

**Exercise-associated Hyponatremia: The Effects of Glycogen and Hydration Status
on IL-6, ADH, and Sodium Concentrations.**

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Exercise-associated Hyponatremia: The Effects of Glycogen and Hydration Status on IL-6, ADH, and Sodium Concentrations.

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Chapter I Introduction

Background

Exercise-associated hyponatremia (EAH) is a rare, but serious life-threatening condition that has been identified in marathoners, ultra-endurance athletes, and others engaging in prolonged, physical activity conducted in a hot environment. Hyponatremia is diagnosed when serum sodium concentrations fall below 135 mmol/L (Siegel, 2006). Normal serum sodium concentrations range from 135 mmol/L to 144.9 mmol/L. Symptoms of EAH may include: gastrointestinal distress such as bloating and nausea, vomiting, wheezing, headaches, swollen extremities, fatigue, confusion, and lack of coordination (Kundrat, 2003; Siegel, 2006). The severity of symptoms increases with decreasing serum sodium concentrations, with severe hyponatremia (usually less than 130 mmol/L) causing seizures, coma, and death.

The symptoms of EAH are very similar to those of dehydration. This can be dangerous because an athlete developing hyponatremia or a clinician treating an athlete for post-exercise symptoms may mistake EAH for dehydration; thus, consumption of additional fluids may further dilute serum sodium levels. The easiest way to distinguish hyponatremia from dehydration is by measuring body weight since weight gain during an event signifies fluid retention and possible EAH, whereas weight loss during an event signifies a loss of fluid and dehydration (Siegel, 2006).

It was initially thought that hyponatremia was caused by excessive sodium loss during exercise since sodium is the primary electrolyte secreted from the body in sweat. Sweat rates vary among athletes, but have been reported as high as 4 L/hr; however, during moderate to high intensity exercise, lower sweat rates of 1 L/hr are typical

(Noakes, 1992). Excessive sweating, which can occur during exercise in hot environments, and the associated sodium loss can reduce the body's absolute sodium content; thus, if these losses are combined with rehydration with hypotonic solutions, EAH due to the dilution of serum sodium concentrations can occur (Noakes, 1992).

Another cause of hyponatremia among endurance athletes may be simply over-hydration without excessive sodium losses (Noakes, 1992). Maintaining body fluid balance is extremely important for athletic performance during prolonged activity. Athletes should rehydrate with a volume of fluid greater than that lost during exercise (about 150% of the volume lost) because not all of the fluid consumed will be absorbed by the body. However, emphasis on the dangers of dehydration may result in athletes consuming more liquid than actually needed, thus creating a dilutional imbalance in electrolyte status. This is a common occurrence not only during exercise, but also during post-competition recovery. When an athlete finishes a race he or she may not yet be hyponatremic, but out of fear of dehydration, the consumption of mass quantities of fluid may cause hyponatremia to develop a few hours after the cessation of exercise (Noakes, 1992).

A more recently identified cause of EAH is abnormal fluid retention, possibly due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH) (Noakes, 2005; Siegel, 2006). Antidiuretic hormone (ADH) is stimulated primarily by hyperosmolality; however, nonosmotic factors such as nausea, hypotension and hyperthermia can also stimulate its release (Montain, Laird, Latzka, & Sawka 1997). When stimulated, ADH secretion conserves water in the body by inhibiting its release into the urine. The ADH

response, in combination with the normal decrease in urine production during exercise, causes abnormal fluid retention and dilutional hyponatremia (Noakes, 2005).

In addition, the failure of the body to mobilize sodium ions from osmotically inactive stores may be another contributing factor to the etiology of EAH (Noakes, Sharwood, Speedy, Hew, Reid, & Dugas, 2005). Osmotically inactive stores are places in the body in which non-dissociated sodium is stored. Such stores may be located in bone, cartilage, or in insoluble compounds throughout the body. The activation of these ions from their stores helps to maintain serum sodium concentrations in the face of sodium loss through sweating during exercise (Noakes et al., 2005).

The cytokine interleukin-6 (IL-6) may also play a role in the development of EAH since it is thought to be a nonosmotic stimulator of ADH (Mastorakos, Weber, Magiakou, Gunn, & Chrousos, 1994; Siegel, 2006). The exact IL-6 mechanism behind the regulation of ADH remains unknown; however, it is well established that IL-6 levels increase during exercise in contracting and damaged muscle. IL-6 levels are also known to increase in response to decreased levels of glycogen in the muscle. (Febbraio & Pedersen, 2002; Ronsen, Bahr, & Pedersen, 2002; Steensberg, Febbraio, Osada, Schjerling, & Pedersen, 2001). Exercising in hot environments has been shown to increase muscle glycogenolysis and can result in even greater IL-6 levels (Jentjens, Wagenmakers, & Jeukendru, 2002). It is possible, therefore, that increases in IL-6 levels due simply to muscle contraction in combination with glycogen depletion cause overstimulation of ADH leading to EAH.

Based upon the multiple factors that have been proposed as causal factors in the development of EAH, it is likely that there is no single cause of this phenomenon. Since

prolonged exercise is associated with all of the conditions that have been proposed as possible causal factors: sodium losses, sweat losses and its replacement with dilute solutions, elevations in ADH, increases in IL-6, and depletion of muscle glycogen stores, it is likely that there is an interaction among these factors that produces the final result of EAH.

Presently, no studies have been conducted to measure the interaction among ADH, IL-6 and sodium concentrations during prolonged exercise. These factors all may contribute to the development of EAH; thus, further study is warranted in order to determine the relationship between these variables.

Purpose

The purpose of this study was to evaluate the effect of hydration status and glycogen level on venous IL-6, ADH, and sodium concentrations during and after prolonged exercise in the heat.

Hypotheses

1. Following rehydration the dehydrated, low glycogen condition will elicit the highest venous concentration of IL-6 and ADH, and the lowest venous concentration of sodium.
2. Following rehydration the euhydrated, high glycogen condition will elicit the lowest venous concentration of IL-6 and the highest venous concentration of sodium.

Project Significance

Exercise-associated hyponatremia is a dangerous condition that can affect ultra-endurance athletes and others engaging in prolonged exercise, especially when exercising in the heat. The development of EAH could be attributed to excessive fluid retention during exercise caused by the abnormal ADH secretion known as the SIADH. Glycogen depletion occurring during endurance exercise causes increases in IL-6 production which may further stimulate the ADH response, which in turn, may exacerbate EAH. Exercise scientists will benefit from the information gained from this study since it will provide a broader understanding of the mechanisms behind EAH. This information may also prove useful to clinicians since it will assist them in the prevention and treatment of EAH.

Chapter II Review of Literature

Exercise-associated hyponatremia (EAH) is a serious health concern to ultra-endurance athletes. EAH, as a documented clinical phenomenon, is relatively new, with the first cases identified among ultra-endurance athletes in the mid 1980's (Siegel, 2006). Since then, numerous case studies and a handful of field studies involving marathon runners and triathletes have been conducted. Hyponatremia is defined as a serum sodium concentration less than 135 mmol/L, with severe hyponatremia beginning with a serum sodium level less than 130 mmol/L (Noakes, 1992; Siegel, 2006). Normal serum sodium levels (normonatremia) range from 136-142 mmol/L. Concentrations above 142 mmol/L are considered hypernatremic (Kundrat, 2003). Symptoms of hyponatremia can include gastrointestinal distress such as bloating and nausea, vomiting, wheezing, headaches, swollen extremities, fatigue, confusion, and lack of coordination (Kundrat, 2003; Siegel, 2006). The exact etiology of EAH remains unknown; however, factors such as hydration status, sweat production and absolute sodium losses, sequestering of sodium stores, muscle glycogen levels, and plasma levels of the cytokine, interleukin 6 (IL-6), and antidiuretic hormone (ADH) stimulation have all been identified as possible regulatory mechanisms (Noakes, 1992). The following sections will cover some of these factors in order to provide a better understanding of EAH.

Hydration Status

Prolonged exercise, especially in hot environments, incurs some level of dehydration depending on individual sweat rates and duration of exercise. Dehydration is of concern for multiple reasons including, thermoregulation and athletic performance.

During exercise, the body faces increased metabolic heat production and a decreased ability to dissipate heat which can result in hyperthermia. Hyperthermia can result in decreased performance and if severe enough can be fatal (Maughan, Leiper, and Sherriff, 1997).

From an athletic performance standpoint, maintaining body fluid balance is crucial, especially when there are repeated bouts of exercise involved or the bouts of exercise are extremely long in duration, such as a marathon. Deficits of up to 3% in body water are not effective enough to stimulate thirst drive; in fact, healthy individuals can tolerate body water losses up to 10% (Maughan et al., 1997). However, a deficit of as little as 1% body water is enough to impair performance (Sherriff and Maughan, 2000). A decrease in plasma volume associated with dehydration limits the amount of nutrient carrying blood that can be delivered to the working muscles, thus impacting work capacity.

There have been many case studies conducted involving endurance athletes, their fluid consumption, and the incidence of hyponatremia. The results of some of these studies suggest that overhydration causes hyponatremia. Noakes and colleagues (1985) reported a series of case studies documenting the effects and possible causes of hyponatremia in ultra-endurance athletes. In each of the four subjects they studied, the estimated fluid intakes ranged from 6 L to 12 L (1.6 gallons to 3.2 gallons) during competitions lasting seven to ten hours. These athletes had post race serum sodium concentrations ranging from 115-125 mmol/L. The researchers postulated that slower runners may be more apt to develop hyponatremia because it takes them longer to complete a race, giving them more time to consume fluids. In addition, they are

exercising at a lower intensity; therefore, they have lower sweat rates with concomitantly lower sodium losses. Their slower pace also allows them to tolerate larger volumes of fluid intake; thus, they may not experience as much nausea and inhibition of gastric emptying as those running at higher intensities (Noakes, Goodwin, Rayner, Branken, & Taylor, 1985). Noakes and colleagues (1988) found that slower runners had the greatest rates of fluid intake and lower rates of fluid loss than faster runners when studying 109 runners from 4 different marathons. They postulated that the greater fluid loss in faster runners was probably attributed to their higher metabolic rate.

Twerenbold et al. studied endurance trained women with different concentrations of sodium in fluids ingested during a four hour run. The women consumed one liter of fluid per hour with randomized fluid intakes. The fluids had sodium concentrations of 680mg/L, 410mg/L, and 0mg/L (Twerenbold, Knechtle, Eser, Muller, and von Arx, 2003). One of the important findings in this study is that a decline in plasma sodium concentration can be minimized or avoided if an overload of fluid is ingested by consuming fluids high in sodium. During the high sodium trial six subjects developed mild hyponatremia, as compared to nine subjects during the low sodium trial and 12 subjects with moderate hyponatremia in the no sodium trial (Twerenbold et al., 2003).

Over-consumption of fluid post-competition can also cause hyponatremia. In a case study on runners in the 2003 London Marathon, Goudie and colleagues (2006) noted that there were often delays between the finish of the race and the onset of hyponatremic symptoms. They also noted that time to finish was not a significant factor in developing EAH. The number of runners with EAH finishing in less than four hours was not statistically different from the total number of finishers with EAH. The results of this

study conflict with the conclusions drawn by Noakes and colleagues (1985) that slower runners are at a higher risk for developing EAH.

It has been documented that excessive fluid retention causes hyponatremia. This fluid retention is associated with a positive weight gain. Armstrong and associates (1992) conducted a case study on a subject who developed hyponatremia while participating in a study in which all subjects underwent eight, one-hour periods of moderate intensity treadmill running in a 41° C environment with 30 minutes rest between bouts. The subject of the case study had a significant increase in mass during the first five hours of the study, experienced fatigue and nausea, and developed a skin rash. The subject's serum sodium concentration was 122 mmol/L and ADH concentration was 4.2 pg/ml which both differed significantly from the rest of the subjects in the study. Almond and associates (2005) also investigated the incidence of hyponatremia among runners at the 2002 Boston Marathon. The results of this study indicated that hyponatremia was associated with substantial weight gain, body mass index extremes, and a long race time (greater than four hours).

