suppressed after 75 min of exercise in the NF trial compared with the FR trial (0.90 vs. 0.86) when dehydration amounted to 2.7% BM loss. Hargreaves et al. (1999) reported a higher RER in males after 60 and 120 min of cycling at 65% VO$_{2\text{peak}}$ with progressive dehydration to 2.9% BM loss versus being euhydrated. The novelty of the present study is that we began to see changes in substrate utilization (increased CHO oxidation) at mild levels of dehydration (~1-2%).

In addition, total muscle glycogen use was greater during the 90 min trial when subjects were 1-2% dehydrated. Hargreaves et al. (1999) demonstrated an increased reliance on muscle glycogen, greater muscle lactate and higher rectal and muscle temperatures after 2 hrs of moderate intensity exercise-induced dehydration to 2.9% BM loss. More recently we reported a 24% increase in
total muscle glycogen use over 2 hrs of cycling at 65% VO$_{2peak}$ in male subjects dehydrated to 2.7% BM loss, with no difference in plasma [EPI] or the energy status of the muscle, but a greater Tc between trials (Chapter six). There appears to be at least three potential mechanisms that may account for the upregulated glycogen phosphorylase (PHOS) activity with dehydration. They include an increased sympathto-adrenal response leading to elevated circulating [EPI] and activation of PHOS, a decreased energy status in the cell manifested by elevated ADPf and AMPf levels, and increased Tm (Febbraio 2000). In the present study there was no difference in plasma [EPI] or the energy status of the cell, which is consistent with the study in Chapter 5 & 6. However, Tc was significantly greater in DEH subjects with 1-2% BM loss. In light of this, we suggest that increased Tm and the Q$_{10}$ effect are the most plausible mechanism to explain the increased muscle glycogenolysis during progressive dehydration in males.

### 7.6.2 Effect of dehydration on Hsp72 response

Heat shock proteins (Hsps) are a group of highly conserved proteins present in all cells and expressed in low concentrations in the basal state. The Hsp70-kDa family is considered to be the most highly inducible form of Hsp and is stimulated by a variety of pathological, physiological, and environmental stressors. The only known measureable form of the Hsp70-kda family in the skeletal muscle is Hsp72, functioning in cytoprotection mediating antiapoptosis, antioxidative activity, neuroprotection, immunoprotection, and as a molecular chaperone (Horowitz & Robinson, 2007). Exercise has been shown to increase Hsp72 gene and protein in serum and contracting skeletal muscle following acute exercise (Febbraio et al. 2002), but the effect of hydration alone on Hsp72 response has not been investigated until now. By measuring serum and muscle Hsp72 we determine if dehydration poses a greater systemic and cellular stress than exercise alone. This may help us explain the poorer performance in DEH, as we speculate that greater circulating Hsp72 exerts greater central feedback, acting as a signal in addition to Tc and RPE, leading to a slowing of self-selected pace to avoid a catastrophic outcome (i.e. heat exhaustion, protein denaturation). Moreover, we hypothesized that serum and muscle Hsp72 protein content would be greater in DEH vs. HYD subjects. We demonstrated that serum Hsp72 was greater during the performance trial with ~2-3% BM loss indicating greater systemic stress with mild dehydration at high intensity exercise. Rodrigues-Krause (2010) suggest that the motoneurons, cells that are very active, very sensitive to heat and potentially one of the most stressed cells during exercise could be the major site for the extracellular Hsp72 function. They suggest that extracellular Hsp72 during exercise can be taken up by the motoneuron
and act as an intracellular chaperone yielding cytoprotection against oxidative damage, heat, and protein denaturation, along with many other stressors to help prevent neurodegeneration (Rodrigues-Krause 2010). Although in this study it was not determined what tissue the serum Hsp72 was being released from, we can speculate based on research done by Febbraio et al. (2002) that the splanchnic tissues release Hsp72 during exercise and is partly responsible for the elevated serum Hsp72 content.

Additionally, there was a trend for greater muscle Hsp72 protein after the TT in DEH vs. HYD subjects suggesting that the muscle cell is under greater stress when dehydrated by ~2-3% BM. Febbraio et al. (2002) suggested that intact muscle cells are not able to release Hsp72 into the circulation, but that stressed muscle cells synthesize Hsp72 in order to protect intracellular proteins from unfolding and denaturation and also exert an anti-inflammatory effect.

7.6.3 Effect of dehydration on TT performance

The results of this study demonstrate that TT performance was significantly compromised when subjects started 2.3% dehydrated and ended at 3.1% BM loss vs. maintaining BM. Performance time was 13% longer in the DEH trial and HR, Tc, Posm, blood and muscle lactate were all significantly higher despite subjects cycling at a lower mean power output compared to the HYD trial. These results are powerful as in reality athletes are losing significant amounts of fluid during competition. Ebert et al. (2007) reported that male and female elite road cyclists during the Tour de France and the Tour de l’Aude cycling tours lost 2.8% and 2.6% BM loss per stage over the 21-day and 10-day tours, respectively. Ultimately, this study demonstrates that high intensity exercise for an extended period of time cannot be sustained when dehydration amounts to ~3% BM loss.

