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Effect Of Acute L-alanyl-l-glutamine (sustamine) And Electrolyte Ingestion On Plasma Electrolytes, Physiologic Measures, And Neuromuscular Fatigue During Endurance Exercise

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EFFECT OF ACUTE L-ALANYL-L-GLUTAMINE (SUSTAMINE^{TM}) AND ELECTROLYTE INGESTION ON PLASMA ELECTROLYTES, PHYSIOLOGIC MEASURES, AND NEUROMUSCULAR FATIGUE DURING ENDURANCE EXERCISE

by

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ABSTRACT

The purpose of this study was to compare the efficacy of two dose levels of L-Alanyl-L-Glutamine in a commercially available sports drink to the sports drink only on time to exhaustion, neuromuscular fatigue and physiological measures during prolonged endurance exercise. Twelve endurance-trained males (23.5±3.7 yrs; 175.5±5.4 cm; 70.7±7.6 kg) performed four trials, each consisting of 1 hr treadmill runs at 75% of VO_2peak followed by a run to exhaustion at 90% of VO_2peak. The trials differed in type of hydration. One trial consisted of no hydration (NHY), another required ingestion of only a sports drink (ET), and two trials required ingestion of a low dose (LD) (300 mg·500 ml^{-1}) and high dose (HD) of L-Alanyl-L-Glutamine (1 g·500 ml^{-1}) mixed in the sports drink. During the fluid ingestion trials 250 ml were consumed every 15 min. Plasma glutamine, glucose, electrolytes, and osmolality were measured prior to the run (PRE), and at 30, 45, and 60 min. VO_2, RQ, and HR were measured every 15 min and surface electromyography (EMG) of the vastus lateralis and rectus femoris were measured every 10 min during the 1 hr run. Time to exhaustion was significantly longer during the LD and HD trials compared with NHY. Plasma glutamine concentrations were significantly elevated at 45 min in LD and HD trials, and remained elevated at 60 min during HD. Sodium concentrations increased with the beginning of exercise and remained stable for the duration of the 1 hr run. At 60 min plasma sodium was significantly lower in all trials compared with NHY. The results from this study indicated that ingestion of the alanine-glutamine dipeptide at either the low or high dose significantly improved time to exhaustion during high intensity exercise compared to a no hydration trial. These differences were not noted between ET and NHY.

Keywords: Alanine-Glutamine, Running Performance, Electrolytes, Electromyography

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

Introduction

A number of factors have been identified that can lead to fatigue during endurance activity. These causes of fatigue range from substrate availability, electrolyte imbalance, dehydration, hyperthermia, and neuromuscular fatigue. Dehydration often leads to a reduced sweat rate and reduction in skin blood flow from the loss of fluid leading to an increase in core temperature (Coyle, 1999; Coyle and Montain, 1992a, 1992b; Gonzalez-Alonso, Mora-Rodriguez, Below, & Coyle, 1995). These effects may be further magnified if dehydration occurs during exercise in a hot environment. Ingestion of fluid during endurance exercise helps maintain circulating plasma volume decreasing the cardiovascular strain and reducing the risk of hyperthermia (Coyle and Montain, 1992a, 1992b). It has been reported that a fluid loss of approximately 2 to 3% of body weight can impact endurance capacity (Coyle, 2004; Goulet, 2013; Montain, 2008). The reduction in plasma volume results in increased heart rate, increased muscle glycogen use, and reduced central nervous system function (Cosgrove et al., 2014).

During prolonged endurance events (lasting longer than 1 hr) substrate availability, the loss of fluid, increase in core temperature, and change in plasma electrolytes are major factors leading to a decrease in exercise performance (Coyle, 1999; Sawka and Noakes, 2007). The ingestion of fluid during prolonged endurance events has been shown to reduce the cardiovascular strain on the individual leading to delayed fatigue (Coyle, 2004). In events lasting less than 1 hr, the benefits of ingesting a carbohydrate solution versus a placebo appear to be equivocal. Several studies have shown an improvement in performance (Ball, Headley, Vanderburgh, & Smith, 1995; Below, Mora-Rodriguez, Gonzalez-Alonso, & Coyle, 1995;

Burke, Wood, Pyne, Telford, & Saunders, 2005; Carter, Jeukendrup, Mundel, & Jones, 2003), while others have shown no improvement (Anantaraman, Carmines, Gaesser, & Weltman, 1995; Desbrow, Anderson, Barrett, Rao, & Hargreaves, 2004; Tsintzas, Liu, Williams, Campbell, & Gaitanos, 1993). In addition to fluid loss through sweating, the loss of electrolytes may play a role in the onset of fatigue during endurance exercise (Bangsbo, Gunnarsson, Wendell, Nybo, & Thomassen, 2009; Cairns & Lindinger, 2008; Nielsen et al., 2004; Nordsborg et al., 2008). Electrolyte loss can impact nerve impulse conduction, muscle fiber contraction, and maintenance of cell membrane permeability (Shier, Butler, & Lewis, 2004). Studies examining electrolyte replacement during exercise have recommended sodium be included in fluids if exercise exceeds 2 h, or for those individuals who lose more than 3 – 4 g of sodium in their sweat (Coyle, 2004; Shirreffs and Sawka, 2011).

It is highly recommended that fluid and electrolyte replacement strategies are used to maintain endurance performance in athletes (Sawka et al., 2007; Shirreffs and Sawka, 2011). Recently, glutamine has been shown to enhance fluid and electrolyte absorption in both animal (Lima et al., 2002; Silva et al, 1998) and human models (van Loon, et al., 1996). Glutamine is a non-essential amino acid and is involved in many physiologic functions including cellular proliferation, acid-base balance, transport of ammonia between tissues, and antioxidant synthesis (Curi et al., 2005; Newsholme, et al., 2003a, 2003b; Rutten, Engelen, Schols, & Deutz, 2005). It has been used in both medical and athletic settings to try to enhance fluid absorption (Hoffman et al., 2010; Hoffman et al., 2011; Hoffman et al., 2012; Mertes et al, 2000; Novak, Heyland, Avenell, Drover, & Su 2002). During prolonged starvation, sepsis, and long duration physical activity, glutamine concentrations may become deficient (Castell, Poortmans, & Newsholme,

1996; Hankard, Haymond, & Darmaun, 1997; Parry-Billings, Leighton, Dimitriadis, Vasconcelos, & Newsholme, 1989; Santos, Caperuto, & Costa Rosa, 2007;). The decrease in plasma glutamine following long duration exercise may be caused by several factors, including an increase in glutamine extraction by the liver to increase gluconeogenesis or for urea formation, and increased rate of utilization by cells, in particular the kidneys and immune system, or a decreased rate of glutamine being released from the skeletal muscles (Newsholme, 1994).

When glutamine is supplemented, there is a problem with absorption due to the low pH in the gut and the low solubility of glutamine (Fürst, 1998; Fürst, Pogan, & Stehl, 1997; Stehle et al, 1989). The addition of alanine to the glutamine molecule increases the stability of glutamine, especially at low pH as seen in the gut (Fürst, 2001). Additionally, alanine is considered the most gluconeogenic amino acid (Klein, Nyhan, & Kern, 2009) and has been identified as a major gluconeogenic precursor in prolonged exercise (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974). Hoffman and colleagues (2010) examined the efficacy of the alanine-glutamine dipeptide in participants that were dehydrated to -2.5% of their body weight and then rehydrated to -1.5% with the dipeptide. Participants were also required to cycle to exhaustion at 75% of VO_2max. The investigators reported significantly greater times to exhaustion in participants that were provided the dipeptide compared to a no fluid trial (Hoffman et al., 2010). Interestingly, time to exhaustion was not significantly different between the no fluid trial and water only trial. In research examining the effect of ingesting three different rehydration fluids (an artificially-flavored placebo, a commercial sports drink, and a rehydration electrolyte drink with glutamine) on endurance performance, Snell and colleagues (2010) saw an improvement in run to

exhaustion time with the rehydration electrolyte drink following a 60 min run at 70 to 75% of VO_2max. The runners ran the 60 min run followed by 60 min of rest, performed the run to exhaustion, rested 60 min during which time fluid was ingested to replace the amount of fluid lost during the 60 min run and then performed one final run to exhaustion.

The efficacy of alanine-glutamine ingestion and athletic performance has also been demonstrated during competitive games. Hoffman and colleagues (2012) studies the effect of the dipeptide on female basketball players. Their results were consistent with the other studies examining the benefits of alanine-glutamine dipeptide in preventing performance decrements during a dehydration stress. Others have shown significant improvements in distance covered when consuming glutamine combined with carbohydrates and water, versus carbohydrates and water alone in soccer players during simulated soccer activities (Favano et al., 2008).

Considering that the alanine-glutamine dipeptide is suggested to enhance water and electrolyte absorption, studies to date have not examined the efficacy of ingesting the combination of the dipeptide with a sports drink containing electrolytes. Thus, the purpose of this study was to evaluate the efficacy of the L-Alanyl-L-Glutamine dipeptide mixed in a commercial sport drink on changes in plasma concentrations of glutamine, sodium, and potassium compared to the sport drink alone during prolonged endurance exercise in male endurance-trained runners. An additional purpose of the study was to examine the physiological effects of the dipeptide on oxygen consumption, heart rate, respiratory quotient and muscle activation patterns and neuromuscular fatigue during prolonged endurance exercise in male endurance-trained runners.