Most recently, researchers described three independent biological mechanisms that cause EAH (Noakes et al., 2005). One of these mechanisms, as mentioned previously, is overhydration. Consuming mass quantities of hypotonic fluid can dilute sodium concentrations. Another mechanism is the inappropriate secretion of the antidiuretic hormone (ADH), which can be caused by the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Release of antidiuretic hormone causes the body to retain fluid. Retaining excessive amounts of fluid will also dilute the body's sodium concentration. The third mechanism is the failure of the body to mobilize sodium

ions from osmotically inactive stores (Noakes et al., 2005). This mechanism will be discussed more in detail later. The researchers claim that the presence of these three mechanisms is necessary for a normonatremic individual to become hyponatremic.

Post-exercise rehydration

Because hyponatremia often develops in the post-exercise recovery period, it is important to consider the factors associated with fluid restoration and sodium balance during the hours immediately following exercise. Important factors to take into consideration concerning rehydration are the volume of the drink ingested, the electrolyte and/or carbohydrate content, and the palatability of the drink. Each of these variables influences the extent and rapidity of rehydration.

The volume of fluid ingested post exercise is extremely important to rehydration. Numerous studies have been done examining the effect of different volumes on rehydration. Shirreffs et al. (1996) tested four different post exercise rehydration volumes (50%, 100%, 150%, and 200% of fluid lost) on men who participated in intermittent cycling until a 2% loss in body mass. The researchers concluded that a drink volume greater than the amount of sweat lost must be consumed to restore fluid balance. However, larger volumes of fluid ingestion will also result in larger quantities of urine excreted (Shirreffs, Taylor, Leiper, and Maughan, 1996). This has led researchers to study the effects of different electrolyte concentrations in post-exercise fluid consumption for rehydration. Ray and colleagues (1998) tested drinks with different sodium concentrations on fifteen men and women. The drinks contained 0mmol/L, 16mmol/L, and 333.8mmol/L of sodium. They consumed 175mL of each drink followed by water at 20 minute intervals. The results of this study indicated that including sodium in post-

exercise fluids increases fluid retention thus, improving rehydration (Ray, Bryan, Ruden, Baier, Sharp, and King, 1998). The osmolality of plasma is 150 mmol/L. Thus, fluids that are less than 150 mmol/L are considered hypotonic. Ingesting large volumes of hypotonic solutions post-exercise can put an individual at risk for the development of EAH. Sports drinks such as Gatorade® and Powerade® are both hypotonic beverages. Even though these beverages are supposed to help replace the electrolytes lost during exercise, ingesting large volumes of these drinks could lead to a dilutional hyponatremia.

Absolute Sodium Losses Through Sweat Production

Ultra-endurance exercise poses many stresses on the body. One such stress is a rise in core temperature that consequently stimulates large sweat losses and the concomitant loss of sodium from the body (Jentjens et al., 2001). As an individual exercises, there is an increase in metabolic rate and associated heat production. These responses are exacerbated by exercising in a hot environment. Jentjens and associates (2001) studied the effects of heat stress during exercise in nine trained male cyclists who performed two bouts of 90 minutes of exercise at 55% of their maximum power output in 16.4 and 35.4° C climates. In the cold condition, core temperature rose steadily for the first 40 minutes of exercise to a level 1.1° C greater than pre-exercise compared to an increase in the hot condition of 1.6° C during the first 60 minutes of exercise. No significant difference between rises in core temperature between conditions were found for the first 60 minutes of exercise; however, core temperature at the end of the hot condition was significantly higher ($p < 0.05$) than in the cold condition.

Metabolic rate determines sweat rates in addition to body temperature (Noakes, 1992). The body regulates increases in temperature by producing sweat. Sweat rates have been reported as high as 4 L per hour, but 0.75 to 1.4 L per hour is more typical for moderate intensity exercise (Noakes, 1992). Hyperthermia results when the body can no longer compensate for excessive heat production. Heat stroke is considered to be an extreme case of hyperthermia and most frequently occurs in younger individuals during exercise in the heat (Brenner et al, 1995). Heat stroke is associated with increases in IL-6 concentrations, a response that will be discussed in a later section (Brenner, Shek, & Shephard, 1995).

Sweating affects the body's hydration status. Hydration status is very important in the development of EAH. As mentioned before, EAH can develop because of overhydration. However, it has been reported that mild hyponatremia has developed in the presence of dehydration (Speedy, Noakes, Rogers, Thompson, Campbell, & Kuttner, 1999). One study tested fluid overload in athletes who have a history of hyponatremia and those that did not (Speedy, Rogers, Noakes, Wright, & Thompson, 2000). All subjects participating in the study consumed 3.4 L of water over a two hour period. Exercise was not included in the study. The water consumption took place during rest. The researchers found that at rest, there was no difference in the incidence of hyponatremia between groups. The results of this study reinforce the hypothesis that EAH results from fluid overload. It has been suggested that hyponatremia resulting from fluid overload could be due to the kidney's failure to excrete fluid as fast as it is absorbed by the intestine. Another possibility is that unabsorbed fluid in the intestine can draw sodium out of the tissues (Speedy et al., 2000). The results of the study also suggest that

having a history of hyponatremia does not increase the likelihood that hyponatremia will develop. Although these are possible factors, other factors may be associated with exercise such as large losses of fluid and/or sodium through sweat, and inappropriate hormonal responses that could increase the risk of developing hyponatremia (Speedy et al., 2000).

Sodium loss through sweat is a controversial factor in the development of EAH. Sweat is a hypotonic solution with a wide range of sodium concentrations typically below 100 mM. The extracellular space of a 70 kg individual is roughly 13 L (Noakes, 1992). For a normonatremic individual with a serum sodium concentration of 138 mmol/L, the extracellular space would contain approximately 1794 mmol of sodium. If the individual's sweat sodium content was 70mmol/L and he or she had a sweat rate of 1 L/hr, completion a four hour marathon hydrating with only water would lead to a serum sodium concentration of 116 mmol/L, which is severely hyponatremic. Sports drinks contain only small amounts (10-20 mmol/L) of sodium (Noakes, 1992). Even rehydration with a sports drink would lead to hyponatremia. Since hyponatremia occurs in less than 0.3% of ultra-marathon runners, there must be another factor contributing to the development of EAH (Noakes, 1992). Noakes and colleagues (2005) believe that under normal circumstances the body activates sodium stores in the body to accommodate these sodium losses and that the failure to do so can lead to the development of hyponatremia. Since the cause of the failure to mobilize inactive sodium stores remains unknown, athletes should be encouraged not to overhydrate prior to exercise (Noakes et al., 2005).

Hew-Butler and colleagues (2006) conducted a study on the effects of electrolyte ingestion during an Ironman triathlon. They randomly assigned 145 triathletes competing in the 2001 Cape Town Ironman to a sodium supplementation group or a placebo group. Participants in the supplementation group were given 40 tablets containing 620 mg of table salt, while the placebo group was given 40 identical looking tablets containing only starch. Both groups were told to take the tablets ad libitum within a suggested range of 1-4 per hour while on the course. They found no significant difference between post race serum sodium levels. There was no difference in performance between the supplementation and placebo group. In addition, there was no difference in the amount of tablets consumed between the groups, indicating that the placebo group was not 'craving' salt.

Muscle Glycogen Levels

Another stress from ultra-endurance exercise that impacts the body is glycogen depletion. Exercise in the heat has been shown to increase rates of muscle glycogen depletion (Hargreaves, Angus, Howlett, Conus, & Febbraio, 1996; Jentjens Wagenmakers, & Jeukendrup, 2001). Jentjens and associates (2001) examined trained male cyclists and showed a 25% increase in muscle glycogen oxidation during a bout of exercise in a heated environment versus a cold environment. This leads to the conclusion that an increase in muscle temperature may cause an increase in muscle glycogen use. The exact mechanisms responsible for increased glycogen oxidation in the heat remain unknown, but may be related to enzymes involving carbohydrate breakdown. It is

possible that during hyperthermia epinephrine levels increase and that this increase in epinephrine plays a role in glycogen breakdown (Jentjens et al., 2001).

Glycogen is stored in the liver and the muscles of the body. In order to store glycogen, the body must also store water at a ratio of three grams of water for each gram of glycogen. Thus, in a glycogen loaded state the body is more hydrated than in a glycogen depleted state.

Interleukin 6

Decreases in muscle glycogen have been shown to elicit increases in serum IL-6 concentrations (Febbraio & Pedersen, 2002; Siegel, 2006; Steensberg, et al., 2001). IL-6 is classified as a type of cytokine which is a marker of inflammation. Contracting skeletal muscle is one of the areas in which IL-6 is produced.

Information regarding IL-6 release during exercise is ambiguous. Febbraio and Pederson (2002) have reported that increases in IL-6 from endurance activity do not show up until later in exercise. However, studies have shown IL-6 production during exercise as early as 6 minutes during maximal intensity rowing (Nielsen, Secher, & Pedersen, 1996). It has been suggested that epinephrine (EPI) secretion stimulates IL-6 production. However, it appears that IL-6 production is related to exercise intensity, muscle recruitment, and endurance capacity, not EPI concentrations (Febbraio & Pederson, 2002).

It has been demonstrated that IL-6 production is related to the pre-exercise glycogen content of the working muscles (Steensberg et al., 2001). In addition, longer rest periods between multiple bouts of exercise seemed to attenuate levels of IL-6

production (Ronsen et al., 2002). Ronsen and colleagues (2002) conducted a study testing the effects of repeated exercise bouts on IL-6 levels in elite male endurance athletes. The subjects participated in two conditions; one in which exercise bouts were separated by three hours, and another in which bouts were separated by six hours. The researchers postulated that the incomplete resynthesis of glycogen associated with the shorter rest period increased the IL-6 response (Ronsen et al., 2002).

In addition to longer rest periods, ingestion of carbohydrate during exercise has been shown to attenuate IL-6 production (Febbraio & Pedersen, 2002). It has been proposed that IL-6 is involved in the regulation of glucose homeostasis as a sensor of carbohydrate availability (Pedersen, Steensberg, Fischer, Keller, Plomgaard, & Wolsk-Petersen, 2004). In cultured hepatocytes, IL-6 inhibits glycogen synthase activity while simultaneously stimulating glycogen phosphorylase activity, resulting in glycogenolysis (Keller, Steensberg, Pilegaard, Osada, Saltin, & Pedersen, 2001).

ADH

In addition to IL-6 concentrations, ADH secretion may also play a role in the development of EAH. Some researchers have described three independent biological mechanisms that cause EAH (Noakes et al., 2005). One of these mechanisms is the inappropriate secretion of antidiuretic hormone (ADH), which can be caused by the syndrome of inappropriate antidiuretic hormone secretion (SIADH). ADH is a hormone secreted by the anterior pituitary gland and is responsible for maintaining the body's extracellular fluid volume by inhibiting the release of water into the urine. It does this by increasing the permeability of the collecting tubules in the kidney to water. Therefore,

water is reabsorbed. Solutes are reabsorbed as well, but the volume of solute reabsorbed is proportionally much less than water. This results in a decrease in urine flow and an increase in urine osmolality (Verbalis, 2007).

ADH is especially important in maintaining fluid volume in the face of large sweat losses occurring in the heat (Norsk, 1996). Maximal excretion capacity of the renal system is about 800 to 1000 mL/hr. High levels of fluid intake by individuals can often exceed the sum of fluid loss from sweat and renal excretion resulting in EAH (Verbalis, 2007). Retaining excessive amounts of fluid will dilute the body's sodium concentration. ADH is largely stimulated by hyperosmolality. It can also be stimulated by nonosmotic factors such as nausea, hyperthermia, and hypotension (Montain, et al., 1997). This was demonstrated by determining the effects of hydration status and exercise intensity in the heat on ADH responses (Montain, et al., 1997). The study included nine male subjects who performed nine bouts of 50 minute continuous running in a warm environment. The subjects performed at 25%, 45%, and 65% of their VO_2 max and were either euhydrated or hypohydrated by 3% or 5% of their baseline body weight. At rest the 5% hypohydration state produced the largest ADH response followed by 3% hypohydration state. ADH concentrations rose during the 65% exercise intensity trials, but no difference in ADH concentrations were observed in the lower intensity trials. The researchers concluded that ADH concentrations increased in a graded manner with hypohydration and that this relationship continued through exercise in a heated environment. In addition, higher exercise intensities elicited higher ADH concentrations.