Our results demonstrated that greater muscle glycogen use is not the reason for the decreased TT performance when dehydrated as both the HYD and DEH trials began the TT with very low and similar muscle glycogen contents (HYD, 159 ± 22 vs. DEH, 140 ± 24). The 90 min of cycling at 65% VO$_{2peak}$ depleted almost all the muscle glycogen in our male subjects, leaving little for use during the TT.

Alternately, the lack of heat transfer to the periphery while sweat rates were the same accounts for the greater Tc in the DEH trial. When dehydrated by ~2-3%, the body may be forced to slow down during high intensity exercise as a means of self preservation to prevent further metabolic heat production and further increases in Tc, which may lead to cellular and protein damage. As well, the combination of a high Tc and low Pvol contributes to a heightened RPE leading to a slowing of self-selected pace. Moreover, this study reveals that the greater glycogen use during exercise with
dehydration does not appear to play an important role in determining performance. Instead we suggest that a critically high Tc is the more domineering factor determining performance when dehydrated by 2-3% BM. We believe that the effect of Tc exerts its effect through feedback to the brain leading to a higher RPE and lower self-selected pace.

7.7 CONCLUSIONS

This study demonstrated that all physiological parameters along with CHO oxidation and muscle glycogen use were greater during 90 min of moderate intensity exercise when subjects progressed from 0.6 to 2.3% dehydration. TT performance was 13% slower when subjects began 2.3% and ended 3.1% dehydrated. Throughout the TT, Tc, Posm, blood and muscle lactate, and serum Hsp72 were higher, even while working at a lower power output. Differences in muscle glycogenolysis with dehydration cannot explain the detriments to high intensity endurance performance as subjects began the performance trials with the same muscle glycogen content in both DEH and HYD trials. Instead, a critically high Tc may account for the performance detriments as the body slows to prevent further heat production.
This thesis set out to answer 4 major questions: 1) Do elite hockey players arrive for a game dehydrated and do they consume enough fluid to prevent dehydration over the course of a game? 2) Is hydration status repeatable between days and can an athlete who arrives dehydrated prior to training or competition become hydrated in the time before the start of activity? 3) What is the extent of dehydration (% BM loss) necessary to change substrate oxidation and skeletal muscle metabolism during exercise in male and female subjects? 4) Will progressive dehydration have a negative effect on endurance performance? The work in this thesis combined a series of practical studies with laboratory based studies to answer these questions and provide some useful practical information for athletes, trainers and coaches.

8.1 Pre-exercise Dehydration

It has been reported that many athletes arrive to training and competition dehydrated (Maughan et al. 2004; Osterberg et al. 2009; Volpe et al. 2009) with only a few known studies investigating pre-game dehydration in ice hockey players. Palmer et al. (2008) were the first to report the prevalence of dehydration in elite male Jr. hockey players during an on-ice practice at a National selection camp. They reported that 55% of the players were dehydrated (USG > 1.020) prior to the start of the try-out. Similarly, Palmer et al. (2010) assessed pre-practice hydration in another group of elite male Jr. hockey players (Ontario Hockey League (OHL) team) and reported that 75% arrived dehydrated to an in-season practice. This was unexpected as one would expect elite athletes to pay closer attention to their pre-practice hydration status, as mild dehydration can have a negative impact on performance. To follow these studies on elite hockey players, this thesis determined if the trend of arriving dehydrated to practice also occurred prior to OHL games. Surprisingly, 41% of players arrived to the game dehydrated, which was an improvement over the practice data, but still a high prevalence. What was surprising was that most of the players tested during the games had been previously hydration/sweat tested during at least one practice, prior to being game tested. They had received a one-page report and oral feedback regarding their own hydration needs prior to and during practice, in order to maintain fluid balance.

However, it should be pointed out that 9 of the 20 players who arrived to the arena in a dehydrated state (USG > 1.020) drank a mean of 0.7 ± 0.1 L of fluid in the 60-90 min prior to the game. Since this is greater than the amount of fluid that the ACSM and NATA position stands recommend (~600 mL) that an athlete should drink prior to a strenuous workout, it is likely these players were properly hydrated before starting the OHL game. This may not be the case prior to OHL
practices, where players often arrive to the arena only ~30 min before practice and dehydrated, and do not have enough time to become hydrated before practice begins.

In light of the prevalence of the pre-exercise dehydration, study two provided a plan for athletes who normally arrive to training or competition dehydrated. It was determined that hydration status (fluid retention and USG measures) could be reversed within 30-45 min after consuming 600 mL of fluid in two boluses of 300 mL, 15 min apart. The hockey players that we assessed in the game study arrived to the arena ~120 min before the game, which gave dehydrated players sufficient time to become hydrated prior to game start. This recommendation, ~600 mL of fluid in the 60 min prior to exercise - should be adopted by athletes and health professionals as it proves to be a very easy and effective approach to combat pre-exercise dehydration. In comparison, when players arrive at the arena with little time left before practice time (~30 min), their pre-practice hydration regimen has to begin before they leave for the arena or on the way to the arena depending on their commute. This will ensure enough time to become hydrated before starting practice.