Hypotheses

1. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink will significantly increase absorption as measured by plasma glucose, electrolytes, and glutamine during prolonged running by endurance-trained males.
2. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink will significantly reduce the cardiovascular strain as measured by oxygen consumption, heart rate, and respiratory quotient during prolonged running by endurance-trained males.
3. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink will significantly improve muscle activation patterns and neuromuscular fatigue as measured by electromyography root mean square signals from the vastus lateralis and rectus femoris muscles during prolonged running by endurance-trained males.

Assumptions (Theoretical)

1. Subjects accurately answered the medical history and activity questionnaire.
2. All subjects gave maximal effort when performing the VO_2peak test and isometric leg extension test.
3. Participants maintained their current training routine throughout the duration of the study.
4. Participants consumed a similar diet prior to each experimental testing session.
5. Participants were well-rested prior to each experimental testing session.
6. Participants were unable to identify which drink was consumed during experimental trials T2 through T4, and there was no influence on effort during the trial.

7. The weight loss during T1 is approximately the sweat rate for that participant, with no consideration to the loss of the metabolic fuel used during the run.

8. The absorption and effect of the dipeptide L-Alanyl-L-Glutamine is the same across individuals.

Assumptions (Statistical)

1. The population from which the samples are drawn is normally distributed.

2. The sample was randomly selected and the treatment order was randomly assigned.

3. The data met the assumption of sphericity. This requires that the repeated measures data demonstrate both homogeneity of variance and homogeneity of covariance.

Limitations

1. Because the participants were male only, this could impact generalizability. Furthermore, the participants were endurance-trained males, which could further impact generalizability.

2. The main recruiting mechanism was in-class announcements through the College of Education courses, which made subject selection not truly random, which could affect internal validity.

3. The sample was made up of volunteers, therefore, not meeting the underlying assumptions of random selection.

CHAPTER II

Literature Review

Glutamine

Glutamine is a naturally occurring nonessential amino acid. In humans, glutamine is the most abundant amino acid in the body, found in all tissues in the body including the plasma, with the largest storage area in skeletal muscle (Felig, 1975). The resting level of glutamine in the plasma has been reported to range between 550 and 750 $\mu mol \cdot L^{-1}$, while glutamine concentrations within skeletal muscle is approximately 20 $mmol \cdot kg^{-1}$ wet weight (Jonnalagadda, 2007). Glutamine is involved in many physiologic functions including cellular proliferation, acid-base balance, transport of ammonia between tissues, and antioxidant synthesis (Curi et al., 2005; Newsholme, et al., 2003b; Rutten et al., 2005). Glutamine supplementation stimulates an increase in protein synthesis in the muscle (Jepson, Bates, Broadbent, Pell, & Millward, 1988; MacLennan, Brown, & Rennie, 1987), improves glycogen resynthesis (Bowtell et al., 1999; Varnier, Leese, Thompson, & Rennie, 1995), and can lead to an improvement in performance (Favano, et al., 2008; Hoffman, et al., 2010). Glutamine has also been shown to enhance fluid and electrolyte absorption in both animal and human models (Lima et al., 2002; Silva, et al., 1998; van Loon, et al., 1996).

During times of severe stress, especially in catabolic states, glutamine requirements are dramatically increased (Ziegler, Smith, Byrne, & Wilmore, 1993). These stresses can be in the form of prolonged starvation, sepsis, and long duration physical activity (Castell et al., 1996; Hankard et al., 1997; Parry-Billings et al., 1989; Santos et al., 2007). When endogenous stores are unable to meet requirements, skeletal muscle becomes the source of glutamine through

muscle catabolism (Ziegler et al, 1993). Low plasma glutamine has been correlated to an unfavorable outcome in critically ill patients (Berg, Rooyackers, Norberg, & Wernerman, 2005) and intravenous supplementation of glutamine has been shown to decrease mortality and morbidity (Novak et al., 2002). Glutamine supplementation in post-operative patients has been shown to decrease morbidity and lead to a shorter hospital stay (Mertes et al, 2000). In an animal model, Silva and colleagues (1998) demonstrated in rabbits that glutamine added to an oral rehydration solution can increase the rate of fluid absorption greater than water alone. In a rat model, Lima et al. (2002) showed that the addition of glutamine to an oral nutritional rehydration solution enhances electrolyte and water absorption. Van Loon and colleagues (1996) demonstrated in a human model, increased water absorption via glutamine supplementation in an oral hydration solution versus the oral hydration solution alone.

Ingestion of supplemental glutamine has been shown to enhance plasma glutamine concentration, with peak values attained approximately 30 to 50 min following supplementation (Castell and Newsholme, 1997; Harris, Hoffman, Allsopp, & Routledge, 2012; Klassen, Mazariegos, Solomons, & Fürst, 2000). Harris, Hoffman, Allsopp, and Routledge (2012) demonstrated with eight healthy males that supplementing with 60 $mg \cdot kg^{-1}$ body weight of glutamine, there was a 179 ± 61 $\mu mol \cdot L^{-1}$ increase in plasma glutamine level within 30 min of ingestion. All eight participants had an increase in plasma glutamine concentration. However, a number of studies have reported that the stability of glutamine in an acidic environment is not consistent (Fürst et al., 1997; Fürst, 1998; Stehle et al, 1989). Additionally, glutamine is unstable during heat sterilization and prolonged storage for later use (Fürst et al, 1997). Due to these factors there were a number of strict guidelines for the preparation of fluids containing

glutamine, including fresh preparation under aseptic conditions, sterilization by membrane filtration, no greater than 2.5% glutamine concentration, and stored at 4°C (Fürst et al, 1997; Fürst 1998).

Alanine

To overcome the problems with acidity and water solubility, the addition of alanine to form a dipeptide (such as L-alanyl-L-glutamine) increases the stability of glutamine, especially at low pH (Fürst, 2001). This has important implications for oral ingestion of the dipeptide. A number of studies have shown that when alanine is combined with glutamine to form the dipeptide L-alanyl-L-glutamine there is an increase in absorption of glutamine into the plasma (Arii, Kai, & Kokuda 1999; Fürst 2001; Harris, et al, 2012). In a study with humans, Harris and colleagues (2012) examined eight male participants who supplemented with 89 $mg \cdot kg^{-1}$ of L-alanyl-L-glutamine and reported a 284 ± 84 $\mu mol \cdot L^{-1}$ increase in plasma glutamine levels. The increase in plasma glutamine following L-alanyl-L-glutamine supplementation was significantly greater than the increase in plasma glutamine following glutamine supplementation alone.

Alanine is considered the most gluconeogenic amino acid (Klein, Nyhan, & Kern, 2009) and has been identified as a major gluconeogenic precursor in prolonged exercise (Ahlborg et al., 1974). Carraro, Naldini, Weber, and Wolfe (1994) examined the alanine flux during exercise in five healthy males utilizing labeled alanine. Participants were examined on two occasions; once while walking on a treadmill at 45% of their VO_2max for 2 hr and a second time that required no exercise and served as a control. Results showed a nearly 50% increase of plasma alanine during the exercise trial compared to the rest trial.

Glutamine and Exercise

The plasma concentration of glutamine during exercise is dependent on the duration and intensity of the activity (Gleeson, 2008). During high intensity exercise lasting less than 1 hr, studies have shown both an increase (Babij, Matthews, & Rennie, 1983; Sewell, Gleeson, & Blannin, 1994) and no change (Robson, Blannin, Walsh, Castell, & Gleeson, 1999) in plasma glutamine concentration. Babij et al. (1983) speculated that the increase in plasma glutamine concentrations during high intensity exercise may be the result of the production of ammonia, which combined with glutamate forms glutamine. The research examining plasma glutamine concentration in prolonged exercise is consistent in showing a decrease in activities lasting longer than 2 hr. Mourtzakis, Saltin, Graham, and Pilegaard, (2006) had six men cycle at 44% of VO_2max to exhaustion and saw an initial rise in plasma glutamine concentrations during the first 2 hr. After 3 hr of exercise glutamine concentrations returned to baseline levels and continued to decrease at the point of exhaustion (3 hr 23 min ± 11 min) with a continued decrease during the 3 hr of recovery. Parry-Billings and colleagues (1992) demonstrated that following a marathon, there was a significant decrease in plasma glutamine concentrations in 24 endurance trained runners.

Hydration and Exercise

During prolonged exercise, even in a thermoneutral environment, there is a need for fluid and electrolyte ingestion to decrease the effects of dehydration (Coyle, 2004). During endurance events dehydration can impact endurance performance with studies showing that weight loss as little as 2 to 3% of body weight can impair performance (Coyle, 2004; Goulet, 2013; Montain,

2008). Dehydration plays a role in the cardiovascular strain during endurance activities, with research showing that for every 1% decrease in body weight, there is an increase in heart rate of 5 to 8 beats·min^{-1} (Cheuvront, 2003; Cheuvront and Haymes, 2001a, 2001b; Coyle and Montain, 1992a,1992b; Sawka et al., 2007). The loss of fluid causes a decrease in blood volume which decreases stroke volume, which can decrease oxygen delivery to the working muscles (Coyle, 2004). In addition to the cardiovascular impact from fluid loss, electrolytes are lost from the plasma, with sodium being the most abundant electrolyte lost in sweat (Maughan, 2000). The sodium and potassium balance is another factor that can lead to fatigue in endurance athletes (Cairns & Lindinger, 2008). The addition of an L-alanyl-L-glutamine supplement could potentially increase fluid and electrolyte absorption, possibly enhancing performance.