ADH may also be stimulated by IL-6. Mastorakos and associates (1994) demonstrated increases in ADH concentration in subjects when recombinant IL-6 was

administered via injection. Subjects in the study were given injections of IL-6 every 8 hours. Each subject received a dose varying from 0.3-30 µg/kg three times per day. ADH levels increased in subjects receiving more than 1.0 µg/kg of IL-6. The ADH concentrations peaked 30 minutes after injection and the concentrations increased with increasing doses of IL-6. The researchers suggested that IL-6 may be the factor that stimulates ADH secretion in SIADH (1994).

The role of ADH release in SIADH as a possible factor involved in the etiology of EAH is uncertain. The majority of literature available in this area sites normal ADH concentrations in athletes who develop hyponatremia (Speedy et al, 2000). Normal ADH concentrations were observed in a large group of ultra-endurance triathletes who developed EAH during an Ironman triathlon (Speedy et al, 1999). They reported no apparent relationship between ADH and serum sodium concentrations and were unable to explain the delay of diuresis in the hyponatremic athletes. One study did report elevated ADH levels in a subject with hyponatremia due to overhydration (Armstrong et al, 1992). The subject consumed 1.9 L per hour over a four hour trial. However, Siegel and colleagues (2007) have recently reported that EAH in marathon runners is in fact caused by SIADH. They compared ADH levels of normonatremic runners in the 2001 Boston Marathon to hyponatremic runners in the 2004 Boston Marathon. They cited that the increases in ADH seen in the runners were due to volume depletion as a result of weight loss which normalized blood sodium levels post-competition. Runners with hyponatremia were normovolemic, but still had detectable levels of ADH (>0.5 pg/mL). This is an inappropriate physiological response in the presence of hypo-osmolality and

fits the criteria for the diagnosis of SIADH (Siegel, Verbalias, Clement, Mendelson, & Mello, et al., 2007).

Fellmann and associates (1989) suggest that regulation of ADH secretion may be altered via endurance training. They studied hormonal, fluid and electrolyte changes during recovery from ultra-endurance exercise. They followed nine male runners for 72 hours of recovery from a 24 hour endurance race. ADH concentrations peaked immediately after the race, fell back down towards baseline levels after 24 hours of recovery and continued to fall 48 hours and 72 hours post-race. They observed no change in sodium concentrations immediately after the race, or in the following days of recovery. The researchers site the subjects' post-race increases in plasma volume as the possible factor responsible for the decreases in ADH concentrations (1989).

Summary

There are many possible causes of EAH. Depletion of glycogen levels, increases in ADH, elevations in IL-6, sodium losses, dehydration and fluid replacement with hypotonic solutions have all been proposed as factors related to its development. Since there are so many potential factors related to the development of EAH it is likely that there is no single cause, and EAH results from a combination of factors.

Information on the interaction of ADH, IL-6 and sodium concentrations is lacking. In addition, information regarding the role of ADH in the development of EAH is ambiguous. It is yet to be determined what causes the abnormal diuresis in athletes who develop EAH.

Chapter III Method

Participants

Ten male participants completed the study. The mean age and percent body fat of the participants were 24.2 ± 7.6 years and $9.28 \pm 4.88\%$. Exclusion criteria included any known disease and/or any contraindications to vigorous exercise based on medical history. Participants were recruited from a university campus in the southwestern United States. The mean VO_2 max of the participants was 3.613 L/min

Experimental Protocol

Human Subjects approval was obtained prior to subject recruitment and data collection. The recruitment process consisted of posting ads on the university's weekly mass emails to the entire student body until ten participants enrolled in the study. Participants were also recruited from local cycling and triathlon clubs via flyers and word of mouth.

Experimental Design

Each participant was to complete four exercise trials: a glycogen depleted, euhydrated condition (DE); a glycogen depleted, dehydrated condition (DD); a glycogen loaded, euhydrated condition (LE); and a glycogen loaded, dehydrated condition (LD). Each condition was completed in a randomly assigned and counter balanced order. Each exercise condition consisted of 90 minutes of cycling at 60% VO_2 max on a cycle ergometer (Ergometer 894E, Monark Exercise AB, Vansbro, Sweden) in a 35°C, 40% relative humidity environment followed by a three hour rehydration period. Seven of the

ten participants completed the full 90 minute exercise protocol for each trial. One participant completed the 90 minutes for 3 trials and terminated one trial at 75 minutes. The last two subjects failed to finish the 90 minute protocol stopping at 66 and 75 minutes for all four trials.

Preliminary Testing. Participants reported to the Texas Christian University Exercise Physiology Lab for an assessment of VO₂ max test using a Parvo Medics metabolic cart (TrueOne 2400 Metabolic, Parvo Medics, Inc, Sandy, Utah). On a subsequent day they reported to the lab and performed a 45-minute acclimation ride in the heat chamber at 35°C at an intensity equal to 60% of his VO₂ max. Core temperature and heart rate were monitored using a polar heart rate monitor, and body masses were taken to determine sweat rates. The sweat rate was calculated from this ride by determining the difference in pre and post exercise body mass per unit time and was used to determine individual rehydration needs for the euhydrated trials. Participants were either naturally heat acclimated since the data collection took place between the months of June and November.

Experimental Testing. Participants then completed each of the four exercise conditions separated by at least one week between conditions. Forty-eight hours before each exercise condition participants reported to the lab for a glycogen depleting exercise bout which consisted of 60 minutes of cycling at an intensity of 70% of VO₂ max followed by a series of six one minute sprints with two minutes rest between sprints. Depending on the condition, after the depletion ride, participants were given either a low CHO (0.5 g/kg) meal or a high CHO (8 g/kg) meal to impair or facilitate glycogen resynthesis. Mean calorie consumption for the high CHO diet was 2793.4 ± 461.8 kcal consisting of a

mean CHO content of 6.891 ± 1.04 g/kg body mass. Mean calorie consumption for the low CHO diet was 2740 ± 452.5 kcal consisting of a mean CHO content of 0.4777 ± 0.09 g/kg body mass. Food for both diets was provided. Prior to the depletion bout of exercise, participants were asked to keep a 3 day food record. That record was used to help construct a diet containing foods the subjects liked and approximated the individual's normal daily caloric intake. The low CHO diet consisted of foods such as cheese, tuna, hamburger, eggs, and bacon. The high CHO diet consisted of foods as pasta, bread, cereal, fruit, and fruit juices. Both meals were isocaloric. The same meals were repeated prior to each exercise trial.

For each exercise trial, participants reported to the lab at the same time of day. Participants were fitted with a rectal thermistor to a depth of 12 cm and Polar heart rate monitor. A catheter was inserted into the participant's antecubital vein for blood sampling and kept patent with 0.9% saline flushes. VO_2 data was collected periodically throughout the exercise to verify workload and RQ. Blood samples were taken pre-exercise, 45 minutes into exercise, and immediately post exercise. Core temperature and heart rate was monitored throughout the duration of the 90-minute exercise bout. If at anytime during any of the exercise trials a participant reached or exceeded a core temperature of 39.5°C the trial was immediately terminated for the participant's safety. During the euhydrated trials participants were given water at 15-minute intervals to maintain 0% body weight loss. The volumes of water given were varied depending on individual sweat rates.

During the 3-hour rehydration period, participants completing the DD and LD conditions consumed a volume equal to 150% of the fluid lost during the ride in 6 post

exercise feedings. An initial feeding of 35% was given immediately post exercise and the remaining fluid was given in five equal volumes every 30 minutes. For participants completing the DE and LE trials, a volume of 50% of fluids ingested during exercise was administered during the 3-hour rehydration period. Feedings were conducted in the same manner as the DD and LD trials with an initial post exercise feeding of 35% and the remaining volume given in five equal feedings at 30-minute intervals. If at any time during rehydration a subject's sodium level dropped below 135 mM, fluid consumption was stopped, and fluid was withheld until a subsequent 30-min analysis showed a return to 135 mM or greater. Blood samples were taken every 30 minutes during this period and analyzed for sodium concentration to ensure the safety of the participants.

Blood Analyses

Blood samples were analyzed for IL-6, ADH, sodium concentrations, and glucose, as well as hematocrit and hemoglobin in order to calculate changes in plasma volume. All blood samples were collected in lithium heparin treated vacutainers. Samples were aliquoted and stored in a -80 °C freezer until assayed. Sodium concentrations were analyzed on fresh whole blood samples using a Radiometer ABL 77 series electrolyte analyzer, Copenhagen, Denmark. Hematocrit and hemoglobin were calculated using the microcapillary microcentrifugation method and cyanomethemoglobin method using whole blood samples (Dill and Costill, 1974). Changes in plasma volume were calculated using the Dill and Costill method (Dill and Costill, 1974). IL-6 concentrations were measured using the Invitrogen Ultra Sensitive human IL-6 ELISA assay. ADH concentrations were measured by Quest Diagnostics

Incorporated in San Juan Capistrano, California. Blood glucose was measured using an enzymatic assay with spectrophotometry.

Other Analyses

Sweat samples were collected at minutes 35-45 and 80-90 of exercise using the arm bag method. In addition, urine samples were collected. Urine and sweat sodium concentrations were measured using the Roche Diagnostics AVL 9180 electrolyte analyzer, Indianapolis, Indiana. In addition, urine volume was measured to calculate total body sodium concentration.

Calculations

Plasma volume was calculated using the Method of Dill and Costill (Dill & Costill, 1974).

Carbohydrate oxidation was calculated using VO_2 data collected during the trials. VO_2 data was collected during 3-5 minutes, 45-48 minutes, and 77-80 minutes of exercise. The respiratory exchange ratios (RER) for the 3 minute segments were averaged to yield one value per three minute period. These values were used to look up the corresponding kcal/L O_2 consumed at that RER. The grams of carbohydrate utilized per minute (gCHO/min) was calculated by multiplying the kcal/L O_2 by the average VO_2 (L O_2 /min) from that time point. The carbohydrate oxidations from each of the three time points were averaged to find the mean carbohydrate oxidation during exercise for each trial.

Total body sodium was calculated by estimating extracellular fluid volume (ECF) and multiplying it by the corresponding plasma sodium concentration: $ECF * [Na^+]_{plasma}$. (Noakes, 1992). ECF was calculated as $0.2 * BM$ (Guyton and Hall, 2006).

In addition to total body sodium, total sodium loss was calculated for each of the trials. This was calculated by multiplying the average sweat concentration by the total sweat loss and adding it to the urine volumes produced multiplied by their corresponding concentration. Total sweat loss was calculated as the change in body mass from pre to post-exercise minus the respiratory water loss and carbon loss during exercise.

To examine Noakes's idea of possible sodium stores within the body, total body sodium concentrations before exercise and post-rehydration were calculated for each trial and compared to the theoretical post-rehydration values. Theoretical values were calculated by subtracting the total sodium loss from the pre-exercise total body sodium concentration and dividing that quantity by the post-rehydration ECF.

Rate of first urine production was calculated by dividing the total volume (in mL) of the first urine sample collected by the time (in minutes) at which it was collected post-exercise.

Statistical Analyses

Systat® software was used to do all statistical analyses. All of the dependent measures were analyzed using a three factor analysis of variance (ANOVA) with repeated measures. The first factor was *hydration*, which had two levels, dehydrated and euhydrated. The second factor was *glycogen status*, which also had two levels, loaded and depleted. The third factor was *time*, which had varying levels depending on the

number of times the variable was measured. Plasma sodium data were analyzed using a 2 X 2 X 9 ANOVA based on pre-exercise, 45 minutes into exercise, post-exercise and the 30, 60, 90, 120, 150, and 180 minutes of rehydration. ADH, IL-6, and blood glucose data were analyzed using a 2 X 2 X 5 ANOVA. Core temperature and plasma volume data was analyzed using a 2 X 2 X 4 ANOVA. Carbohydrate oxidation, change in body mass, rate of first urine production, time to first urine, and total urine volume were analyzed using a two factor ANOVA using only the hydration and glycogen status factors. Differences detected by the ANOVA were isolated using a Newman-Keuls *post hoc* test. Significance was accepted at the $p < 0.05$ level. Possible correlations between the three main factors were studied using correlation and probability matrices.

Chapter IV
Results

Fluid Intake

Due to the safety restriction of withholding fluid when plasma sodium dropped below 135 mM, the actual volume of fluid consumed by the subjects in the various conditions varied from the precise volume protocol described in the methods section.

Table 1. Fluid Volumes During Rehydration. All volumes are reported in liters.