Another interesting finding was that there was no difference in the type (W, W-S, vs. CES-L, vs. CES) of fluid consumed on USG response or total Uvol over the 60 min rehydration period. We predicted that fluid retention would be greater after consuming Na\(^+\) containing fluid (CES, W-S, CES-L), as it has been demonstrated that the amount of fluid retained is directly proportional to the [Na\(^+\)] of the fluid consumed when dehydrated following exercise (Shirreffs et al. 2007). However, our results reported only a trend for fluid retention to be slightly greater in the CES trial (76% retention), followed by the W-S (72%), and then the W and CES-L trials (68%) \((p > 0.05)\). It is possible that the volume of fluid and the electrolyte (Na\(^+\)) concentration of the W-S and CES were not high enough to differentiate between fluid types in USG or fluid retention over a 1 hr period. Also, given that 68% of the fluid was retained in the W trial, there was not a lot of room for improvement in the CHO and salt solution trials. Most of the studies reporting greater rehydration with an electrolyte containing solution have been conducted after exercise once a BM loss of 1.5-2% had been achieved and then rehydrating with a fluid volume of 150% of BM loss over a 3-4 hr recovery period (Shirreffs et al. 2007; Valiente et al. 2009; Utter et al. 2010). It seems more logical that after a dehydrating bout of exercise a beverage containing a combination of fluid and electrolytes would be more superior at replacing sweat loss leading to greater fluid retention than water alone. Instead our research focused on a mild dehydration situation (≤1% BM loss) that may be more representative of the normal living situation prior to training for the average athlete. In light of this, any of the fluids tested here in a volume of 600 mL (2 x 300 mL) 45-60 min prior to the start of exercise were effective at restoring
pre-exercise dehydration.

One potential limitation to this study was measuring USG alone without measuring plasma osmolality. Although USG is a reliable field measurement, it can provide misleading information regarding hydration status if measured during rehydration periods (Sawka et al. 2007). If a dehydrated athlete consumes large volumes of hypotonic fluids, they will have large urine production long before euhydration is reestablished (Sawka et al. 2007). As a result, urine samples collected during this period will be light in color and have USG values that reflect euhydration when in fact the person remains dehydrated (Sawka et al. 2007). Our study tried to control for this by having subjects consume a mere 600 mL of fluid in 2 x 300 mL boluses 15 min apart. As well, by having four different fluid types, three of which contained sodium, our study tried to prevent acute diuresis and differentiate between fluid types in retention and changes to hydration status. It would be of value to repeat this study and measure Posm to confirm if subjects were actually euhydrated 30, 45, and 60 min after fluid consumption.

In addition, we determined that morning hydration assessments were repeatable between days. This is important for athletes, coaches and practitioners using morning hydration measurements to assess hydration status. Athletes can use morning BM (upon waking after voiding) as a way of assessing hydration status on a daily basis. Consistency is the key, as deficits in BM from day to day indicate dehydration. This easy approach of measuring BM proves effective during consecutive days of training and competition such as training camps and stage races such as the Tour de France.

8.2 DEHYDRATION DURING A HOCKEY GAME

It has been demonstrated that many athletes allow themselves to become dehydrated during competition due to high sweat loss with inadequate fluid intake. Study 1 reported that 33% of male Jr. hockey players were mildly dehydrated (1.8-3.1% BM) by the end of an in-season game. The results for Studies 3 and 5 demonstrated with male subjects that progressive dehydration of 1.5-2% BM exacerbated the physiological responses to exercise (Tc, HR, RPE, Pvol loss) and accelerated CHO oxidation and muscle glycogen use, while decreasing high-intensity endurance performance. In light of this, the 8/24 players who lost between 1.8-3.1% BM throughout the game may have compromised their performance in the third period. Ultimately, even though elite male Jr. hockey players are hydrated prior to games, many are not consuming enough fluid to prevent dehydration during a game, and as a result, their performance may suffer. The sport of hockey is very unique in that although the game is played in a cold environment (10-12°C), the body is covered with protective
equipment that prevents sweat evaporation and the cooling of the skin surface. The equipment worn creates a hot micro-environment increasing sweat loss, while the face is the only part of the body exposed to the cold environment. Therefore, in addition to monitoring fluid balance in hockey players, it would be of interest to measure skin temperature under the equipment, Tc, and Tm, to monitor the changes in skin, core, and muscle temperature as the game progresses in hydrated and dehydrated players.

Furthermore, the ability to measure pre-exercise hydration status (USG) and fluid balance during field practices and games (sweat loss, fluid intake, BM loss) provides feedback for each athlete on how successful their attention to staying hydrated is. Ultimately, the goal is to educate athletes about the importance of hydration to performance, assess their individual fluid habits and needs, and provide them with a practical strategy to follow.