Electrolytes during Exercise

Plasma electrolytes play an important role in cellular homeostasis (Bangsbo, Gunnarsson, Wendell, Nybo, & Thomssan, 2009; Nordborg et al., 2008;). In the resting muscle cell, extracellular sodium levels are higher than intracellular levels with the opposite being true for potassium, intracellular levels are higher than extracellular levels (McArdle, Katch, & Katch, 2010). During exercise, venous plasma potassium levels increase with the intensity of exercise (Medbo & Sejersted, 1990). The increased extracellular potassium concentration during exercise can be explained by the increased electrical activity in the exercising muscle (Medbo & Sejersted, 1990). This increased extracellular potassium may cause fatigue during exercise due to impaired membrane excitability (Fitts, 1994).

Extracellular sodium concentration may increase at the onset of endurance exercise (due to hemoconcentration) and then remain constant during moderate duration exercise depending on exercise intensity and fluid loss (Cairns & Lindinger, 2008; Fortney, Vroman, Beckett, Permutt, & LaFrance, 1988). During prolonged endurance exercise, especially in a warm environment with heavy sweat loss, plasma sodium concentration may eventually decrease (Coyle, 2004). There appears to be a large safety margin when looking at the peak force and extracellular sodium relationship (Cairns & Lindinger, 2008). A decrease in extracellular sodium concentration of 50% results in a 10 to 15% decrease in peak tetanic force production (Cairns, Buller, Loiselle, & Renaud 2003; Jones & Bigland-Ritchie, 1986; Overgaard, Nielsen, & Clausen, 1997). A decrease in extracellular sodium alone is not likely to cause muscular fatigue, although there is a possibility of an impact on the action potential, with smaller action potentials seen, skipping or propagation failure, which would leave some fibers unexcitable (Bezanilla, Caputo, Gonzalez-Serratos, & Venosa, 1972; Cairns et al., 2003; Duty & Allen, 1994). The interaction of sodium and potassium on muscular fatigue appear to be additive (Cairns, 2005; Cairns & Dulhunty, 1995). Moderately raised extracellular potassium with lowered sodium can reduce peak tetanic force by as much as 67% in mouse muscle, demonstrating the synergistic impairment on force production (Cairns & Lindinger, 2008).

Fluid Ingestion and Plasma Electrolytes

Several investigations have been conducted examining fluid ingestion, plasma electrolytes, and exercise performance in events lasting approximately 1hr. Fallowfield, Williams, Booth, Choo, and Growns (1996) examined whether water ingestion during a treadmill

run to exhaustion at 70% of VO_2max (in a thermoneutral environment, 20°C) can limit dehydration and improve endurance capacity when compared to no fluid ingestion. Plasma electrolyte responses were similar between the trials with plasma potassium increasing a significant 21% ($p < 0.01$) (4.00 ± 0.18 to 4.83 ± 0.15 mM) during the no fluid replacement trial and a significant 23% ($p < 0.01$) (4.08 ± 0.10 to 5.00 ± 0.32 mM) during the fluid replacement trial. There was no difference between the trials. Plasma sodium concentrations were not different from pre to post for the individual trials or between the trials. During the no fluid trial the plasma sodium went from 140 ± 0.7 to 142 ± 0.8 mM and during the fluid replacement trial went from 139 ± 0.7 to 142 ± 1.3 mM. There was a significant difference ($p < 0.01$) in the mean run time (no fluid trial = 77.7 ± 7.7 min fluid replacement trial = 103.0 ± 12.4 min).

In research examining endurance capacity, Snell and colleagues had runners complete a 60 min run at 70 to 75% of VO_2max followed by 60 min of rest, perform a run to exhaustion lasting 7 to 10 min, rest for 60 more min during which time fluid was ingested to replace the amount of fluid lost during the 60 min run and then perform one final run to exhaustion. The rehydration drinks were an artificially-flavored placebo, a commercial sports drink, and a rehydration electrolyte drink with glutamine. The results revealed that the run to exhaustion following ingestion of the rehydration electrolyte solution was significantly better than the other two drinks, nearing the baseline performance in a euhydrated state. Unfortunately, plasma electrolytes, glucose, or glutamine were not measured (or reported) in this investigation.

In a study designed to examine cycling performance at 85% of VO_2max while ingesting three different fluids (non-electrolyte placebo, carbohydrate drink with electrolytes, and placebo with electrolytes), Powers and colleagues (1990) found that there was no difference in plasma

sodium or potassium between the trials. Although no specific data was presented, interpreting the data from the figures, plasma sodium increased from approximately 142 $mEq \cdot L^{-1}$ at rest to approximately 145 $mEq \cdot L^{-1}$ throughout the exercise bout. Plasma potassium increased across all trials from an initial concentration of approximately 3.9 $mEq \cdot L^{-1}$ at rest to approximately 5.0 $mEq \cdot L^{-1}$. Robinson et al. (1995) conducted an investigation to examine whether an attempt to replace a large amount of fluid loss during intense exercise improves performance in moderate temperatures. Eight cyclists rode as far as possible in 1 hr either with no fluid or an artificially sweetened water. There were no differences in the increases in plasma potassium concentrations between trials. During the first 5 min of exercise the increases in plasma sodium concentrations were the same. From the 5 min mark on, there was a significant difference ($p < 0.005$) in plasma sodium concentrations between trials, with the fluid replacement trial remaining lower than the no fluid trial at all time points. Plasma osmolality followed a similar pattern as sodium. There were similar initial increases in plasma osmolality during the first 5 min. From the 20 min point on, there was a trend toward a difference ($p = 0.054$) between trials, with the fluid replacement trial lower than the no fluid trial.

McConell, Stephens, and Canny (1999) investigated whether fluid ingestion volume had an impact on heart rate, plasma osmolality, plasma electrolytes, and performance during an intense endurance exercise in a moderate environment (21°C). Eight cyclists/triathletes completed three trials in which they rode for 45 min at 80% of VO_2max then completed a 15 min performance trial during which they were instructed to complete as much work as possible. The three trials consisted of no fluid, a trial where they consumed enough fluid (water) to replace 50% of the fluid lost, and a trial in which they consumed enough fluid (water) to replace 100%

of the fluid lost. There were no differences in the work performed during the three trials. Plasma sodium was not different across the trials during the first 30 min, but tended ($p = 0.07$) to be higher in the no fluid trial late in the exercise. Plasma potassium was not different across the trials, increasing in a similar fashion.

It appears that during endurance exercise lasting approximately 1 hr, plasma potassium concentrations increase regardless of fluid ingestion. The pattern of plasma sodium concentration changes appear to differ in that regardless of fluid ingestion, there is an initial increase in extracellular sodium concentration. Following the initial rise in sodium concentration, during fluid ingestion trials sodium appears to remain constant. With no fluid ingestion the data is equivocal, some investigations reporting an increase in sodium concentration while others report a constant sodium concentration.

Methods to Enhance Hydration

There are a number of methods that have been proposed to enhance rehydration during exercise, including ingestion of glycerol, betaine, and alanine-glutamine. A number of investigations have been conducted with glycerol to examine fluid retention and thermoregulation. The data appear to be equivocal with several studies showing an improvement in performance with glycerol ingestion (Dini, Corbianco, Rossi, & Lucacchini, 2007; Hitchins et al., 1999; Montner, et al., 1996; Ohkuwa, Miyamura, Andou, & Utsuno, 1988), while others reporting no improvement in performance (Inder, Swanney, Donald, Prickett, & Hellemans, 1998; Magal et al., 2003; Marino, Kay, & Cannon, 2003; Murray, Eddy, Paul, Seifert, & Halaby, 1991). Dini and colleagues (2007) examined glycerol ingestion in competitive rowers and

found an approximate 37 m improvement in performance. In cycling trials, Montner et al. (1996) and Hitchins et al. (1999) showed improved performance with glycerol ingestion, although in the Hitchins' study, the improvement in performance (higher power output) was attributed to a lower perception of effort. In a running trial, Ohkuwa and colleagues (1988) found significant improvements in performance following glycerol ingestion. In contrast, Inder et al. (1998) showed no improvement in performance when glycerol was provided to triathletes. In a study examining glycerol ingestion during tennis performance, Magal et al. (2003) found an improvement in hydration status and expanded plasma volume from the glycerol ingestion compared to water only but no improvement in tennis-related performance. In addition, Murray and colleagues (1991) found no significant improvement in cardiovascular or thermoregulatory responses during 90 min of cycling at 50% of VO_2max even though glycerol ingestion attenuated the normal decrease in plasma volume when compared to a water placebo and carbohydrate electrolyte drink.

Betaine is an organic osmolyte and is thought to protect cells under stress including hydration stress. Armstrong and colleagues (2008) showed a non-significant 16% improvement in a run to exhaustion at 84% of VO_2max following betaine supplementation. Millard-Stafford et al. (2005) examined eight trained cyclists who cycled for 120 min in a warm environment which was followed by a 15 min time trial. In addition, participants were also required to perform an isometric knee extensor test. Participants hydrated with either a 6% carbohydrate solution, 7% carbohydrate solution with betaine, or a placebo. There were no time trial performance differences between the treatments, but during the betaine trial, participants were reported to have significantly higher isometric knee extensor strength.