Subj #	DD			DE			LD			LE		
	Rx Vol	Act Vol	% vol	Rx Vol	Act Vol	% vol	Rx Vol	Act Vol	% vol	Rx Vol	Act Vol	% vol
2	2.008	2.008	150	0.377	0.377	50	1.83	1.83	150	1.03	1.03	50
3	2.069	2.069	150	1.24	0.918	37	1.983	1.467	111	1.485	1.292	43.5
4	5.55	4.829	130.5	2.749	1.319	24	5.247	4.565	130.5	2.875	2.125	37
5	3.57	3.38	142	1.63	1.206	37	2.974	2.974	150	1.508	1.508	50
6	3.748	3.261	130.5	1.239	1.239	50	3.27	3.27	150	1.23	1.23	50
7	3.57	3.106	130.5	1.81	0.633	17.5	3.93	3.93	150	1.345	1.345	50
8	2.161	2.161	150	0.748	0.651	43.5	1.983	1.983	150	0.661	0.661	50
9	3.117	2.712	130.5	0.961	0.711	37	2.85	2.85	150	1.091	0.949	43.5
10	2.548	2.217	130.5	0.909	0.791	43.5	2.972	2.972	150	1.007	0.876	43.5
11	3.87	3.87	150	1.23	0.59	24	3.9	2.886	111	1.369	0.479	17.5
Average	3.2211	2.9613	139.45	1.2893	0.8435	36.35	3.0939	2.8727	140.25	1.3601	1.1495	43.5

Rx Vol is the prescribed volume to be ingested. Act Vol is the actual volume consumed. Target percentages were 150% for the DD and LD conditions and 50% for the DE and LE conditions.

Table 2. Fluid Feedings Missed During Rehydration.

Subj #	DD	DE	LD	LE
2	0	0	0	0
3	0	0	2	1
4	1	4	1	2
5	0.59	3	0	0
6	1	0	0	0
7	1	5	0	0
8	0	1	0	0
9	0.33	2	0	1
10	1	1	0	1
11	0	4	2	5
Total	4.92	20	5	10

Core Temperature

Core temperature (T_c) had a significant hydration by time interaction ($p=0.000$). Post hoc testing showed the DD and LD conditions to have significantly higher T_c than the DE and LE conditions at the 60 minute and final time points (Figure 1). In addition, post hoc testing showed that there was a significant difference between the pre and 30 minute time points and between the 30 and 60 minute time points for the dehydrated and euhydrated conditions.

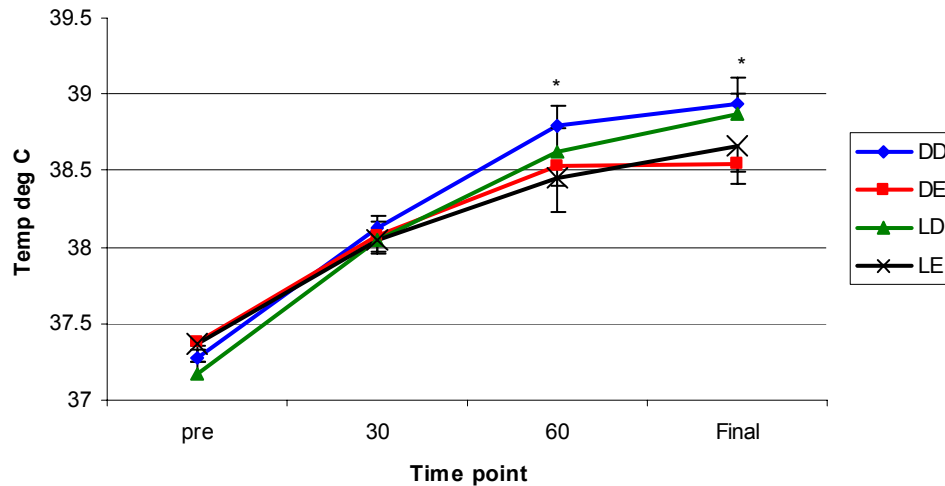


Figure 1. T_c Responses. The * indicates that the DD and LD conditions are different from the DE and LE conditions.

CHO Oxidation

For CHO oxidation, there was a significant main effect for glycogen status ($p=0.000$). The average rates of CHO oxidation in the LD and LE conditions (2.298 and 2.333 g CHO/min, respectively) were significantly greater than those observed in the DD and DE conditions (1.714 and 1.609 g CHO/min, respectively) (Figure 2).

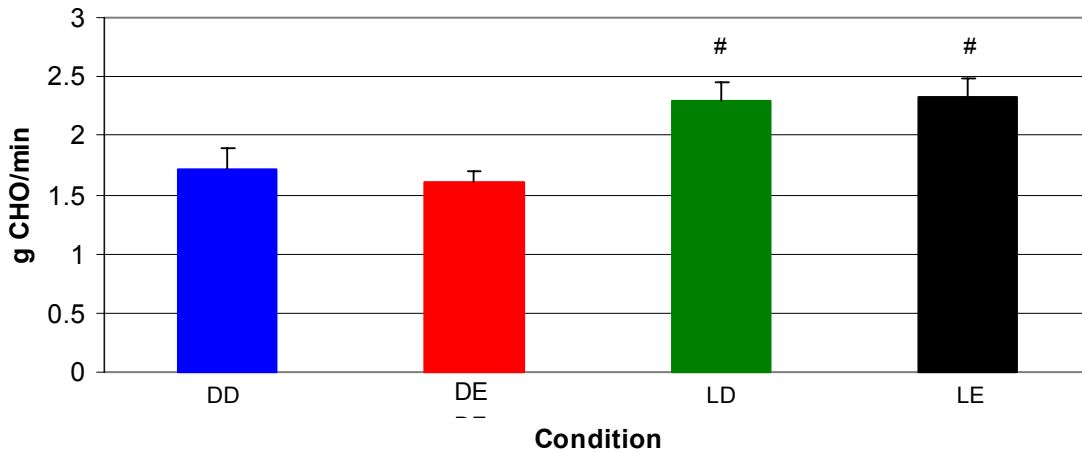


Figure 2. Carbohydrate Oxidation. The # indicates that the LD and LE conditions are significantly different from the DD and DE conditions.

Blood Glucose

There was a significant glycogen status by time interaction ($p=0.000$) for plasma glucose levels. There was no significant difference in plasma glucose levels prior to exercise for any of the conditions. The LD and LE conditions had significantly higher plasma glucose concentrations than the DD and DE conditions at all time points except for pre-exercise. In addition, the pre exercise and post exercise glucose levels were significantly different for the glycogen depleted trials, but not for the glycogen loaded trials (Figure 3).

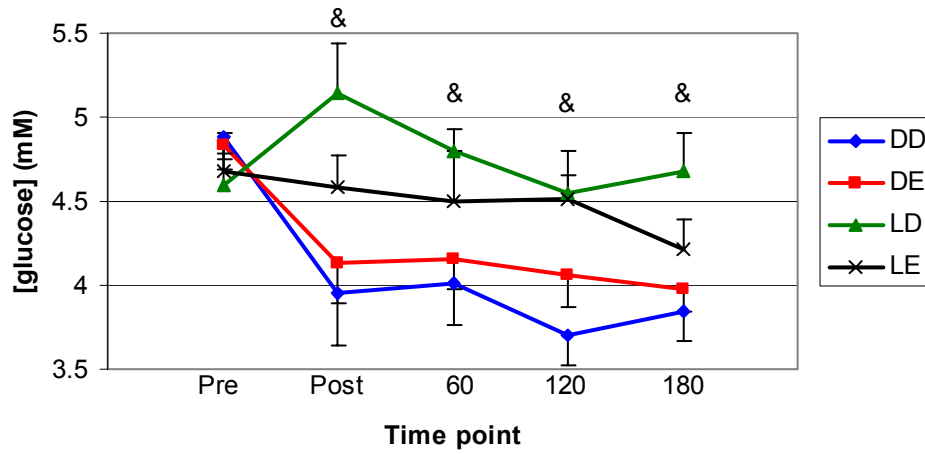


Figure 3. Plasma Glucose Responses. The & indicates that the LD and LE conditions are different from the DD and DE conditions.

Hydration Responses

There was a significant main effect for time ($p=0.000$) for changes in plasma volume. Post hoc testing showed the post exercise time point to be the only time point that was significantly different from all other time points. There was also a significant main effect by hydration ($p=0.004$). The LD and DD conditions produced the largest changes in post exercise plasma volume with decreases of 13.82% and 12.29%. Plasma volume upon completion of the 3 hour rehydration period was lower than pre exercise levels in all four conditions with the LD condition being the lowest (Figure 4).

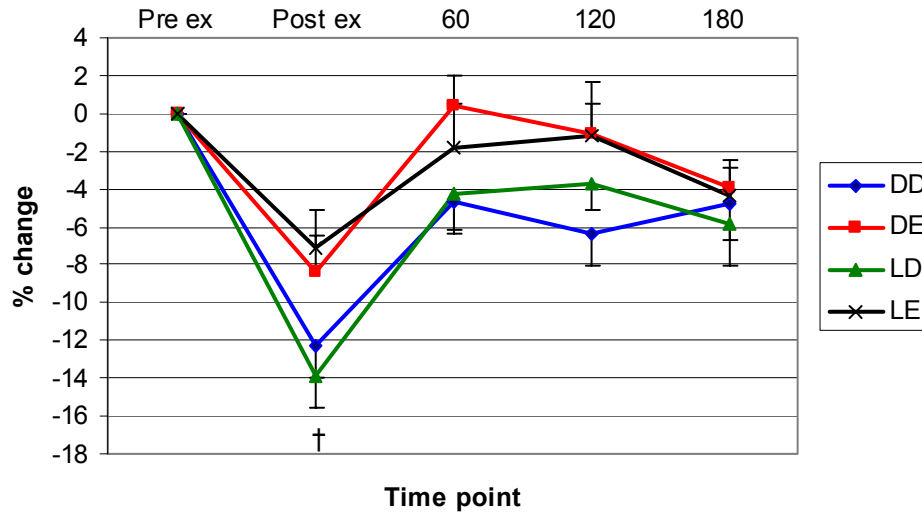


Figure 4. Plasma Volume Responses. The † indicates which time points are significantly different from all other time points.

Changes in body mass measured over the entire exercise and rehydration periods were larger in the DE and LE conditions (-0.616 kg and -0.913 kg) than in the DD and LD conditions (-0.353 kg and -0.458 kg). The largest total urine production occurred in the LE condition with an average total production of 1.333 L of urine (Figure 7). There was a significant glycogen by hydration interaction ($p= 0.031$) for rate of first urine production. Rate of first urine production was highest in the LE condition with an average of 7.593 mL/min (Figure 5). In addition, there was a significant glycogen by hydration interaction ($p=0.034$) for time to first urine production, in which the LE condition average time was the shortest at 50.2 min post exercise (Figure 6).

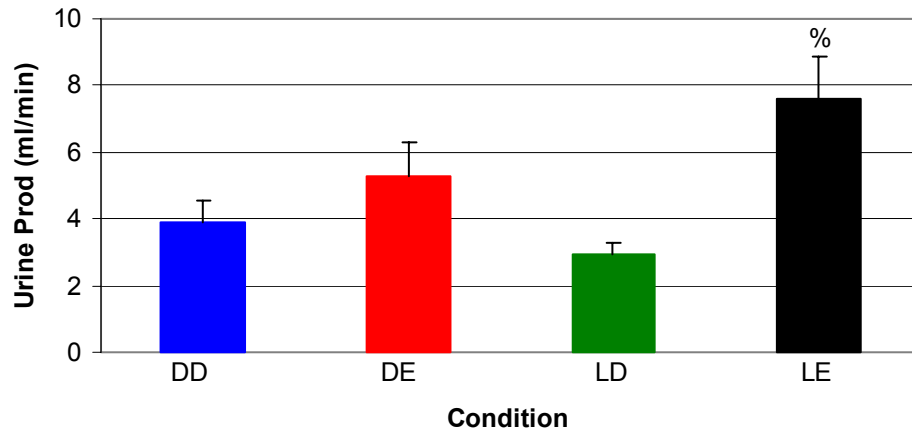


Figure 5. Rate of First Urine Production. The % indicates that the LE condition is significantly different from the dehydrated trials.