8.3 PHYSIOLOGIC AND METABOLIC CONSEQUENCES TO DEHYDRATION

The third research question of the thesis was to determine how quickly the physiologic and metabolic changes that occur during prolonged exercise were exacerbated by progressive dehydration. The consequences of exercising mildly dehydrated were extremely pronounced as subjects had an increased RPE, Pvol loss, HR, Tc and probably Tm. HR was increased, most likely to compensate for the decreased stroke volume and to maintain cardiac output, and contracting muscle blood flow. Heat dissipation was compromised as Tc was elevated in the face of maintained sweat rates, suggesting that skin blood flow was reduced when dehydrated. Whole body CHO, muscle glycogen use and muscle lactate production were increased, as were serum and muscle Hsp72 content. The studies of this thesis demonstrate that normal hydrated physiologic and metabolic responses during exercise are exacerbated when dehydration amounts to ~1-2% BM loss.

8.3.1 The Physiologic Consequences of Dehydration

It is well known that exercise increases metabolic heat production. In order to control or limit the extent of the exercise-induced increase in Tc, skin blood flow (SBF) is increased to deliver heat to the periphery and sweating dissipates heat via evaporation to cool the body. When exercising in a dehydrated state, SBF is reduced in an attempt to decrease central venous pressure (CVP) in the face of a reduced Pvol (Nadel et al. 1971). Consequently, heat dissipation via sweat evaporation is reduced causing Tc to rise. This was evident in the studies of the thesis as Tc increased with only
small levels of DEH (1% BM loss), while sweat rates were the same as the HYD trials, suggesting that SBF was reduced and heat transfer to the periphery was reduced.

It was interesting that although dehydration increased Tc, sweat rate was the same between trials. It might be argued that, if sweat rate responds to increases in Tc, an increase in sweat rate would be expected in the DEH trials, but this was not the case. It appears that in the HYD state, the first physiological priority is to limit the increase in Tc via heat loss through increased SBF and sweating. However once a certain Pvol loss has been attained due to sweating and inadequate fluid intake, the priority shifts to the preservation of CVP achieved by reducing skin blood flow, peripheral heat transfer, and increasing Tc. It is speculated that during dehydration when blood volume drops and Tc rises to a critical level, the brain perceives work to be harder (increased RPE) causing the body to slow down to prevent any further increases in Tc and potential cellular damage. In the DEH studies, the critical Tc when a higher RPE began to be reported, was ~38.3-38.5°C, indicating that the brain slows down the body to prevent further increases in Tc. Moreover, it is speculated that all the exacerbated physiological responses with DEH (increased Tc, HR, Pvol loss, CVP, Hsp72) provide feedback to the brain, and contribute to a greater RPE.

8.3.2 The Metabolic Consequences of Dehydration

The availability of CHO as a substrate for the muscle is critical for the performance of both intermittent high-intensity and prolonged aerobic exercise (Burke 2010). Typically, exhaustion of muscle and or liver glycogen stores limits exercise performance and it was expected that any accelerated glycogen use during exercise when dehydrated by 1-2% BM loss would contribute to earlier fatigue. The present results demonstrated that dehydration of ~1-2% BM increased whole body CHO oxidation and accelerated muscle glycogen use compared to being hydrated. However, there was no indication that liver glycogen stores were depleted as blood glucose was maintained when dehydrated in all studies.

In terms of the increased muscle glycogen use, it was surprising that EPI was not increased with dehydration in spite of the elevated Tc. There was also no change in the powerful allosteric regulators of glycogen PHOS, free ADP and AMP. Consequently, as suggested previously (Chapter 5), it appears that the increased Tc and presumably, Tm with DEH increased the activity of PHOS and increased glycogenolysis.

In addition, of interest was that whole body CHO oxidation was greater during exercise at low levels of BM loss (~1%) in two of three DEH studies. This coincided with a 1% BM loss and Tc
of ~38.5°C in females at 60 min (Chapter 5), and a 1% BM loss and Tc of ~38.2°C at 30 min in males (Chapter 7). This suggests that the critical Tc where we begin to see greater CHO use is ~38°C. In Chapter 6, it was unexpected that total CHO oxidation was not greater in DEH males, but muscle glycogen use was greater over the entire 2 hr trial. When comparing the two male studies, DEH subjects in Chapter 7 had a higher Tc at 45 min (38.7 ± 0.3) vs. Chapter 6 (38.1 ± 0.1 °C, p < 0.05) and 90 min (38.9 ± 0.2 vs. 38.5 ± 0.1 °C, p < 0.05) compared to DEH subjects in Chapter 6 (>38.5°C at both time points) suggesting that subjects in Chapter 6 may not have reached a high enough Tc & Tm in the first 60 min of cycling to see changes in substrate oxidation and glycogen use. It is speculated that the activity of PDH is more sensitive to a higher Tm during dehydration accounting for the greater CHO oxidation seen in Chapter 7 (vs. Chapter 6) due to more pyruvate being oxidized and less converted to lactate. This was surprising since the mean %VO\textsubscript{2} peak and laboratory conditions (temperature and relative humidity) were the same between studies (~65% VO\textsubscript{2} peak), but the later study implemented four hill climb intervals, which must account for the difference in Tc between studies. Regardless, there appears to be no solid explanation for this discrepancy.