There have been few studies examining endurance performance with alanine-glutamine supplementation. During a cycling trial to exhaustion, Hoffman and colleagues (2010) examined 10 physically active males cycling at 75% of VO_2max to exhaustion following a dehydration protocol. Participants were dehydrated to -2.5% of body weight at the beginning of the protocol and then rehydrated to -1.5% of body weight prior to the cycling trial. Exercise performance (time to exhaustion) was significantly lower during the no hydration trial when compared with the rehydration trials when provided the alanine-glutamine dipeptide, but not different between the water and dipeptide trials. The authors concluded that supplementing with alanine-glutamine provided a significant increase in time to exhaustion (during a mild hydration stress) and the effect was likely due to enhanced fluid and electrolyte uptake. In research where glutamine levels were not measured, Favano and colleagues (2008) demonstrated in nine male soccer athletes a significant increase in distance covered (22% increase) in treadmill activity simulating soccer activities following ingestion of a carbohydrate-glutamine peptide drink. The soccer athletes had lower Borg scale feelings of perceived exertion during the first two batteries (of three) of the trials. The trials were comprised of 3 x 25 min batteries of various exercises simulating a soccer match.

Neuromuscular Fatigue and Economy

Neuromuscular fatigue is defined as the inability to produce or maintain maximal voluntary force (Kent-Braun, 2009). Neuromuscular fatigue is a complex phenomenon in which there is an interruption of the muscle activation signal somewhere between the brain and muscle. The interruption could be central in nature, with a disruption in recruitment or rate coding of the

signal. It could be in the peripheral nervous system, in which conduction velocity, membrane excitability, or the neuromuscular junction is affected. Or the interruption could be in the muscle where the action potential is disrupted at some point affecting the contractile function of the muscle. The disruption could be in the excitation-contraction coupling, caused by a change in the calcium concentration in the muscle, or caused by a change in the cross-bridge function of the muscle (Kent-Braun, 2009).

As described by Cadore et al. (2011b), neuromuscular economy can be defined as the lower muscle activation represented by a lower EMG signal amplitude that is necessary to move the same absolute load. The load could be weight on a leg extension machine, resistance on an isokinetic machine, the resistance (in wattage) on a cycle ergometer, or the speed on a treadmill. The person with the lower signal amplitude would be considered more economical at the same load (resistance).

Neuromuscular economy can be measured utilizing both a strength measure (e.g., an isokinetic machine or leg extension machine) and an endurance measure (e.g., running on a treadmill or cycling on a cycle ergometer). To measure neuromuscular economy using an endurance measure, participants would cycle at the same wattage or run at the same speed with EMG being measured. The more economical cyclist would have a lower EMG signal. Cadore and colleagues (2011b) explained that the more economical cyclist may be recruiting fewer motor units to perform the set workload, resulting in a lower EMG signal. Also, the more economical cyclist would rely more on type I motor units, which have a lower activation threshold, leading to the lower EMG signal (Cadore et al., 2011a). Heise, Morgan, Hough, and Craib (1996) explained that more economical runners may rely more on the use of a bi-articular

muscle (rectus femoris) to contribute to the dual function of hip flexion and knee extension in contrast to using mono-articular knee extension. The decrease in electrical activity in the quadriceps muscle during aerobic activity indicates that fewer motor units were recruited for the same load, suggesting that economy of movement can occur (Cardore et al., 2011a).

Hanon, Thépaut-Mathieu, and Vandewalle (2005) examined the onset of fatigue in the major muscles involved in running. The authors had nine well-trained male runners (VO_2max = 76.1 ± 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$) run on a motorized treadmill utilizing a discontinuous protocol of 4 min stages of increasing speed to exhaustion. The runners were outfitted with surface electromyography to record the EMG signals for the vastus lateralis, rectus femoris, biceps femoris, tibialis anterior, gastrocnemius, and gluteus maximus. The major finding from this study was that during running, the hip-mobilizing muscles (rectus femoris and biceps femoris) become fatigued earlier than the other lower limb muscles analyzed in this study.

In a study designed to evaluate the changes in leg-spring behavior and the associated modifications in the lower limb muscular activity utilizing EMG, Rabita and colleagues (2013) had 12 trained runners (VO_2max = 60 ± 6.4 $ml \cdot kg^{-1} \cdot min^{-1}$) run at their VO_2max velocity to exhaustion. The authors recorded the EMG signals for eight leg muscles of the right leg, including the soleus, gastrocnemius medialis, gastrocnemius lateralis, tibialis anterior, vastus medialis, vastus lateralis, rectus femoris, and biceps femoris. The results indicate that the plantarflexors were more affected by the run than the knee extensors, and the biarticular muscles, rectus femoris and biceps femoris seem to play an important role in order to maintain the preset velocity during the latter part of an exhaustive, high velocity run.

Zuniga and Malek (2013) examined the individual patterns of response of the three superficial thigh muscles (vastus lateralis, rectus femoris, and vastus medialis) utilizing EMG in a treadmill running test of increasing speed. The authors had nine physically active men run one min continuous stages on a motorized treadmill until voluntary exhaustion. The authors averaged the last three complete EMG bursts during the stance phase during the last 10 s of each stage to get their EMG amplitude values. The authors found that the patterns of response of the EMG signal amplitude across running velocities were consistent for all three quadriceps muscles. They further concluded that the normalized EMG amplitude did increase as running velocity increased, but this was independent of muscle group.

No published studies are known that examined the effect of adding the alanine-glutamine dipeptide to a low-calorie sports drink during an endurance event in euhydrated participants. This research will examine serum electrolyte concentrations, blood glucose levels, EMG economy, and running performance in euhydrated endurance-trained males. The outcomes of this study will contribute to the scholarly knowledge in the exercise science community. In particular, does a dipeptide enhance absorption of glucose and electrolytes during a 1 hr run at 75% of VO_2max and if so, does that improve running performance and muscle recruitment activity during a subsequent run to exhaustion at 90% of VO_2peak in endurance-trained male runners.

CHAPTER III

Methods

Research Design

The research design of this study was a double-blind, randomized, placebo-controlled, cross-over study. Participants were asked to report to the Human Performance Lab (HPL) on six separate occasions. The first two visits were preliminary visits (PV1 and PV2) followed by four experimental trial visits (T1 – T4). During PV1, participants completed the Confidential Medical and Activity questionnaire, Physical Activity Readiness Questionnaire (PAR-Q), and informed consent form and any questions were addressed. Prior to PV2, participants completed a 24 hr food log, which was considered their pre-testing diet, and participants were asked to replicate this diet prior to all experimental trials.

During PV2 participants were weighed and asked to provide a urine sample to measure baseline euhydration levels. Participants were provided with a specimen cup to use for urine collection. Each sample was analyzed for osmolality (U_{osm}) and specific gravity (U_{sg}). U_{osm} was measured by freezing point depression (Model 3320 MicroSample Osmometer, Advanced Instruments, Inc., Norwood, MA) and U_{sg} by refractometry (Human Urine Refractometer, MISCO Refractometer, Cleveland, OH). These measures were used to document euhydration on all testing days. Participants were considered euhydrated if $U_{sg} \leq 1.020$. During PV2 participants also performed a lactate threshold (LT) and VO_2peak test to determine the treadmill speed for T1 – T4.