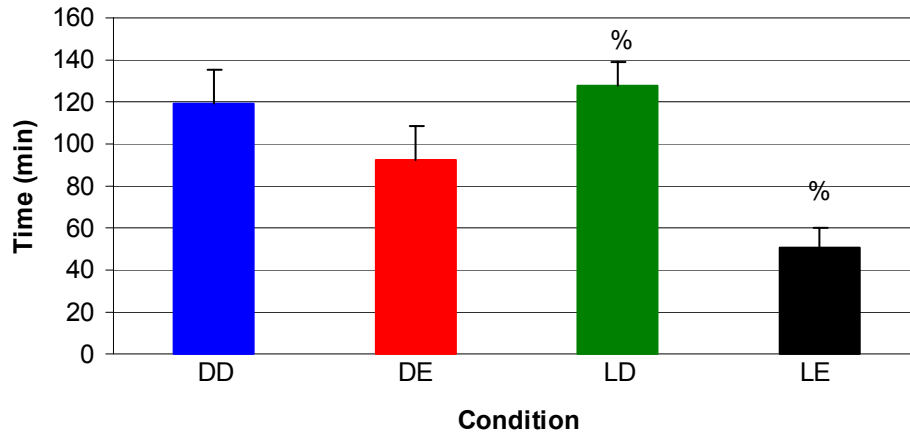


Figure 6. Time of First Urine. The % indicates that the LD and LE conditions are significantly different from each other.

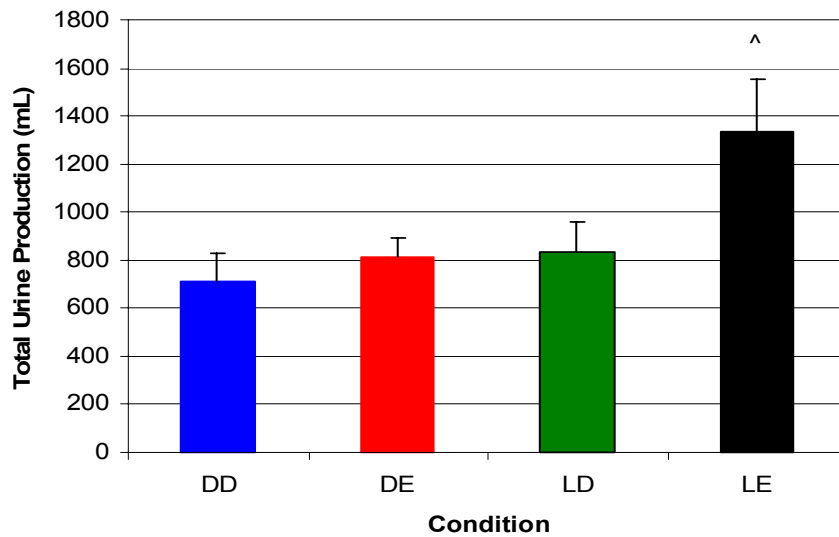


Figure 7. Total Urine Production. The ^ indicates that the LE condition is statistically different from all other conditions.

Sodium Responses

Across the entire exercise and rehydration protocol, plasma sodium concentrations decreased in each of the four conditions; however, a comparison of the change in plasma sodium from pre-exercise to post-rehydration revealed no significant differences (Figure 9). For this analysis, a glycogen by hydration interaction approached significance ($p=0.062$) (Figure 8).

There was a significant glycogen by hydration by time interaction for plasma $[Na^+]$ ($p=0.022$) indicating significant differences within the protocol. The LD and DD conditions showed the greatest overall $[Na^+]$ changes from post exercise to post rehydration (-6.85 and -6.7 mmol/L, respectively) compared to the DE and LE conditions (-1.45 and 0.10 mM, respectively) (Figure 10). The LD and DD conditions showed the highest increase in $[Na^+]$ post exercise (141.65 and 141.7 mmol/L), whereas the LE and

DE conditions showed decreases in $[Na^+]$ post exercise (135.9 and 136.35 mmol/L). Post Hoc testing determined that $[Na^+]$ in the dehydrated conditions was significantly higher than the euhydrated conditions at the mid, post, 30, 60, and 90 time points. In addition, at the 30 minute time point the DD condition produced a significantly higher $[Na^+]$ compared to all the conditions but LD, and DE was significantly lower than all conditions except LE. At the 60 minute time point all conditions were significantly different except for DD and LD. At the 90 minute time point only LD and DE were significantly different from each other.

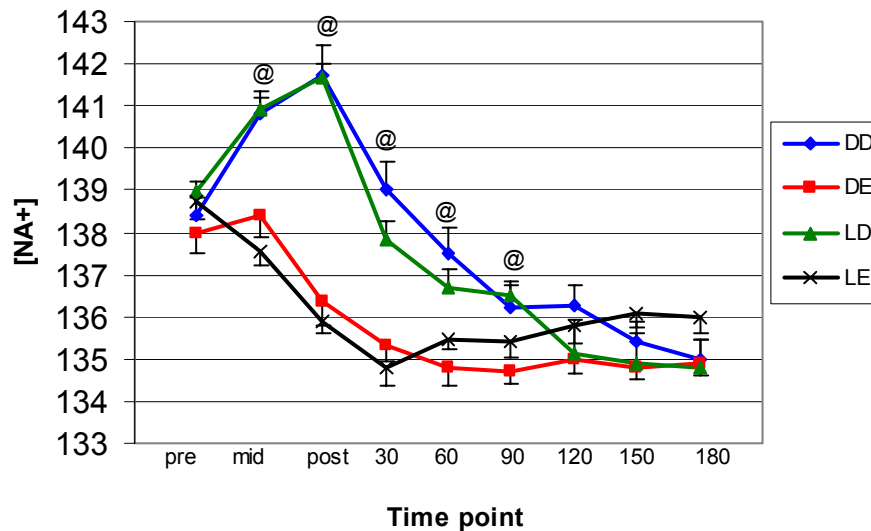


Figure 8. Plasma Sodium Responses. The @ indicates a significant glycogen by hydration by time interaction (see text for clarification).

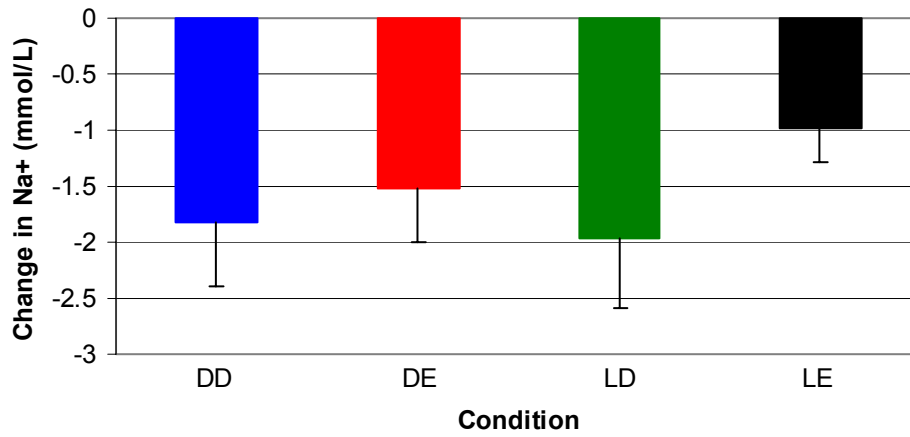


Figure 9. Change in Sodium Pre-exercise to Post-rehydration.

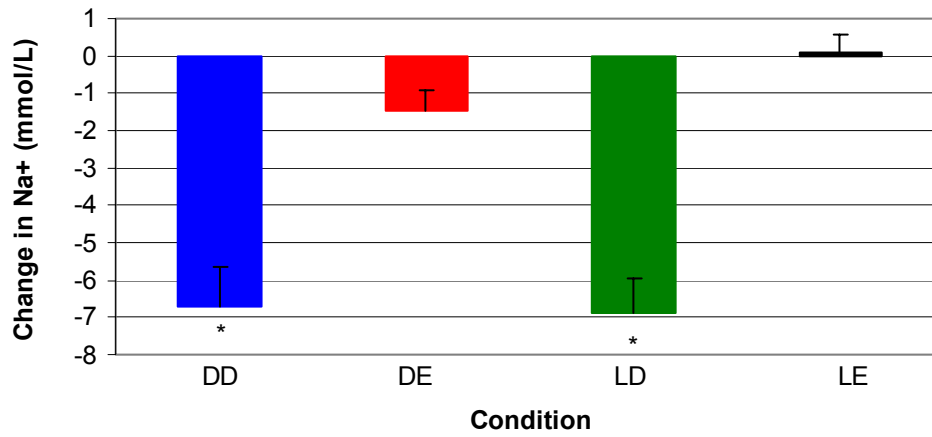


Figure 10. Change in Sodium from Post-exercise to Post-rehydration.

The * indicates conditions that are significantly different from the euhydrated conditions.

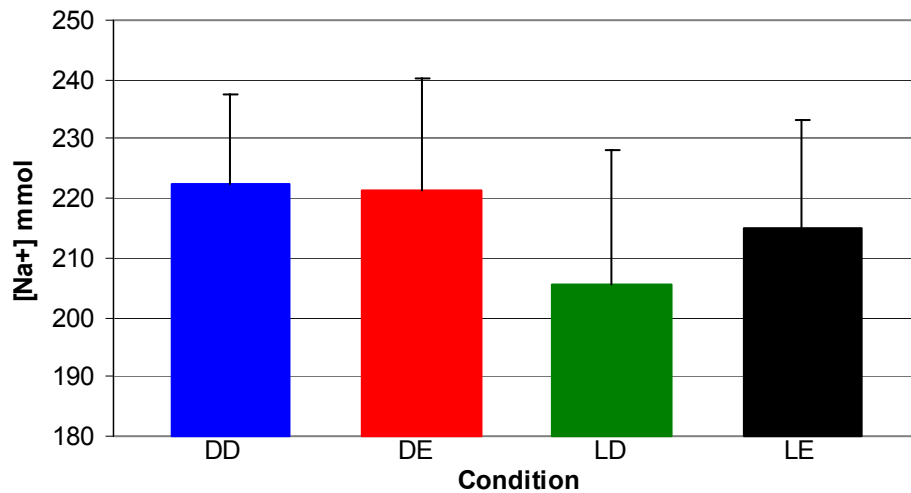


Figure 11. Calculated Total Body Sodium Losses.

Prior to exercise, average total body sodium was 2183.6 ± 95.8 , 2186.1 ± 92.3 , 2152.138 ± 90.8 , 2166.8 ± 92.6 mmol for conditions LE, LD, DE, and DD respectively. After completion of each trial, total body sodium dropped to 2115.5 ± 91.9 , 2108.0 ± 90.0 , 2087.0 ± 86.6 , and 2105.5 ± 90.4 mmol for conditions LE, LD, DE, and DD. After taking the post rehydration total body sodium and dividing it by the new ECF we calculated the theoretical post rehydration $[\text{Na}^+]_{\text{plasma}}$. The graph below shows the theoretical values and the actual measured values for $[\text{Na}^+]_{\text{plasma}}$ (Figure 12).

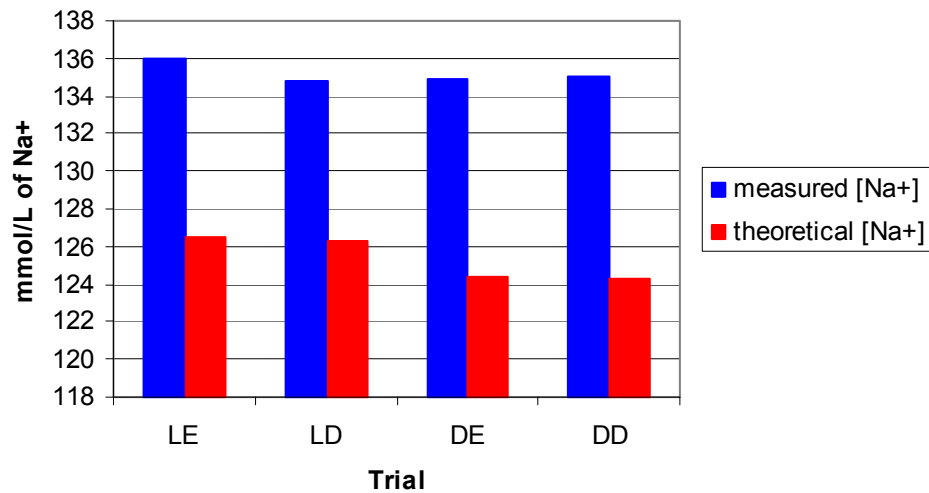


Figure 12. Observed Post-rehydration Total Body Sodium vs. Theoretical Post-rehydration Total Body Sodium.