Ultimately, findings of this thesis suggest that the reason for the accelerated glycogen use during dehydration is an elevated Tc (~38.5°C) and Tm increasing the activity of PHOS. It may be that a higher Tm during progressive dehydration and prolonged exercise, favors glycogen as a substrate in an attempt to liberate bound water from stored glucose as it has been shown that the breakdown of 1 g of CHO liberates 2.7 g of water (Hall & Guyton, 2011). The amount of water liberated from glycogen may not be substantial, but may be enough to prevent heat damage to the muscle or facilitate cytoplasmic transportation to move more substrate into and out of the myocyte and around the cytosol.

It has been established that during exercise when dehydrated fluid is moved from the intracellular compartment of non-active muscle to the extracellular compartment in an attempt to maintain plasma volume (Nose et al. 1988). In light of this, it is speculated that the changes in the intracellular volume of non-active muscle during exercise when dehydrated by 1-2% BM loss may accelerate whole body CHO oxidation in an attempt to liberate bond water and replace intracellular fluid in non-active muscle. Leading with the speculation that muscle water content is decreased, it may be that the water necessary for the hydrolysis of ATP is diminished slowing the rate of ATP breakdown and energy production, which would contribute to a slowing of self-selected pace. However, all of the discussed speculations above are merely a teleological argument as there is no shown basis or mechanism supporting this speculation.
In addition, the significant reduction in Pvol by up to ~11% due to progressive dehydration in all three of the metabolic studies of this thesis changed the distribution of blood flow to maintain CVP. As a result, it is speculated that there may have been a reduction in blood distribution to adipose tissue reducing the breakdown of triglycerides and the entry of FFAs into the circulation. Although there were no difference in our venous measurements of plasma FFAs there may have been greater arterial FFAs in hydrated subjects and greater uptake of FFAs at the muscle resulting in the venous FFA concentration to be similar between trials. The only way to elucidate this speculation would be to repeat the study with arterial-venous FFA measurements and determine if there was a difference in arterial plasma FFA concentration and uptake at the muscle between trials. As well, it would be of interest to measure the activity of hormone sensitive lipase to determine if mild dehydration during exercise reduces the breakdown of triglycerides.

8.4 THE EFFECTS OF DEHYDRATION ON PERFORMANCE

The increased muscle glycogen use with DEH in the first male and female 2 hr cycling studies suggested that endurance performance would be decreased during a high intensity bout of exercise at the end of a prolonged exercise session. In the final study, subjects were asked to complete a TT in the shortest amount of time, and DEH increased the time to complete the TT by 13%. However, muscle glycogen was essentially depleted in both the DEH and HYD trials, by the time the subjects began the TT. As mentioned previously, it appears that the hill intervals must have accelerated glycogen use as there was no difference in mean %VO$_2$ or laboratory conditions compared to the first male DEH study. In any case, the almost total depletion of muscle glycogen in both trials removed it as a differentiating factor in the HYD and DEH TTS. It also argues that other factors must be responsible for the poorer performance in DEH. Tc differences between trials during the TT were substantial (mean HYD 37.8 ± 0.3 vs. DEH 38.9 ± 0.2°C) indicating that Tc suggests a major role in influencing performance. For example, a lower power output (214 ± 15 vs. HYD, 252 ± 18 W) and higher Tc (38.3 ± 0.4 vs. HYD, 37.6 ± 0.3°C) were reported in the DEH trial at the first intermediate measurement when the athletes had completed only 15% of the TT work and when Tc was already elevated. This once again suggests that reaching a Tc in the low 38s is where a slowing of self-selected pace first appeared.

The question then becomes, how does an elevated Tc reduce self-selected pace? Recent work by Noakes (2010) exploring the complex regulation of human exercise performance directs attention to the anticipatory central governor model (CGM). This model describes exercise as a
behavior that is regulated in anticipation by complex intelligent systems (the brain), the function of which is to ensure that whole body homeostasis is protected under all conditions (Noakes 2010). Noakes suggests that failure to maintain homeostasis either directly in the active muscles (peripheral fatigue) or indirectly in the central nervous system (central fatigue) causes both fatigue and termination of exercise. According to the CGM, during exercise there is continuous feedback from all the organs in the body, which inform the central command of the state of fuel reserves, the rate of heat accumulation, and the hydration state, as well as other variables. The continual feedback from multiple organs act to regulate exercise behavior by modifying motor unit recruitment in contracting skeletal muscle. Ultimately, feedback allows the brain to anticipate a future failure and modify that behavior specifically to ensure that homeostasis is protected and a catastrophic failure prevented (Noakes 2010). For example, there are multiple studies reporting the presence of anticipatory pacing, with the specific goal being to ensure that a thermoregulatory failure does not occur during demanding exercise; thus, behavior modification guarantees that homeostasis is protected under all conditions (Tatterson et al. 2000; Marino 2004; Tucker et al. 2004, 2006). In light of this model, the increased Tc, HR, Pvol loss, Posm, and possibly serum Hsp72, during the DEH performance trial provided the physiological feedback leading to a reduction in self-selected pace in an attempt to prevent a catastrophic failure (i.e. heat exhaustion, protein denaturation).