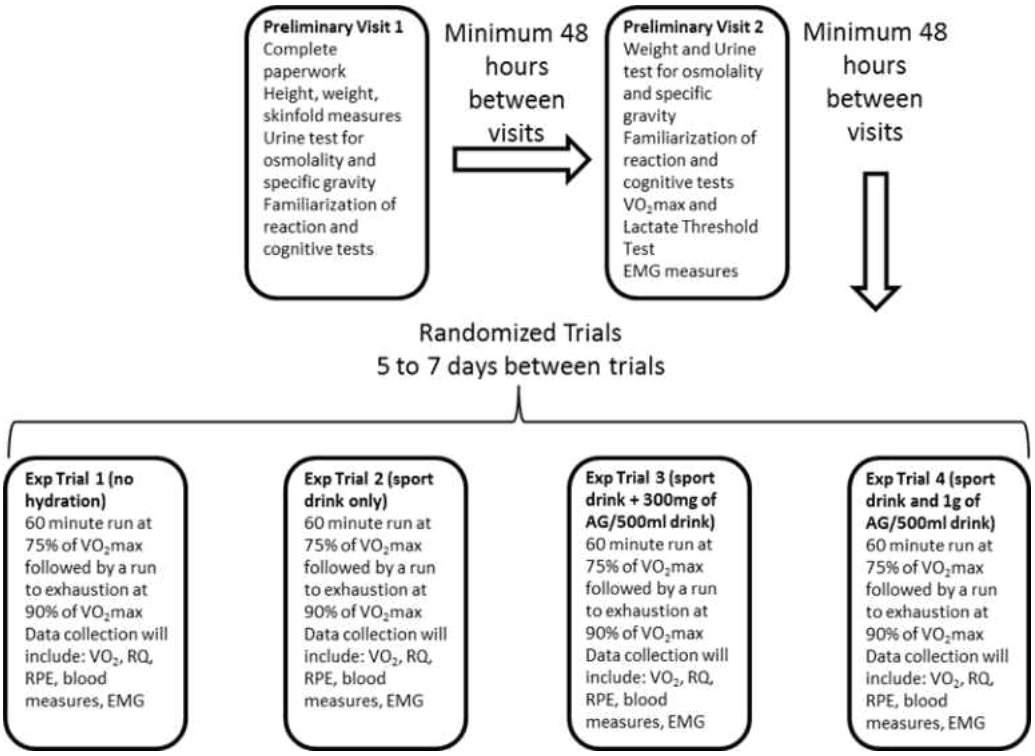

Figure 1. Study Protocol

Data collection for T1 – T4 occurred on four separate occasions separated by a minimum of 7 days with a mean of 8.4 ± 3.3 days (Figure 1). Prior to each experimental trial, participants were weighed in running shorts. During each experimental trial, participants completed a 60 min run at 75% of their previously measured VO_2peak. Following the 60 min run, participants were towel dried, put on dry running shorts, and were weighed to measure sweat loss. The participants then completed a run to exhaustion with treadmill speed adjusted to produce 90% of their VO_2peak. T1 was performed without any rehydration (NHY). The fluid loss during this session was used to determine the participant's sweat rate ($L \cdot hr^{-1}$). To continue in the study, the participant's sweat rate needed to be or exceed 1.3 $L \cdot hr^{-1}$. All participants met or exceeded 1.3 $L \cdot hr^{-1}$ of sweat loss, with a mean sweat loss of 1.68 ± 0.22 $L \cdot hr^{-1}$. During T2, T3, and T4, participants were provided 250 ml of sport drink every 15 min. The sport drink was a

commercial product containing 21 calories, 4.9 g of carbohydrate, 113 mg of sodium, and 32 mg of potassium per 250 ml (Gatorade G2, PepsiCo, Purchase, NY). During one of these trials, participants consumed only the sport drink (ET), while during the other trials participants consumed the alanine-glutamine supplement (Sustamine™) mixed in the same flavor sport drink at either a low (300 mg·500 ml^{-1}) or high dose (1 g·500 ml^{-1}) (LD and HD, respectively). Trials T2, T3, and T4 were performed in a randomized order. A laboratory worker not involved in the investigation mixed the drinks to ensure a double-blind protocol was maintained. Participants performed a maximal effort isometric contraction of the knee extensor muscles prior to T1 through T4 utilizing an isokinetic machine (System 4 Pro, BIODEX Medical Systems, Inc., Shirley, NY). During the maximal effort isometric contraction, electromyography (EMG) root mean square (RMS) amplitude values were recorded. During each experimental trial, the EMG RMS signal was recorded for 2 min every 10 min and throughout the run to exhaustion portion of the trials. The values obtained every 10 min and throughout the run to exhaustion were calculated and presented as a percent of maximum EMG RMS signal.

Participants

Twelve male endurance-trained athletes (23.5 ± 3.7 yrs; 175.5 ± 5.4 cm; 70.7 ± 7.6 kg) volunteered for the study. All participants were recruited via word of mouth or flyer advertisement throughout the university and local running community. All participants were free of any physical limitations as determined by the Confidential Medical and Activity questionnaire and PAR-Q. University Institutional Review Board approved all experimental protocols and signed informed consent was obtained from each participant.

VO_2max and Lactate Threshold Testing

During PV2, participants performed a LT and VO_2peak test. The LT testing protocol consisted of six to eight discontinuous 5 min stages of increasing speed beginning at approximately 40 to 50 $m \cdot min^{-1}$ below estimated 10K racing speed. Expired gases were analyzed with a metabolic cart (TrueOne, ParvoMedics, Sandy, UT) for oxygen consumption and carbon dioxide production. Prior to the test, the participants estimated their 10 km race pace and this time was converted to a running pace in $m \cdot min^{-1}$. During the run, participants reached their estimated 10 km running pace in the fourth or fifth stage. Running velocity was increased by 10 $m \cdot min^{-1}$ each stage. At the completion of each stage, participants straddled the treadmill belt and a finger prick (Tenderlett Finger Incision Device, ITC, Edison, NJ) was utilized to collect 50 μL of blood to test for blood lactate via automated analysis (Analox GM7 Enzymatic Metabolite Analyzer, Analox Instruments USA, Lunenburg, MA). Blood lactate levels were plotted against running speed with LT being defined as a minimum increase of 1.0 $mmol \cdot L^{-1}$ above baseline followed by another increase greater than 1.0 $mmol \cdot L^{-1}$ (Coyle et al., 1983). The treadmill speed was increased 10 $m \cdot min^{-1}$ until a clear LT was established. Following the final stage of the LT test, participants were allowed a 10 min rest period. Once the participant was back on the treadmill, the treadmill speed was set at the estimated 10 km racing speed. Treadmill speed was increased 10 $m \cdot min^{-1}$ every min until the participant could no longer continue. The highest VO_2 measure averaged over one min was considered VO_2peak. This measure was used to determine running velocity for the 60 min run (75% of VO_2peak) and the run to exhaustion (90% of VO_2peak).

Electromyography Testing

EMG measures were recorded during T1 – T4 to measure muscle activity of the right vastus lateralis and rectus femoris. Prior to all experimental trials, a maximum isometric leg extensor measurement was made with an isokinetic machine (System 4 Pro, BIODEX Medical Systems, Inc., Shirley, NY). Participants performed three trials of 6 sec in duration with the highest value recorded as their maximum value. Lab personnel provided verbal encouragement throughout each trial. Participants were provided a 3 min rest between trials. Participants were seated and securely strapped into the isokinetic machine with a hip angle of 90° and lower leg extended 110°. All participant positioning measurements were recorded and repeated for each subsequent trial.

To measure the EMG activity, a bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick Prep Electrodes, Cardiology Shop, Boston, MA) arrangement was placed over the right vastus lateralis (VL) and rectus femoris muscles (RF). Electrodes over the VL were placed two-thirds of the distance between the anterior, superior iliac spine (ASIS) and the lateral patella, and 5 cm lateral to this line with the participant in a standing position. Electrodes over the RF muscle were positioned halfway between the inguinal fold and the patella, with the participant's hip and knee flexed 90°. The RF muscle was palpated to ensure placement over the belly of the muscle. A ground electrode was placed over the ASIS. All electrode measurements were recorded and repeated for each trial. To ensure proper signal conductance, skin around the marked areas was shaved, rubbed with alcohol, and once the electrode was placed on the skin, the center of the electrode was spun causing a final abrasion of the skin. A 2-channel wireless EMG transmitter (BIONOMADIX Dual-channel Wireless EMG Transmitter, BIOPAC Systems,

Inc., Santa Barbara, CA) was used to transmit the EMG information to the receiver/amplifier (MP150 BIOPAC Systems, Inc., Santa Barbara, CA). The transmitter was strapped to the thigh approximately 3 cm above the top electrode. The electrodes and transmitter wires were wrapped with cohesive bandage (Fisherbrand Cohesive Wrap Bandage, Thermo Fisher Scientific, Inc., Waltham, MA) to prevent wire slap and electrode movement.

During the experimental trials, EMG signals were recorded for the final 2 min of each 10 min period during the 60 min run and continuously during the run to exhaustion portion of the trial. All EMG signals were expressed as RMS amplitude values (μVrms) by software (AcqKnowledge v4.2, BIOPAC Systems, Inc., Santa Barbara, CA). The RMS values were reported as the percentage of maximal leg extension value for that day.

The EMG RMS value from each maximal isometric contraction was analyzed using methods described by Cadore, et al. (2010). The middle four seconds of the 6-second signal were visually scanned for the maximum signal. The one second surrounding this peak was averaged and used as the maximum RMS signal for that day. During the 1 hr run, the average RMS value of the middle one minute of each two minute recording was computed and then compared to the maximum RMS value determined from the maximal leg extension trials.

Blood Measurements

During each experimental trial, baseline (BL) blood samples were obtained with additional blood samples drawn following 30, 45, and 60 min during the 60 min run. All blood samples were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a 3-way stopcock with a male luer lock adapter. Cannula placement and blood draws were

performed by personnel trained in phlebotomy. BL blood samples were drawn following a 15 min equilibration period (participant lying supine) prior to exercise. Every effort was made to test subjects on the same day of the week and same time each day during the experimental trials to eliminate any diurnal variation in performance.

Blood samples were drawn into sodium heparinized treated tubes. Blood samples were analyzed in triplicate for hematocrit and hemoglobin via microcapillary technique. The remaining whole blood was centrifuged for 10 min at 3000 g at 4ºC. Resulting plasma and serum was aliquoted and immediately analyzed for glucose, lactate, sodium, potassium, and osmolality. All plasma measures were performed in duplicate. The remaining plasma was stored at -80°C for future analysis of glutamine. Plasma concentrations of glucose and lactate were measured in duplicate via automated analyzer (Analox GM7 Enzymatic Metabolite Analyzer, Analox Instruments USA, Lunenburg, MA). Plasma glutamine concentrations were determined via assay (Glutamine Assay Kit, Abnova Corporation, Taiwan). Plasma sodium and potassium concentrations were determined via ion-selective electrodes (EasyLyte, Medica Corporation, Bedford, MA). Plasma osmolality was measured by freezing point depressions (Model 3320; Micro-Sample Osmometer, Advanced Instruments, Inc., Norwood, MA). Samples that were frozen were thawed only once.