IL-6 Responses

There was a significant main effect by time ($p=0.00$) (Figure 13). Post hoc testing showed that the post-exercise plasma concentrations of IL-6 were significantly greater than the rest of the time points for each condition except for the 60 min time point. In addition, all time points except for 180 min were significantly greater than the pre-exercise values. The DD condition had the greatest [IL-6] post exercise (5.33 pg/mL); however, this was not statistically significantly different from the rest of the conditions. By the end of the three hour rehydration period IL-6 concentrations were lower than the post exercise levels, but had not yet returned to baseline.

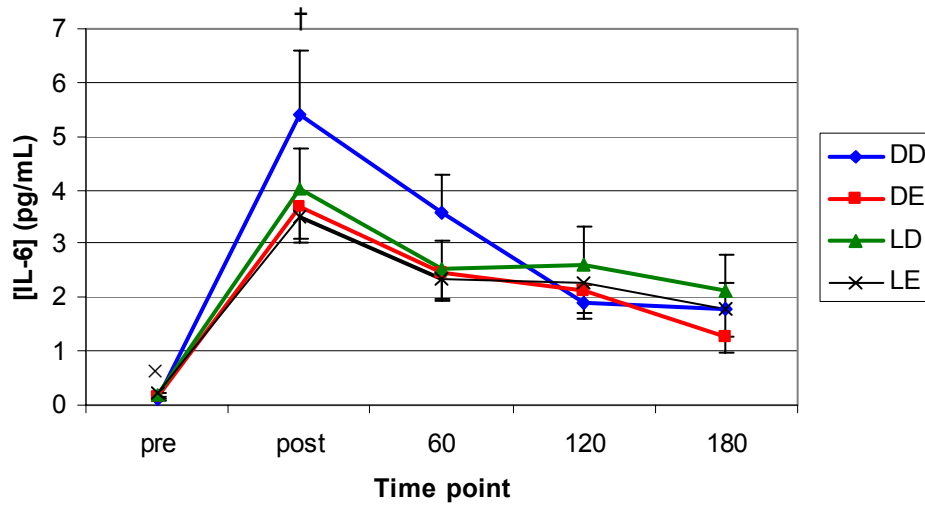


Figure 13. Plasma IL-6 Responses. The × indicates time point is significantly different from all time points except 180. The † indicates time point is significantly different from 120 and 180.

ADH Responses

There was a significant hydration by time interaction ($p=0.000$) with the post exercise time point being significantly different from all other time points (Figure 14). The dehydrated conditions showed the largest post-exercise increase in ADH concentrations with the DD conditions having an average ADH concentration of 50.16 ± 40.552 pg/mL and the LD conditions having an average of 55.46 ± 68.124 pg/mL.

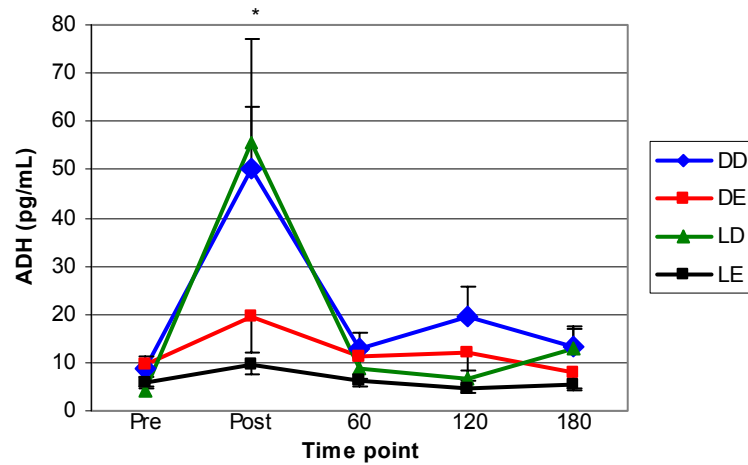


Figure 14. Plasma ADH Responses. The * indicates time point at which euhydrated conditions are significantly different from dehydrated conditions.

Correlation and Probability Analyses

Table 3. Correlations and Probabilities for all conditions.

DD CONDITION	Post-Ex [IL-6]		30 Post-Ex [Na+]		Post-Ex Glu		CHO OX		ΔBM	
	r	p	R	P	r	p	r	p	R	P
Post-Ex [IL-6]	1	0								
30 Post-Ex [Na+]	-0.109	0.764	1	0						
Post-Ex Glu	-0.227	0.528	-0.681	0.03	1	0				
CHO OX	0.063	0.863	0.342	0.334	0.228	0.527	1	0		
ΔBM	0.086	0.813	0.827	0.003	-0.781	0.008	0.123	0.735	1	0
Post-Ex [ADH]	0.254	0.479	0.493	0.148	-0.523	0.121	0.06	0.87	0.791	0.006
Total Urine	0.012	0.974	-0.691	0.027	0.743	0.014	0.106	0.77	-0.907	0
DE CONDITION	Post-Ex [IL-6]		30 Post-Ex [Na+]		Post-Ex Glu		CHO OX		ΔBM	
	r	p	R	P	r	p	r	p	R	P
Post-Ex [IL-6]	1	0								
30 Post-Ex [Na+]	-0.777	0.008	1	0						
Post-Ex Glu	0.315	0.375	-0.449	0.193	1	0				
CHO OX	0.088	0.809	0.137	0.707	-0.072	0.844	1	0		
ΔBM	-0.82	0.004	0.612	0.06	-0.552	0.098	-0.102	0.778	1	0
Post-Ex [ADH]	-0.277	0.439	0.098	0.788	-0.625	0.053	-0.123	0.735	0.282	0.43
Total Urine	0.775	0.008	-0.588	0.074	0.419	0.228	0.253	0.481	-0.615	0.059
LD CONDITION	Post-Ex [IL-6]		30 Post-Ex [Na+]		Post-Ex Glu		CHO OX		ΔBM	
	r	p	R	P	r	p	r	p	R	P
Post-Ex [IL-6]	1	0								

30 Post-Ex [Na ⁺]	-0.149	0.682	1	0						
Post-Ex Glu	0.194	0.591	-0.381	0.277	1	0				
CHO OX	0.255	0.477	0.169	0.641	0.617	0.057	1	0		
ΔBM	-0.372	0.289	0.188	0.603	0.36	0.306	0.305	0.391	1	0
Post-Ex [ADH]	-0.076	0.835	0.11	0.762	0.468	0.172	0.773	0.009	0.123	0.735
Total Urine	0.648	0.043	-0.075	0.837	-0.004	0.992	-0.041	0.91	-0.422	0.225
LE CONDITION	30 Post-Ex [IL-6]		30 Post-Ex [Na ⁺]		Post-Ex Glu		CHO OX		ΔBM	
	r	p	R	P	r	p	r	p	r	P
Post-Ex [IL-6]	1	0								
30 Post-Ex [Na ⁺]	0.068	0.853	1	0						
Post-Ex Glu	0.47	0.17	-0.039	0.915	1	0				
CHO OX	0.386	0.271	-0.287	0.436	0.544	0.104	1	0		
ΔBM	-0.55	0.099	-0.153	0.673	-0.661	0.037	-0.237	0.51	1	0
Post-Ex [ADH]	-0.014	0.97	-0.627	0.052	0.07	0.847	0.131	0.718	0.257	0.474
Total Urine	0.355	0.315	0.361	0.305	0.714	0.02	0.143	0.694	-0.88	0.001

Bolded numbers indicate significant correlations. Bolded and Italicized numbers indicate correlations approached significance (<0.06).

There was a significant correlation between post-exercise [IL-6] and 30 minutes post-exercise [Na⁺] ($r=-0.077$ $p=0.008$) in the DE condition (Table 3). There were also significant correlations between change in body mass and total urine volume in the DD and LE conditions ($r=-0.907$ $p=0.000$ and $r=-0.88$ $p=0.001$, respectively). The LE condition had a correlation that approached significance between post-ex [ADH] and 30-minutes post-exercise [Na⁺] ($r=-0.627$ $p=0.052$).

Chapter V Discussion

Our experimental design was constructed in order to examine possible relationships between glycogen status, hydration status, IL-6, ADH, and plasma sodium. In addition, in order to create differences in hydration status and the concomitant plasma sodium levels, we examined the timing of fluid intake with large volumes of fluid ingested either during exercise, or during a three-hour rehydration period. Based on this design, we hypothesized that we would be able to observe mechanistic relationships between IL-6, ADH, and plasma sodium levels. The differences in blood glucose and CHO oxidation suggest that our dietary manipulation was successful in producing differences in CHO availability. Hydration status was also clearly different based on the differences in body mass at various points during the trials. Although there were increases in IL-6, the increases that occurred were due to exercise alone, and were not impacted by the glycogen or hydration manipulations. In addition, sodium and ADH levels also varied, but only in response to exercise and hydration status, and not due to glycogen status. Finally, we did not observe differences between or relationships among any of the dependent measures that would allow us to elucidate mechanistic processes.

CHO Status

There was a significant difference in the average CHO oxidation between the glycogen loaded and glycogen depleted conditions. In addition, blood glucose levels were lower in the depleted compared to the loaded conditions. These measures serve as control variables to verify the effect of the pre-trial exercise and dietary manipulation that we implemented. Although muscle biopsies with subsequent analysis of glycogen levels

would have been a direct means of confirming CHO status, given all of the other invasive and demanding measures that we made on our subjects, it was not feasible to add this measure. Our intent was to create glycogen depleted and glycogen loaded states that would simulate what would occur with the energy and carbohydrate status of individuals engaging in exercise durations longer than 90 minutes. Given the conditions we required the participants to exercise, it is unlikely that we could find individuals who were able to exercise much longer than 90 minutes in the environmental conditions and at the exercise intensity we specified. The glycogen loaded trials were to represent the start of a long exercise bout when an individual would start with ample amounts of glycogen stored in the muscle and liver. The glycogen depleted trials represented the end of a long exercise bout. The low CHO diet given in this case was intended to impair glycogen synthesis so that the muscles were in a state of depletion simulating the end of a very long bout of exercise. The decrease in the markers of carbohydrate status—CHO oxidation and blood glucose— in the current study were similar to the responses reported by Arkinstall and colleagues (2004) when decrements in muscle glycogen were observed.

Since previous researchers (Steensberg et al., 2001) have shown that glycogen depletion provides a stimulus for greater IL-6 release from the muscle, our intent was to determine the effects of the manipulation of CHO status on this cytokine. In addition, since each gram of glycogen is stored with three grams of water, the relationship between CHO status and hydration status was also of interest. The possible interaction with CHO status and both of these dependent variables will be discussed in a later section.

Hydration Responses

We examined changes in body mass before and after exercise and throughout the three-hour rehydration period. The significant main effect of hydration was evident with the euhydrated conditions exhibiting a greater decrease in body mass than the dehydrated conditions when examining the changes in body mass pre-exercise to post-rehydration. This somewhat paradoxical result may be due to the fact that the subjects had maintained near 0% body weight loss after the euhydrated exercise trials so there was not a physiological need to retain the water that was given post exercise; whereas, after the dehydrated trials the subjects were dehydrated and fluid retention was greater throughout rehydration.

The analysis of body mass changes during exercise showed a 2.77% and 2.66% dehydration for the DD and LD conditions, respectively; whereas the DE and LE conditions showed percent dehydrations of 0.25% and 0.38%, respectively. Despite this difference, and achieving very close to euhydration in the DE and LE conditions, the percentage dehydration observed in the DD and LD conditions is not very severe. Studies have been done where individuals have exercised until levels of dehydration of 7% and greater were achieved (Francesconi et al., 1985). Had the exercise been longer or more demanding, the level of dehydration may have been much greater, and disturbances in related physiological responses would also have been much greater. When individuals complete ultraendurance events in a competitive situation, they are likely to reach much greater levels of dehydration and experience greater disturbances in other physiological measures. The lack of significant differences between conditions,

and the lack of significant correlations between key dependent variables may have been due to the moderate level of physiological strain imposed by our experimental conditions.