Figure 1.2 depicts the proposed mechanism to account for the reduction in self-selected pace during the DEH performance trial. It is hypothesized that the physiological parameters that are exacerbated during exercise (Pvol loss, Posm, HR, Tc) when mildly dehydrated are sensed by barroreceptors, osmoreceptors, and thermoreceptors located in the skin and hypothalamus, providing feedback to the brain. When the brain perceives these responses to be threatening and anticipates a future thermal injury if exercise continues at this intensity, it is believed that the brain alters neurotransmitter release resulting in an increased RPE and reduced motivation resulting in a slowing of self-selected pace. The result being the avoidance of thermal injury, however the consequence is a decrease in performance. By manipulating the release of the excitatory neurotransmitter dopamine and serotonin from the pre-synaptic neuron, the brain can control mood and motivation. It is speculated that when the central nervous system perceives sufficient homeostatic imbalance and anticipates thermal injury as seen in our dehydrated trials, dopamine and serotonin secretion from pre-synaptic neuronal vesicles is decreased resulting in less motivation, potential irritability, and increased RPE, leading to a slowing of self-selected pace and performance.
Another point of interest was how dehydration combined with high intensity cycling (as seen during TT) resulted in greater Hsp72 protein (blood and muscle). It is speculated that Hsp72 are a consequence of increased physiologic stress (HR, Tc, Tm, Pvol loss) when DEH manifesting itself through an increased RPE leading to a slowing of self-selected pace. By measuring serum Hsp72 we were able to confirm that dehydration of ~2-3% in males elicited greater systemic stress than HYD, and an elevated muscle Hsp72 indicates that the contracting skeletal muscle is under greater stress during exercise compared to being HYD.

Moreover, dehydration of 1-2% BM loss exacerbates physiological and metabolic responses, however which one or which combination of effects (Tc, Pvol, HR, glycogen use) actually lead to the decrease in performance seen in the TT? Based on our data the major factor affecting performance when dehydrated is Tc. This thesis determined that a critical Tc of ~38°C increased RPE which in turn reduced self-selected pace compromising performance. A future study needs to replicate study 7 with the addition of a third trial where subjects consume a CES to prevent the possibility of glycogen depletion during the 90 min trial and then have subjects complete the TT to assess the impact of the accelerated glycogenolysis with dehydration on performance. Not including a CES trial to study 7 was a drawback to this thesis, as it was not anticipated that subjects would be glycogen depleted by the end of the 90 min trial. However, having solely a water trial allowed us to study just one parameter, being fluid, independent of CHO and electrolytes.

Ultimately, the goal for an athlete is to prevent the increase in Tc during exercise by consuming an adequate volume of fluid to prevent dehydration (1-2%). Hydration testing for athletes during training and competition is a relatively easy method to assess individual fluid needs to prevent dehydration, which can be the difference between a podium performance and a mere finishing result. Coaches and team physiologists need to make hydration testing a priority throughout the season as individual variability in sweat rate calls for customized hydration plans to ensure each athlete is meeting fluid requirements for the prevention of performance detriments due to voluntary dehydration. This involves educating the athlete about the consequences of dehydration. In addition, pre-exercise dehydration can be easily combated by consuming 600 mL of fluid 45-60 min prior to exercise, meanwhile tracking morning BM measurements can help an athlete ensure they begin the day in fluid balance. In sport, it all comes down to performance, and hydration is a factor commonly ignored. Proper hydration assessment, education, and training, can equip an athlete with the knowledge and application skills necessary to use hydration as a means of optimizing performance. As well, the use of cooling vests prior to endurance events have proven effective in delaying the rise in Tc and has
been adopted by many world class athletes before competing in hot environments (Bogerd et al. 2010; Kenny et al. 2011).

8.5 SEX DIFFERENCES

This thesis demonstrated that dehydration affects recreationally active male and females differently. Dehydrated females in Chapter 5 had higher Tc, HR, RPE, CHO oxidation, and muscle glycogen use, at a lower %BM loss, while exercising at the same relative exercise intensity compared to the males of Chapter 6. These effects appeared earlier in the females when dehydration was ~0.5-1% BM loss, while the effects did not became apparent in the male subjects until subjects lost ~1.5-2% BM loss. It appears that females are more sensitive to low levels of dehydration compared to males. It took female subjects 60 min of moderate intensity cycling to lose ~1% BM and Tc rose by ~1.4°C. Conversely, the male subjects lost ~1% BM loss within 40 min of cycling at the same relative intensity and Tc rose by only ~1.0°C. Ichinose-Kuwahara et al. (2010) documented significant differences in sweat rate between genders during the same relative intensity exercise suggesting that women are less efficient at dissipating metabolic heat leading to greater heat storage. Kilding et al. (2009) determined the sweat rate of elite female soccer players during two game-specific training sessions and reported that mean sweat losses were less than previously reported in male soccer players. Ultimately, these authors suggest that the female sweating response differs from that of males, and sweat rates were significantly lower for the same relative exercise intensity in females even after adjustment for anthropometric measures (Kilding et al. 2009). In our dehydration studies, the females (Chapter 5) sweat rate was ~11 mL/kg BM/hr while the males (Chapter 6) had a sweat rate of ~15 mL/kg/hr at 65% VO2peak for 2 hrs of cycling. This difference in sweat rate would account for the lower %BM loss and the greater Tc increase in the female subjects over the same time period as the men. The overall message from the last three Chapters was that physiological responses to exercise were exacerbated at low levels of dehydration ~1-2% BM. Surprisingly, in females the physiologic and metabolic changes were already apparent at only ~0.5-1% BM loss and only 20-40 min of exercise. HR, Tc, Pvol loss, RER, CHO oxidation, blood and muscle lactate, and muscle glycogen use were all greater when dehydration was ≤ 1%. In comparison, our male subjects required ~1.5-2% BM loss to see the same exacerbated responses. In light of the difference in sweat rate between genders, it is speculated that testosterone enhances the sweating response and is responsible for the higher sweat output reported in males, however future studies need to investigate the relationship between reproductive hormones and sweat gland function.
8.6 CONCLUSIONS