Statistical Analysis

All data are reported as mean ± standard deviation. All data were analyzed utilizing a two-way (time x treatment) repeated measures analysis of variance (ANOVA). When the analysis produced a significant interaction, follow-up analysis was via one-way ANOVA

comparison among treatments and if significant, then a LSD test was used for post hoc comparison. When a significant main effect for time was found, then follow-up repeated measures ANOVA with LSD post hoc was used to examine each treatment for time effect. An alpha level of $p < 0.05$ was used to determine statistical significance. All statistical analyses were conducted utilizing the Statistical Package for Social Science (SPSS) software for Windows version 20 (IBM Corp., 2012).

CHAPTER IV

Results

Running Performance

The temperature and relative humidity for all trials was consistent (22.92 ± 0.28 °C and 44.19 ± 1.33%). All participants completed each trial and each participant consumed all 250ml of fluid every 15 min (total = 1L) during the fluid replacement trials. A significant difference [$F_{(3,33)} = 50.09$, $p < 0.001$] in body mass loss occurred during the 1 hr run (Figure 2).

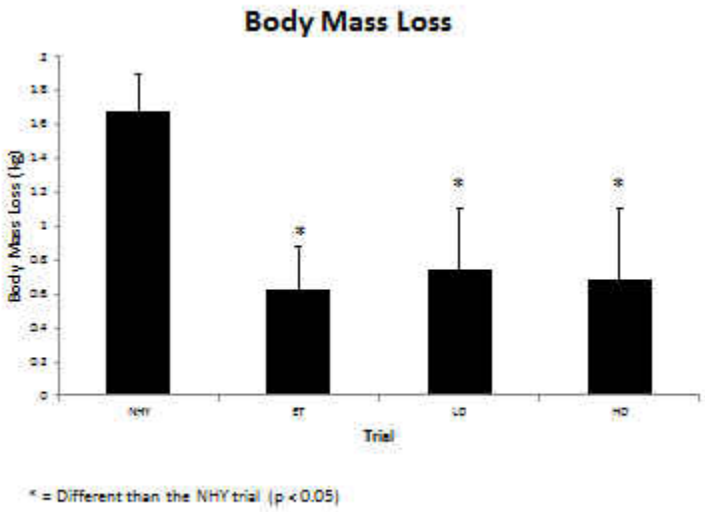

Figure 2. Body mass loss during 1 hr run.

Post-hoc analysis showed a significant difference between the NHY trial (1.68 ± 0.23 kg; 2.4 ± 0.36% of body weight (BW)) and ET (0.63 ± 0.26 kg; 0.9 ± 0.35% BW, $p < 0.001$), LD (0.74 ± 0.39 kg; 1.1 ± 0.55%, $p < 0.001$), and HD trials (0.68 ± 0.44 kg; 1.0 ± 0.62%, $p < 0.001$).

A significant difference [$F_{(3,33)} = 3.22$, $p = 0.035$] was observed in run-to-exhaustion performance at 90% of VO_2peak following a 1 hr run at 75% of VO_2peak (Figure 3). Post hoc analysis revealed a significant difference between the NHY trial (368.33 ± 197.92 sec) and the LD trial (528.67 ± 196.76 sec, $p = 0.025$) and HD trials (562.17 ± 293.11 sec. $p = 0.023$). Although a trend was seen between NHY and ET (499.00 ± 161.53 sec. $p = 0.086$) trial, no other significant differences were observed.

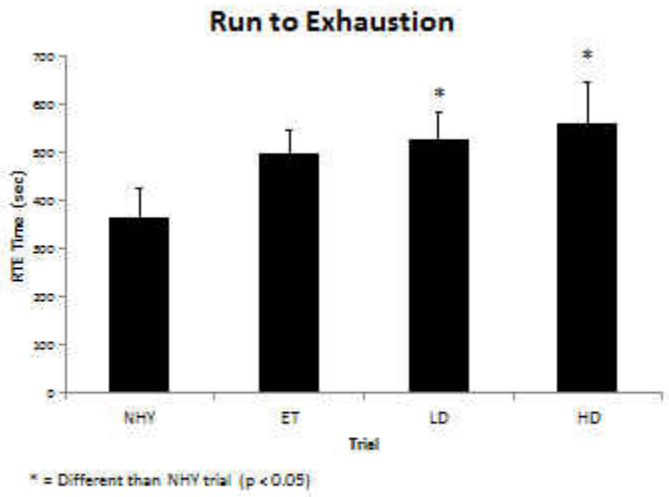

Figure 3. Run to exhaustion performance.

Metabolic Measurements

The mean VO_2peak for the participants was 55.94 ± 5.92 ml·kg^{-1}·min^{-1}. The mean percent of VO_{2peak} utilized across the 1 hr run for all trials was 74.0 ± 3.1%. The mean percent of VO_{2peak} utilized during the RTE portion of all trials was 89.0 ± 5.8%. There was no significant effects for time [$F_{(3,30)} = 2.84$, $p = 0.055$], treatment [$F_{(3,30)} = 2.61$, $p = 0.070$], or time x treatment interaction [$F_{(9,90)} = 0.99$, $p = 0.457$] for VO_2 (ml·kg^{-1}·min^{-1}) measured every 15 min during the 1 hr run (Figure 4).

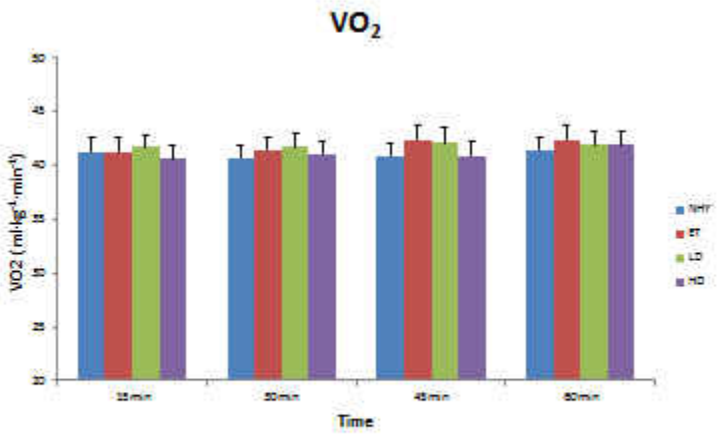

Figure 4. VO_2 during 1 hr run.

Comparison of changes in RQ between trials can be observed in Figure 5. There was a significant main effect for time for RQ across the 1 hr run [$F_{(3,30)} = 3.12$, $p = 0.041$]. Analysis of the time means (collapsed across treatments) revealed significant differences between the 15 min and 60 min RQ ($p = 0.014$) and between the 45 min and 60 min RQ ($p = 0.045$). Follow-up analysis of time differences for each treatment showed that during the LD trial the 15 min RQ was significantly higher than the 60 min RQ ($p = 0.049$) and during the ET trial the 15 min RQ was significantly higher than the 60 min RQ ($p = 0.009$). There was no significant effect for treatment [$F_{(3,30)} = 2.02$, $p = 0.132$] nor any time x treatment interaction for RQ [$F_{(9,90)} = 1.55$, $p = 0.145$].

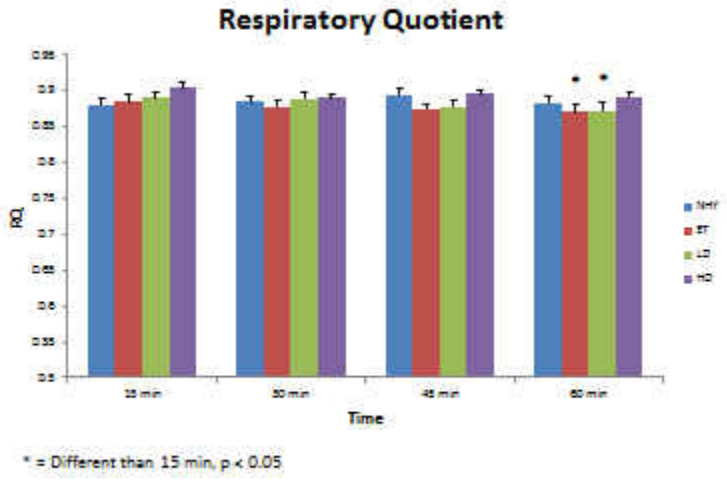

Figure 5. Respiratory Quotient during 1 hr run.

Comparisons of changes in heart rate between trials are depicted in Figure 6. There was a significant main effect for time [$F_{(1.44,14.39)} = 18.34$, $p < 0.001$], a significant main effect for treatment [$F_{(3,30)} = 3.95$, $p = 0.017$], and a significant time x treatment interaction [$F_{(3.16,31.58)} = 3.00$, $p = 0.043$]. Significant differences were noted between heart rate at 15 min, and the heart rate at all other time points for all trials (30 min, $p < 0.001$; 45 min, $p = 0.001$; 60 min, $p = 0.001$). In addition, during the LD trial the heart rate at 60 min was significantly higher than at 30 min ($p = 0.05$). During the ET and HD trials, heart rates at 30, 45 and 60 min were significantly lower than the heart rates seen during NHY ($p < 0.05$).