The significant main effect of hydration for plasma volume was also in line with the expected outcomes given that our fluid intake manipulation maintained close to 0% body weight loss in the euhydrated trials. The participants were well hydrated at the end of exercise during the LE and DE conditions and were dehydrated after completing the LD and DD conditions. The changes in plasma volume seen in this study are similar to those of other studies (Mitchell et al., 2002). Even though there was a significant main effect for time, post hoc testing showed only the post-exercise time point to be statistically different from all other time points. This is because all of the subjects received water after exercise during the rehydration period; thus, their plasma volume began to recover quickly. This recovery, in combination with the normal reversal of exercise-induced shifts brought all of the values within about 1% of baseline by the 60 minute time point. Even though participants received more water during the rehydration period in the dehydrated conditions, because of the greater maintenance of plasma volume in the LE and DE conditions, the fluid overload in the LD and DD conditions was not enough to cause a significant difference in plasma volume. Further, the total volume consumed in all four conditions was the same, with only the timing of ingestion being different. Our results suggest that regardless of when the fluid is consumed, during or after exercise, the final level of hydration of the vascular space ends up the same. This response has significant implications for plasma sodium since the concentrating and dilutional effects of fluid consumption will show up as either hyper- or hyponatremia.

In addition to changes in plasma volume, we looked at rate of urine production, time to first urine production, and total volume of urine produced as additional markers of fluid balance. There were significant hydration by glycogen interactions for both rate of first urine production and time to first urine production with the dehydrated conditions having a longer time to first urine production than the euhydrated conditions. The LE condition had the shortest time to first urine and the largest total urine volume. We speculate this may be due to the water storage in association with glycogen. When the body stores glycogen, it also stores water in a ratio of 3 water molecules to every 1 glycogen molecule. During exercise, as the body is utilizing the stored glycogen for fuel, the water molecules are released and may be the source of the increased urine volume seen in this condition. In addition, the euhydrated trials resulted in significantly higher rates of urine production than the dehydrated trials. This may also have occurred because the participants consumed 2/3 of their total water during exercise for these trials; thus, during the initial stages of rehydration, their tissues were already well hydrated so the kidneys began eliminating excess fluid. During the dehydrated trials the participants were given the entire volume of water post-exercise; thus, it might be assumed that there would be a great deal of fluid unloading. The fact that urine production was delayed indicates that the tissues were able to rapidly assimilate the large fluid intake. Although there was a higher level of ADH present in the LD and DD conditions post-exercise, it is not clear how long the differences persisted since by 60 min, there was no longer a difference. A continued elevation in ADH during the first hour of rehydration would have influenced the rate of urine production in the dehydrated conditions.

Core Temperature Responses

For all trials, there was a significant elevation in T_c ; however, only the hydration manipulation produced significant elevations during the latter part of the trials. This is not surprising since the participants were exercising in a hot environment at an intensity and duration that has been shown to lead to substantial heat accumulation, and T_c has also been shown to be elevated in a dehydrated state (Montain and Coyle, 1992). This may have been due to decreased sweating towards the end of exercise in the dehydrated trials as a result of large fluid loss without fluid replacement. Montain and Coyle (1992) have shown that dehydrated individuals and those deprived of fluid during exercise have higher increases in core temperature and greater cardiovascular drift than individuals that remain hydrated while exercising in the heat. The greater cardiovascular drift would be due to impaired venous return due to reduced plasma and blood volume with a concomitant depression in stroke volume. We did not assess cardiovascular function, but it is likely the dehydration and the greater elevation in T_c were paired with greater elevations in heart rate. The fact that there were no differences due to glycogen status indicates that, even though it could be hypothesized that the water stored with glycogen could enhance hydration status, this effect was not great enough to alter thermoregulatory responses.

Sodium Responses

The significant difference in $[Na^+]_{\text{plasma}}$ seen between the dehydrated and euhydrated conditions is consistent with what we would expect for the mid and post-exercise time points. Since the participants in the dehydrated conditions did not receive

any water during exercise, their plasma volumes decreased and consequently their plasma sodium levels became more concentrated. On the other hand, plasma sodium concentrations in the LE and DE conditions fell during the mid and post-exercise time points even though we only gave them enough water to maintain 0% BWL; that is, they were not hyperhydrated. This may be due to the fact that the decreases in plasma volume in those conditions were not as great as those seen in the dehydrated conditions, meaning that the individuals were not as haemoconcentrated. Therefore, giving fluid to maintain body mass during the euhydrated conditions may have slightly diluted the plasma sodium levels. A drop in sodium, possibly to clinically hyponatremic levels, has been associated with the consumption of large fluid volumes, especially hypotonic solutions. It should be pointed out that in field studies where hyponatremia has been diagnosed, the large fluid consumption often results in post-exercise weight gain (Noakes, 1992). Our findings suggest that it is possible to significantly lower plasma sodium levels during a relatively short exercise session even without fluid-induced weight gain. Based on the downward trend in sodium during the exercise segment, it is possible that plasma sodium levels would have continued to decline to clinically hyponatremic levels.

In addition, the sodium levels in the LE and DE conditions never came back up to baseline after the full 180 minutes of rehydration even though these conditions had a shorter time to first urination and a higher rate of first urine production than the dehydrated conditions. One possible explanation is that plasma volumes returned to baseline levels by 60 minutes post-exercise. This return to baseline would mean that the individual is no longer haemoconcentrated, and this increase in plasma volume, secondary to the fluid consumed, prevented the plasma sodium levels from returning to

their baseline values. This response may be suggestive of selective plasma volume restoration, a phenomenon observed previously (Mitchell et al., 2000), but with an electrolyte-containing beverage.

Many of the participants became clinically hyponatremic ($[\text{Na}^+] < 135 \text{ mmol/L}$) during the rehydration period of many of the trials, but in every instance the subject remained asymptomatic. When this happened we simply withheld the fluid feedings until plasma sodium levels reached 135 mmol/L or greater. An analysis of the number of curtailed feeding regimens shows that participants missed an average of 0.5 feedings in the DD and LD conditions, one feeding in the LE condition, and two feedings in the LD condition. The total number of feedings missed was 4.92, 5, 10, and 20 for the DD, LD, LE, and DE conditions, respectively.

In this study, we observed notable variability in individual resting Na^+ levels, ranging from 135 mmol/L to 141 mmol/L. For an individual with a resting Na^+ of 141 mmol/L, a loss 1 or 2 mmol/L may not be physiologically relevant. However, if an individual with a resting Na^+ of 135 mmol/L loses 1 or 2 mmol/L, they become clinically hyponatremic, even though they may be asymptomatic. Under these circumstances one could argue that the overall change in Na^+ may be more important in the development of symptomatic hyponatremia than an absolute threshold. In fact, for those who normally have a low plasma sodium level, the clinical threshold may be even lower. For example, one of our subjects reported to the lab with a resting level of 139 mmol/L and dropped to 128 mmol/L during rehydration, yet remained asymptomatic. Even a change of 11 mmol/L in his case was not physiologically detrimental; thus, for

clinical purposes a combination of an absolute level along with a total change would likely be of the greatest value.

The mechanism behind EAH has been a source of some controversy for the past several years. Absolute sodium losses and/or the dilutional effects of consuming large volumes of hypotonic solutions have both been proposed as key factors in the development of EAH. Noakes (1992) has proposed the concept of mobilization of sodium stores within the body. Using the calculations proposed by Noakes, the theoretical post-rehydration $[\text{Na}^+]_{\text{plasma}}$ values were much lower than the actual measured post-rehydration $[\text{Na}^+]_{\text{plasma}}$ values. Since all participants in the study were rehydrated with water only, no sodium was replaced either during exercise, or during the three-hour rehydration period. The only source of the sodium, therefore, would be from stores inside the body; thus, our data fit with Noakes' idea that the body must activate stores of sodium in the face of substantial losses during exercise. Unfortunately, we did not measure ECF volume and therefore used a calculation to estimate ECF which is a limitation regarding these data, however, Noakes also used an estimation of ECF for his calculations. If one can rely upon the assumption of a standard ratio of ECF volume to body weight to arrive at this type of analysis, there may be some validity to the assumption of mobilization of previously osmotically inactive sodium stores.

IL-6 Responses

The post-exercise [IL-6] was significantly greater than the pre-exercise levels for all conditions. The magnitude of the response, however, was not as great as we expected, nor was it as high as some researchers have reported following exercise of this duration

(Ronsen et al., 2002). Some of the participants were unable to finish the 90 minutes of exercise or maintain the correct intensity throughout the exercise, especially during the glycogen depleted trials. This decline in intensity may be why the IL-6 response was lower than anticipated. Starkie and colleagues (2001) reported post-exercise IL-6 levels of about 7 pg/mL after only 60 minutes of cycling at lactate threshold while the greatest post-exercise IL-6 level in the present study was 5.4 pg/mL where participants completed 90 minutes of exercise in a glycogen depleted state. Researchers have reported that IL-6 may be involved in glucose homeostasis (Pedersen et al., 2004) and that IL-6 production may be related to pre-exercise muscle glycogen content (Steensberg et al., 2001). In addition, IL-6 has been shown to stimulate glycogen phosphorylase to facilitate glycogen breakdown in cultured hepatocytes (Keller et al., 2001). In light of this previous research, it seems logical to expect higher post-exercise IL-6 levels in the glycogen depleted conditions; however, the data obtained from this investigation did not show a significant main effect for pre-exercise glycogen status. One possible explanation may be the failure to maintain exercise intensity towards the end of the glycogen depleted trials as participants became fatigued. In addition, cycling has not been shown to elicit as great of an IL-6 response as other modes of exercise (i.e. running). Exercise that utilizes more muscle mass has been linked to increased IL-6 levels (Febbraio and Pedersen, 2002). Starkie and colleagues (2001) saw a greater post-exercise IL-6 concentration after running (9 pg/mL) than after 60 minutes of cycling (7 pg/mL). Another way we might have increased post-exercise IL-6 levels is by increasing the duration of exercise. We considered this during the initial planning of the study, but decided on 90 minutes

because we saw substantial increases in IL-6 with 90 minutes of cycling in the research literature (Fischer, 2006).

The main aim of this study was to determine if there was a relationship between IL-6 and ADH. Mastorakos and colleagues (1994) proposed that IL-6 may be a nonosmotic stimulator of ADH and play a role in SIADH; therefore, increased levels of IL-6 should produce some increase in ADH levels. Theoretically, the increased ADH would then prevent urinary water loss and contribute to the dilution of sodium. Although, in the present study, ADH was elevated immediately post-exercise, regardless of condition, it did not stay elevated long enough to prevent urine production once a substantial amount of fluid had been consumed. A major difference between the current results and the work of Mastorakos et al (1994) is that their study used injections of recombinant IL-6, not systemically produced IL-6. Unfortunately, the limited IL-6 response in the current study made any correlations between these two substances difficult to establish.

ADH Responses

The significantly greater ADH responses in the dehydrated conditions was simply due to the fact that the subjects received no water during their 90 minute exercise in these trials; thus, their kidney function was operating in an expected fashion to retain maximal fluid volume. The plasma ADH levels returned to near baseline 60 minutes post-exercise and remained there for the rest of the rehydration period. The differences observed were only present immediately post-exercise; thus, it is obvious that the stimulus for ADH release is rapidly reversed with even partial fluid compartment restoration. Since ADH

release is strongly tied to plasma osmolality, restoration of the plasma compartment would reduce the stimulus for release. At the 60 minute time point there was still a -4% change in plasma volume from baseline. Although we did not measure plasma osmolality, at 60 minutes post-exercise the osmolality was likely below the threshold for ADH secretion since ADH levels returned to near baseline at that time point. The post-exercise ADH levels from this study are much higher than levels seen in the literature. A study done by Montain and colleagues (1997) only showed an increase in plasma ADH levels of about 2 pg/mL after exercising until achieving 5% body weight loss. In this study, participants achieved an average of 2.7% body weight loss during both the DD and LD conditions, but had average plasma ADH changes of over 41 pg/mL and 35 pg/mL, respectively. Kenefick and colleagues (2007) saw ADH concentrations of 25 pg/mL after exercising in the heat until 4% dehydration.