Sweat and hydration testing for athletes during training and competition is a relatively easy and important method to predict an aspect of performance that the athlete needs to be aware of and can control. Coaches and team physiologists need to make hydration and sweat testing a priority as individual variability in sweat rate and sweat \([\text{Na}^+]\) calls for customized hydration plans to prevent a heightened Tc and early fatigue due to voluntary dehydration.

The studies of this thesis demonstrated that dehydration of ~1-2% exacerbated all physiological parameters and increased the reliance on CHO oxidation and muscle glycogenolysis during exercise. Based on our data the major factor affecting performance when dehydrated is Tc. This thesis determined that a critical Tc of >38°C increased RPE which in turn reduced self-selected pace compromising performance.

8.7 FUTURE DIRECTIONS

In light of the fact that this thesis includes three of the four known studies investigating dehydration and muscle metabolism, there are several questions regarding dehydration and the impact on muscle metabolism and performance still unanswered. For example, Chapter 5 represents the only known study investigating the impact of progressive dehydration on muscle metabolism in females. From this thesis we have seen that the female metabolism is more sensitive than males to low levels of dehydration. Ultimately, mildly dehydrated (~1%) women used significantly greater whole body CHO and muscle glycogen compared to being hydrated. The reason for this was not determined in this study, but it is speculated that due to a greater mean fat mass found in women and the fact that fat acts as a thermal insulator, more heat would be stored in the female body during exercise resulting in greater core temperature at the same relative intensity compared to a male counterpart. Ultimately, the greater heat storage would accelerate glycogenolysis and whole body CHO oxidation by increasing the activity of key enzymes such as PHOS, phosphofructokinase (PFK), and PDH. More muscle measurements including intramuscular triglycerides, pyruvate dehydrogenase activity, hormone sensitive lipase, and muscle temperature, need to be done to investigate the impact of dehydration on CHO and fat metabolism in females. It is speculated that the greater thermal storage in female subjects would increase PDH and PHOS activity due to higher Tm accounting for the heightened glycogenolysis. Meanwhile the activity of hormone sensitive lipase catalyzing the breakdown of triglycerides to fatty acids and intramuscular triglycerides may be decreased due to a preferential increase in carbohydrate at a higher Tc and Tm. It would be of interest to repeat the last study of this
thesis (Chapter 7) on females with two subject groups. Both subject populations would be of the same fitness level (recreationally active) and body mass, but one subject population would be women with greater fat mass (18-22% body fat) while the other group would be women with a body fat content of <18%. It is hypothesized that women with a higher % body fat would have a greater Tc throughout exercise as the greater fat mass would act as a heat insulator, storing more heat and reducing core to skin heat transfer, leading to an increase in Tc above that seen in the leaner female subjects. As well, it is proposed that sweat rate may not be different between body compositions but the ability to dissipate heat from the core to the periphery is reduced due to greater thermal storage with increased fat mass. Additionally, the last study of this thesis (Chapter 7) could be replicated in females to reassess the impact of progressive dehydration on metabolism with additional blood and muscle measurements to elucidate the mechanism(s) accounting for the greater glycogenolysis when dehydrated and also the impact of 1-2% dehydration on TT performance.

There is little known research investigating the impact of training status (trained vs. untrained) on sweat rate and physiological responses to dehydration. It is suggested that trained individuals sweat more relative to untrained as their sweat glands are more active, and therefore, trained athletes are better able to regulate Tc during exercise (Ichinose-Kuwahara et al. 2010). The drawback being that trained athletes may be more predisposed to greater BM loss and faster dehydration however this has not been studied. As well, it is suggested that trained athletes can tolerate a higher Tc for a longer period of time based on RPE measures, however this is merely speculative and there is no concrete research to support this. With this in mind it is of interest to replicate Chapter 6 with four subject groups; trained males, untrained males, trained females, and untrained females, to determine the impact of training status and gender on physiological responses to exercise at 65% VO_{2peak} (HR, Tc, blood parameters), substrate oxidation, and muscle glycogen use. It is hypothesized that trained will sweat more than untrained subjects leading to greater dehydration and Tc which may accelerate muscle glycogen use.