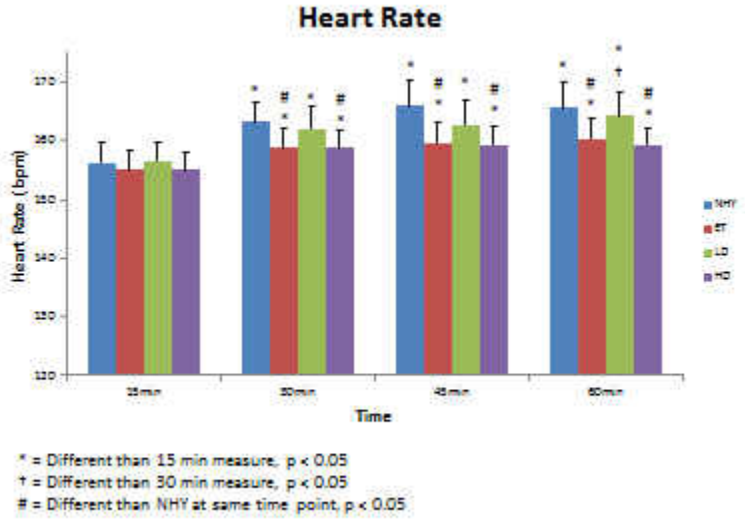

Figure 6. Heart rate during 1 hr run.

Blood and Plasma Analysis

Changes in plasma glutamine are shown in Figure 7. A significant increase in plasma glutamine concentration was observed between PRE and 45 min for both LD ($p = 0.003$) and HD ($p = 0.017$). At 60 min, during the HD trial, the plasma concentration of glutamine remained significantly higher than the PRE value ($p = 0.05$). In addition, significant differences were also noted between 30 min and 45 min during LD ($p = 0.013$), and between the 30 and 60 min measures during the HD trial ($p = 0.006$).

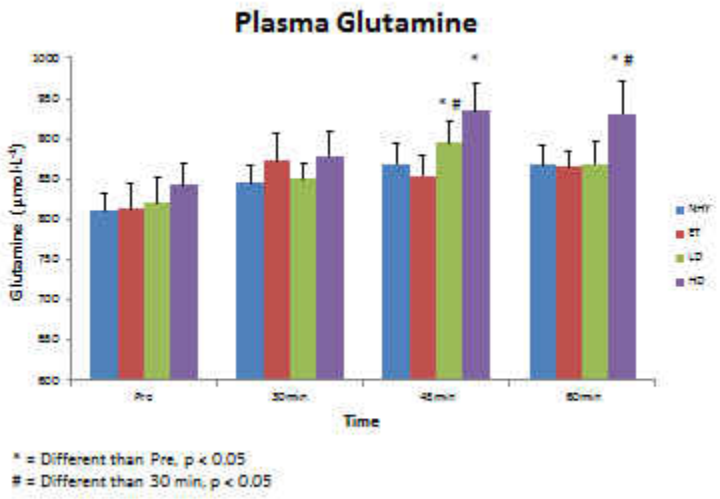

Figure 7. Plasma glutamine changes during 1 hr run.

Changes in plasma sodium and potassium concentrations can be observed in Figures 8 and 9, respectively. There was a significant time x treatment interaction [$F_{(9,72)} = 3.98$, $p < 0.001$], significant main effect for time [$F(3,24) = 91.81$, $p < 0.001$], and a significant main effect for treatment [$F(3,24) = 5.17$, $p = 0.007$] for sodium across the 1 hr run. Plasma sodium concentrations were significantly elevated from PRE to 30, 45, and 60 min for all trials ($p < 0.05$). During the NHY trial, plasma sodium concentrations increased significantly across each time point ($p < 0.05$). Plasma sodium concentrations during NHY were significantly greater than those seen during ET and HD at 45 min ($p < 0.05$). In addition, plasma sodium concentrations at 60 min were significantly greater during NHY compared to all other trials, while plasma sodium concentrations during LD were significantly greater than ET and HD ($p < 0.05$).

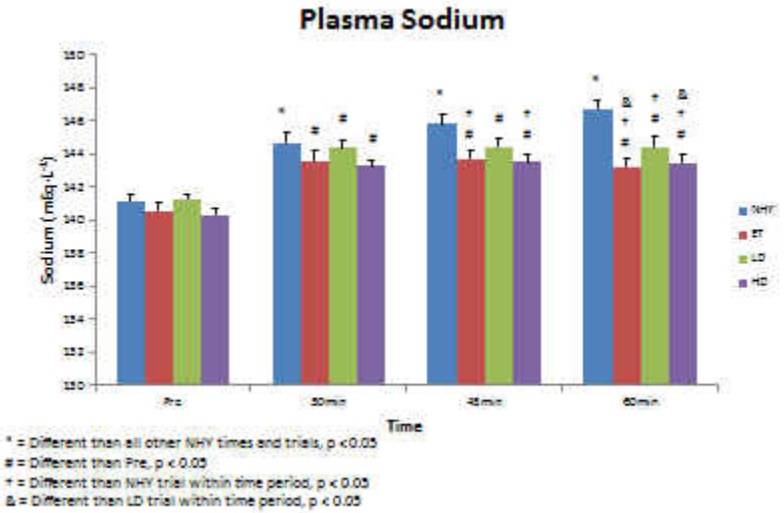

Figure 8. Plasma sodium during 1 hr run.

A significant main effect for time was observed for plasma potassium concentrations [$F_{(1.84,14.74)} = 98.68$, $p < 0.001$]. PRE measures were significantly lower than all other time points for all trials (Figure 8). During NHY, plasma potassium concentrations at 45 and 60 min were significantly higher than seen at 30 min. During the ET trial the 60 min measure was significantly higher than the 30 min measure, while plasma potassium concentrations at 60 min during LD and HD were significantly higher than the 30 and 45 min measures.

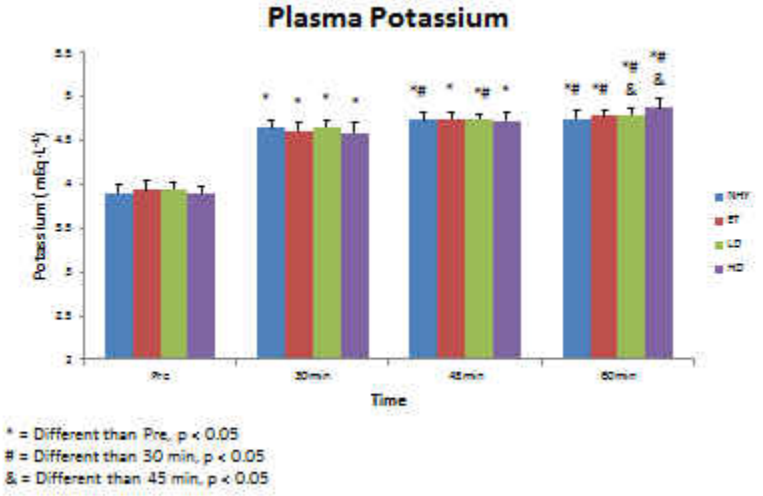

Figure 9. Plasma potassium during 1 hr run.

Plasma glucose and plasma osmolality are shown in Figures 10 and 11, respectively. A significant time x treatment interaction [$F_{(3.21,28.87)} = 3.01$, $p = 0.044$] and a significant main effect for time was seen for glucose [$F_{(1.66,14.92)} = 26.27$, $p < 0.001$]. Plasma glucose concentrations at

30, 45, and 60 min for all trials were significantly higher than the PRE measure ($p < 0.05$). Further, during the ET trial a significant decrease in plasma glucose concentrations was seen at 60 min. Plasma glucose concentrations were not significantly different between trials at any time point.

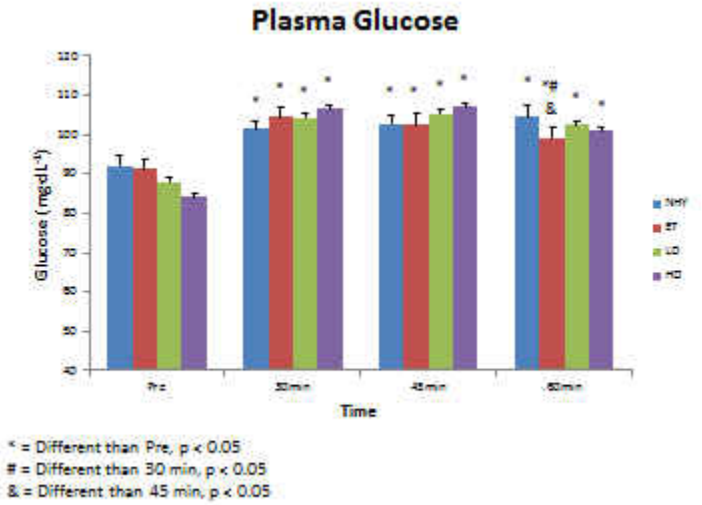

Figure 10. Plasma glucose during 1 hr run.

A significant main effect for time [$F_{(3,27)} = 61.85$, $p < 0.001$] and for treatment [$F_{(3, 27)} = 5.92$, $p = 0.003$] was observed for plasma osmolality, but no time x treatment interaction [$F_{(9,81)} = 1.37$, $p = 0.214$] were noted. When collapsed across trials, plasma osmolality was significantly greater at 30, 45, and 60 min compared to the PRE measure. In addition, plasma osmolality was significantly higher at 45 min compared to 30 min during NHY, ET, and HD, and a significantly higher plasma osmolality was noted at 60 min compared to 30 min during the NHY trial. Comparisons between treatments showed that plasma osmolality was significantly elevated at 45 min during NHY compared to ET and LD, while plasma osmolality at 60 min during the NHY trial was significantly higher than all other trials.