None of the participants in this study appeared to exhibit signs of SIADH. Even though sodium levels continued to fall below baseline during the rehydration period, ADH levels returned to near baseline, not seeming to cause the asymptomatic hyponatremia observed in some participants. Since we do not have any ADH data during this time we cannot be sure how quickly or slowly ADH levels returned toward baseline. It is possible that ADH levels could have been inappropriately elevated between the post-exercise and 60 minute post-exercise time points; however, none of the individual responses are indicative of an abnormally high ADH levels leading to a precipitous drop in sodium.

Correlations

Based on the proposed relationship between IL-6, ADH, and the resulting effects on plasma sodium levels, it could be expected that there would be significant correlations between these variables. However, no significant correlations between IL-6 and ADH nor ADH and sodium were found in any of the conditions. Only the LD condition had a significant correlation between IL-6 and sodium. The negative correlation between these variables (as IL-6 levels increase, plasma sodium concentrations decrease) fits in part with the proposed model. Unfortunately, there was no correlation between either of these variables with ADH to complete the proposed model. The LE condition showed a correlation between ADH and sodium which approached significance, but was missing correlations with IL-6. There were significant correlations between total urine volume and change in body mass in the DD and LE conditions. Both correlations were negative, indicating that as one variable increased the other decreased. As total urine production increases, change in body mass decreases and vice versa. This means that water ingested was either not being reabsorbed into the body and excreted as urine, reducing the change in body mass or the fluid was retained, increasing the change in body mass and decreasing total urine volume. Some of the significant correlations detected may be due purely to coincidence as there seems to be no physiological relevance (ie. post-glucose concentration and total urine production).

Summary

The purpose of this study was to investigate the effects of glycogen content and hydration status on ADH, IL-6 and plasma sodium concentrations. It has been proposed

that increased IL-6 concentrations can stimulate ADH secretion possibly resulting in SIADH and decreased plasma sodium concentrations. We had two hypotheses for this study. We hypothesized the DD condition would elicit the highest IL-6 and ADH responses and have the lowest $[\text{Na}^+]_{\text{plasma}}$ and that the LE condition would have the lowest IL-6 and ADH responses and the highest $[\text{Na}^+]_{\text{plasma}}$. The results of this study did not support our hypotheses. Despite our glycogen, hydration, and exercise manipulations we did not observe a relationship between these variables. Glycogen status had no significant impact on IL-6 and ADH concentrations. We only observed a significant time effect for IL-6 concentrations and a significant hydration status and time effect for plasma sodium and ADH concentrations.

This study investigated the short term shifts in body fluid and sodium induced by exercise and large fluid volume consumption. Despite finding no clear relationship between ADH and IL-6 in this study, there were few key findings. A volume of 100% fluid replacement during exercise, and a rehydration volume of 150% of fluid lost during exercise, (both of which have been recommended in the literature for hydration during and after intense exercise, respectively), were enough to cause a tendency toward decreased plasma sodium concentrations after each of the conditions performed by our participants. In the euhydrated conditions, the decrease in sodium at the end of exercise did not recover; thus, our short-term model demonstrates the ease with which hyponatremic tendencies can be achieved. In the dehydrated conditions, the similar level of sodium at the end of rehydration suggests that, likewise, hyponatremic tendencies can also be achieved with excessive post-exercise water consumption. These responses could possibly be more pronounced with longer duration and more intense exercise such as a

marathon or ultra-endurance triathlon. In addition, the fluid intake of those competing in ultra-endurance events may not be as carefully regulated as the fluid intake in our study; thus, those athletes may be at even greater risk for significant sodium disturbances. The data from this study do support Noakes' idea of osmotically inactive sodium stores within the body since the observed post-rehydration plasma sodium levels were substantially higher than the theoretically calculated sodium concentrations. However, further investigation may be required with more severe exercise conditions to fully elucidate the effects of IL-6 on ADH and plasma sodium concentrations.

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ABSTRACT

Exercise-associated Hyponatremia: The Effects of Glycogen and Hydration Status on IL-6, ADH, and Sodium Concentrations.

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Background: Exercise-associated hyponatremia (EAH) is a rare, but serious life-threatening condition that has been identified in marathoners, ultra-endurance athletes, and others engaging in prolonged, physical activity conducted in a hot environment. Hyponatremia is diagnosed when serum sodium concentrations fall below 135 mmol/L. **Purpose:** The purpose of this study was to evaluate the effect of hydration status and glycogen level on venous IL-6, ADH, and sodium concentrations during and after prolonged exercise in the heat. **Method:** Ten male participants completed four trials: a glycogen depleted, euhydrated condition (DE); a glycogen depleted, dehydrated condition (DD); a glycogen loaded, euhydrated condition (LE); and a glycogen loaded, dehydrated condition (LD). Each condition consisting of cycling at 60% VO_2 max in a 35°C environment for 90 minutes followed by a 3 hour rehydration period. Body mass was measured before and after exercise and after rehydration. Blood and sweat samples were collected during exercise. Blood and urine samples were collected during the rehydration period. Blood was analyzed for glucose, IL-6, ADH, and Na^+ . Sweat and urine samples were analyzed for $[\text{Na}^+]$. **Results:** The LD and LE conditions had significantly higher plasma glucose concentrations than the DD and DE conditions for all time points except for pre-exercise. There was a significant glycogen by hydration by time interaction for plasma $[\text{Na}^+]$ ($p=0.022$). The LD and DD conditions showed the greatest overall $[\text{Na}^+]$ changes from post exercise to post rehydration (-6.85 and -6.7 mmol/L, respectively) compared to the DE and LE conditions (-1.45 and 0.10 mM, respectively). $[\text{Na}^+]$ in the dehydrated conditions was significantly higher than the euhydrated conditions at the mid, post, 30, 60, and 90 time points. There was a main effect for time ($p=0.00$) for IL-6 with the highest concentrations occurring immediately post-ex. There was a significant hydration by time interaction ($p=0.000$) for ADH with the post exercise time point being significantly different from all other time points. The dehydrated conditions showed the largest post-exercise increase in ADH concentrations with the DD conditions having an average ADH concentration of 50.16 ± 40.552 pg/mL and the LD conditions having an average of 55.46 ± 68.124 pg/mL. Significant correlations were detected between post-exercise $[\text{IL-6}]$ and 30 minutes post-exercise $[\text{Na}^+]$ ($r=-0.077$ $p=0.008$) in the DE condition and between change in body mass and total urine volume in the DD and LE conditions ($r=-0.907$ $p=0.000$ and $r=-0.88$ $p=0.001$, respectively). **Summary:** Despite our glycogen, hydration, and exercise manipulations we did not observe a relationship between these variables. Glycogen status had no significant impact on IL-6 and ADH concentrations. We only observed a significant time effect for IL-6 concentrations and a significant hydration status and time effect for plasma sodium and ADH concentrations. A volume of 100% fluid replacement during exercise and a rehydration volume of 150% of fluid lost during exercise were enough to cause a tendency toward decreased plasma sodium concentrations.

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01/06-12/07 **TEXAS CHRISTIAN UNIVERSITY** Fort Worth, TX
Dept. of Kinesiology-Graduate Departmental Assistant
Exercise Physiology Laboratory Research Assistant
Teaching Assistant/Instructor

01/04–05/04 **UNIVERSITY OF WISCONSIN-MADISON** Madison, WI
Dept. of Zoology-Undergraduate Research Assistant

HONORS/AWARDS

2000-2004 Wisconsin Academic Excellence Scholarship
2003-2004 Dean's List 4 semesters
2003-2004 University of Wisconsin Scholar Athlete
2003-2004 Big Ten Scholar Athlete Award

PROFESSIONAL AND ACADEMIC ORGANIZATIONS, AND CERTIFICATIONS

American College of Sports Medicine - Texas Chapter
American Red Cross – CPR/AED-Adult and Standard First Aid Certification

TEACHING EXPERIENCE

1/06 – 12/07 **Texas Christian University Department of Kinesiology**
Teaching Assistant: Undergraduate Personal Fitness
Teaching Assistant: Undergraduate Anatomical Kinesiology
Teaching Assistant: Undergraduate Exercise Physiology

Instructor: Beginning Soccer
Instructor: Body Conditioning
Instructor: Beginning Jogging

COURSES TAKEN

Anatomy, Physiology, Biochemistry, Organic Chemistry, Physics, Genetics, Bacteriology, Exercise Physiology, Cardiopulmonary Physiology, Biomechanics, Motor Behavior, Statistics, Clinical Exercise Testing, Nutrition and Disease

LABORATORY PROFICIENCIES

Maximal Oxygen Uptake Testing

- Treadmill
- Cycle Ergometer

Blood and Tissue Processing

- Venipuncture
- Hematocrit and hemoglobin
- Glucose
- Lactate
- Creatine kinase
- HDL and LDL cholesterol
- ELISA
- Electrolyte and Hematology analysis
- Adipose tissue processing
- DNA/RNA/Protein Isolation
- Electrophoresis
- PAGE
- RT-PCR

Other Laboratory Assessments

- Oral Glucose Tolerance Testing
- Blood pressure
- 12 lead ECG-stress testing
- Wingate testing
- Resting Metabolic Rate
- Body Composition -skin fold measurement

Field Experience

- Involved in the development and implementation of resistance and aerobic exercise programs for elite athletes, obese individuals, and elderly populations.

PRESENTATIONS AND PUBLICATIONS

Presentations:

Hubing, K., Quebedeaux, L., Mitchell, J., Phillips, M., Barbee, J., Kirk, M., Killoren, G., Herrera, R., Mitchell, K., Menzies, R., and Hein, R.. Hyponatremia: Field Based Data Analysis of Cyclists Treated in the Medical Tent After the Hotter 'N Hell 100, and Laboratory Based Pilot Work. Presented 2007 at Texas ACSM Chapter Annual Conference

Quigg, L.R., Quebedeaz, L.P., **Hubing, K.A.**, Mitchell, J.B., and Upton, D.E. The Effect of Two Concurrent Training Programs with Different Inter-Session Recovery on Muscular Strength. Submitted for presentation at the 2008 ACSM National Conference, Indianapolis, IN.

Rogers, M.M., **Hubing, K.A.**, Quigg, L.R., Bassett, J.T., Quebedeaux, L.P., Patrizi, R.M., Byrd, K.L., Upton, D.E., and Mitchell, J.B. Fatigue During High Intensity Exercise: The Interaction Between pH and Thermal Stress. Submitted for presentation at the 2008 ACSM National Conference, Indianapolis, IN.

Hubing, K.A., Rogers, M.M., Quigg, L.R., Bassett, J.T., Quebedeaux, L.P., Patrizi, R.M., Byrd, K.L., Upton, D.E., and Mitchell, J.B. The Relationship Between Lactate, pH, and Bicarbonate Levels During Maximal Graded Exercise. Submitted for presentation at the 2008 ACSM National Conference, Indianapolis, IN.

Publications:

Rogers, M.M., **Hubing, K.A.**, Quigg, L.R., Bassett, J.T., and Mitchell, J.B., The Relationship Between Lactate, pH, and Bicarbonate Thresholds. (Manuscript in preparation).

Mitchell, J.B., Petrasic, J.R., **Hubing, K.A.**, and Rogers, M.M. The Effect of a Pre-exercise Meal on the Rate of Gastric Emptying. (Manuscript in Preparation)

Mitchell, J.B., Phillips, M.D., Yellot, R.C., and **Hubing, K.A.** The Effect of Mode of Exercise on IL-6 Responses and Post-Exercise Glucose Uptake. (Manuscript in preparation).

Hubing, K.A., Quigg, L.R., Bassett, J.T., Barbee, J., and Mitchell, J.B. The Effects Hydration and Glycogen Status on ADH, IL-6, and Sodium Concentrations in the Development of Exercise-associated Hyponatremia. Gatorade Sports Science Institute Grant Recipient, (Study in progress- data collection to be completed, Nov. 2007.)

Phillips, M.D., Patrizi, R.M., **Hubing, K.A.**, Quigg, L.R., Barbee, J., and Mitchell, J.B.

Influence of Resistive Exercise Training on Inflammatory-Related Markers in the Blood and Adipose Tissue, and Glucose Intolerance in Obese Post-Menopausal Women. Study in progress data collection to be completed, Mar. 2008.)

Rogers, M.M., **Hubing, K.A.**, Quigg, L.R., Bassett, J.T., and Mitchell, J.B. Fatigue During High Intensity Exercise: The Interaction Between pH and Thermal Stress. (Manuscript in preparation).

REFERENCES

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