In regards to performance, it is speculated that trained will do better than untrained subjects at a TT to exhaustion performed at the same relative exercise intensity. It is hypothesized that the trained subjects, despite being more dehydrated, will be able to tolerate a greater %BM loss and Tc without as much detriment to performance. It is believed that the drive and motivation to succeed in a trained athlete will push them through mild dehydration and rises in Tc to perform better than the untrained athlete who is dehydrated to a smaller amount. The central mechanisms responsible for this hypothesis are unknown but may be due to memory. For example, a trained athlete may have been
dehydrated during competition on multiple occasions, which at the first occurrence may have led to a slowing of self-selected pace and performance. However, after the first occurrence the brain’s anticipatory effect of a potential thermal injury may be dampened since a catastrophic event did not happen during a similar situation in the past. Thus, performance or self-selected pace would not be as affected due to the memory of not having a thermal injury at this intensity, hydration state, and Tc in past exercise situations.

In addition, future studies need to examine other measurements in the muscle to help elucidate the mechanisms responsible for the increased glycogenolysis and decreased fat metabolism seen during prolonged exercise when mildly dehydrated. For example, study 4 could be repeated with large enough muscle biopsies to measure muscle glycogen, metabolites, IMTGs, CPT1, PHOS and PDH activity, as well as hormone sensitive lipase in the blood to determine if dehydration effects the mobilization of free fatty acids from triglycerides. Tm needs to be measured to ascertain how hot the working muscle gets when dehydrated versus hydrated to elucidate whether the muscle is undergoing thermal damage as a result of a Tm ≥ 40°C. By measuring IMTGs we can determine whether fatty acid availability in the muscle is reduced when dehydrated since it is speculated that the availability of substrate may be the limiting factor to fat metabolism when dehydrated. As well, measuring the activity of CPT1, PHOS, and PDH would clarify how Tm is affecting fat transport into the mitochondria, and determine if PHOS and PDH activity is sensitive to rising Tm increasing glycogenolysis and glycolysis. By measuring Tm we could determine the Tm when changes in muscle metabolism occur, specifically the breakdown of IMTGs and liberation of intramuscular fatty acids, transport of the fatty acids into the mitochondria via CPT1, and the activity of glycogen PHOS and PDH. It is hypothesized that as Tc rises to ≥ 38°C, Tm would be ~39.5-40°C, which is the approximate temperature when we start to observe changes in skeletal muscle metabolism to preferentially use more carbohydrate, but a future study needs to elucidate this. As well, it is speculated that the small reduction in muscle water content when dehydrated may slow down the translocation of fatty acids & pyruvate across the cytoplasm to the mitochondria, which would limit the availability of substrate for beta-oxidation and the Kreb cycle respectively. Ultimately, a future study needs to elucidate the mechanism(s) for greater glycogenolysis during mild dehydrated and answer the question why we preferentially use carbohydrate over fat during exercise when mildly dehydrated.

The research of this thesis suggests that Tc is the culprit responsible for performance detriment when exercising dehydrated, but what if there was a study designed to control Tc to evaluate
the impact of dehydration alone on performance. In light of this, a future study needs to investigate the relationship between Tc and performance when Tc is controlled. Subjects would be asked to ride for ~90 min at 65-70% VO$_{2\text{peak}}$ to progressively dehydrate to ~2-3% BM loss and then complete a TT. Subjects in the DEH trial would be asked to wear a cooling vest throughout the 90 min trial to keep Tc around the same temperature as the HYD trial. DEH subjects would start the TT when Tc is the same as the HYD trial. Skeletal muscle biopsies would be taken at 0, 45, 90 min, and post-TT to determine the effect of controlling Tc with dehydration on muscle metabolism. To keep the experimental trials randomized, the HYD practice trial pre-TT Tc would be the temperature needing to be attained by DEH subjects before starting the TT in the experimental trial. Performing the TT with the same Tc would eliminate Tc as a factor affecting performance when dehydrated and determine the influence of dehydration alone on metabolism and TT performance. It is hypothesized that performance would be improved when Tc is maintained compared to not being controlled. However, it is predicted that performance with mild dehydration would still be compromised compared to hydrated subjects even when Tc is the same between trials. The impact of a fluid deficit on blood volume is substantial which is believed to provide enough central feedback to increase perceived exertion and slow self-selected pace compromising performance. As well, the reduction in blood volume alone may be a limiting factor to the delivery of substrate and oxygen to the working muscle impairing muscle metabolism.

Moreover, the research investigating the impact of dehydration on muscle metabolism, central drive, and performance is in infancy. Future studies need to investigate the impact of consuming a CES throughout exercise versus no fluid on muscle metabolism and performance, the impact of dehydration on performance in females, and how training status influences substrate oxidation and muscle glycogen use with progressive dehydration. More muscle measurements (IMTG, PHOS, PDH, & HSL activity, TM) are needed to elucidate the mechanism(s) responsible for the changes in metabolism with dehydration. As well, a future study needs to control for Tc during exercise with progressive dehydration, through the use of a cooling vest, to determine the impact of dehydration alone on metabolism and performance.
REFERENCES