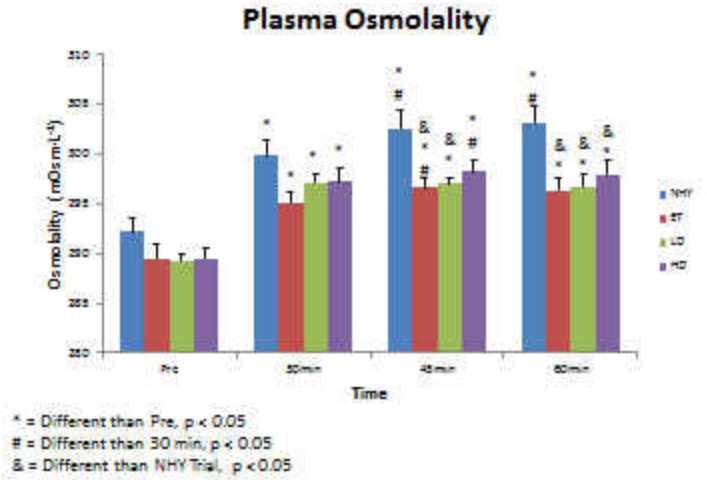

Figure 11. Plasma osmolality during 1 hr run.

Blood lactates are depicted in Figure 12. Blood lactate at 60 min was significantly higher during NHY trial than ET and HD trials (p = 0.05). No other differences were noted between trials at any time point.

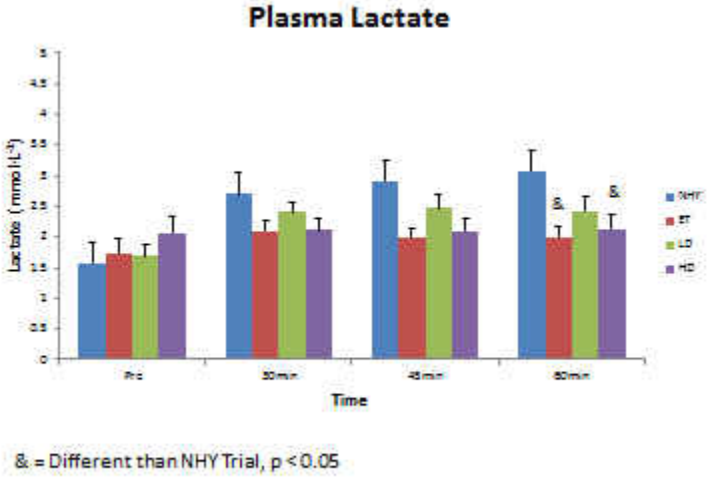

Figure 12. Blood lactate during 1 hr run.

EMG Analysis

Muscle activation of the vastus lateralis and rectus femoris during the 60 min run are depicted in Figures 13 and 14, respectively. No significant differences in muscle activation were noted between the trials in either muscle group.

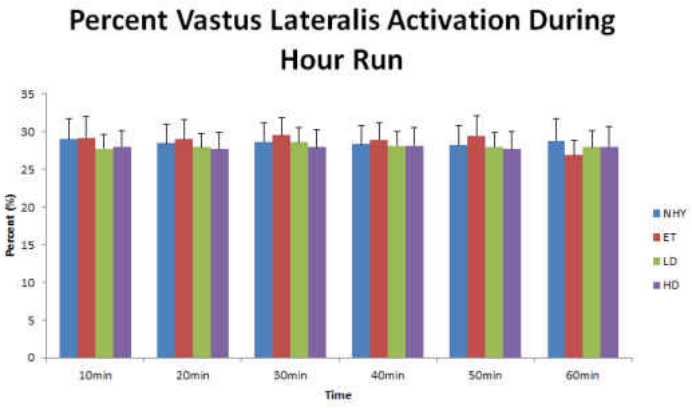

Figure 13. Average EMG percentage utilized during 1 hr run for vastus lateralis.

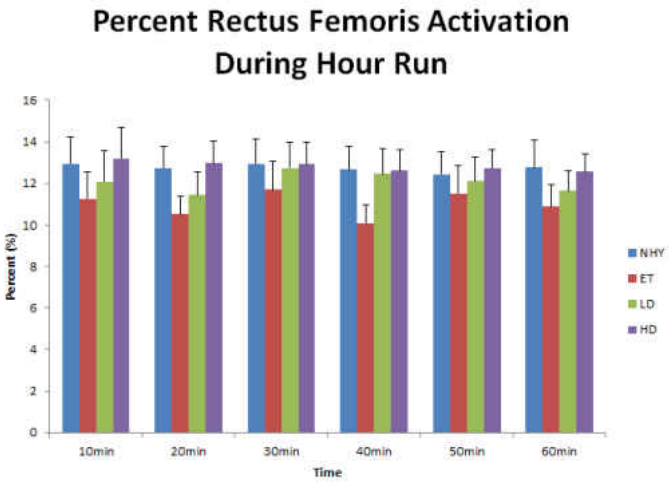

Figure 14. Average EMG percentage utilized during 1 hr run for rectus femoris.

CHAPTER V

Discussion

Ingestion of the alanine-glutamine dipeptide resulted in significant elevations in plasma glutamine that were similar to previous studies that reported glutamine appearance to occur about 30 to 50 min following ingestion (Castell and Newsholme, 1997; Harris, et al., 2012; Klassen et al., 2000). The decrease in glutamine concentration in the LD trial at 60 min suggests that the lower dose ($300mg \cdot 500ml^{-1}$) may not be sufficient to sustain plasma glutamine concentrations for the duration of an endurance event lasting longer than 45 min. However, the performance results of the present study indicated that the ingestion of either a low (LD) or high dose (HD) of the alanine-glutamine dipeptide mixed in a sport drink during a 1 hr, moderate intensity run, was able to significantly improve time to exhaustion (43.5% and 52.6%, respectively) compared to a no fluid ingestion (NHY) trial. Although ingestion of the dipeptide resulted in a 12.7% greater time to exhaustion compared to the sport drink (ET) only, this was not statistically different. These results support the previous investigation of Hoffman et al. (2010), in which ingestion of both a low and high dose of the alanine-glutamine dipeptide also resulted in significantly greater times to exhaustion compared to a no hydration trial. Similarly, no differences were seen between the water only trial and the no hydration trial. In the present study, the ET and NHY trial were not significantly different, however the 35.5% difference in time to exhaustion between those two trials trended towards a difference ($p = 0.086$). Interestingly, these results contrast from Fallowfield and colleagues (1996) who reported a significant difference in time to exhaustion while running at 70% VO_2max, between a water ingestion trial and no fluid intake trial. The difference between these studies may be related to

the training experience of the participants. The present study utilized endurance trained runners while the Fallowfield and colleagues (1996) examined recreationally active participants. It is possible that endurance trained runners are more accustomed to training with little hydration and the differences between running without taking fluid and taking water or low carbohydrate electrolyte beverage are not great enough to be detected (Coyle, 2004).

A possible explanation of the lack of any significant performance differences between the sport drink with the dipeptide (both LD and HD trials) and ingestion of the sport drink without the dipeptide (ET trial) may be related to the length or duration of the 1 hr run and its subsequent effect on plasma electrolyte concentrations. Cairns and Lindinger (2008) suggested that plasma electrolytes would have to change dramatically to affect muscle force production. Significantly greater elevations in plasma sodium concentrations were seen at 45 and 60 min during NHY compared to all fluid ingestion trials. Changes in plasma potassium concentrations did not differ between trials. Although speculative, it is possible that a greater electrolyte absorption occurring during the fluid ingestion trials stimulated a greater sodium uptake within the muscle, maintaining muscle performance (Carlsen et al., 1996).

The electrolyte response seen during the trials was similar to previous studies (Fallowfield et al., 1996; Robinson et al., 1995). Plasma sodium concentrations have been reported to continuously elevate during a 1 hr bike ride at 85% of VO_2max with no fluid replacement (Robinson et al., 1995). When fluid is provided during this duration of exercise sodium concentrations tend to increase and then plateau (Fallowfield et al., 1996; Robinson et al., 1995), similar to the response seen in the present study. During the HD trial, the sodium concentration at 60 min was significantly lower compared with the NHY and LD trials.

Hoffman and colleagues (2010) speculated that the lower sodium concentration during the glutamine trials may be indicative of a greater sodium uptake by the muscles when compared with NHY trial. Plasma potassium concentrations increased approximately 17% from PRE to 60 min. This is within the range (10% - 23%) reported by others during a similar duration and intensity of exercise (Fallowfield et al., 1996; Hoffman et al., 2010; Robinson et al., 1995). Elevations in plasma potassium may be indicative of enhanced electrical activity in the muscles (Medbo & Sejersted, 1990), enhanced mobilization of muscle glycogen (Cairns & Lindinger, 2008), or of muscle fatigue (Fink and Lüttgau, 1976; Nielsen et al., 2004). Considering the duration of exercise performed at the participant's sub-lactate threshold running pace, it is not surprising fatigue during the moderate intensity run was not a factor. This is supported by the EMG results noted in this study. No differences were seen in the percent activation of the vastus lateralis (VL) or rectus femoris muscles (RF) between trials. Differences in the percent of muscle activation between the VL and RF is similar to previous research (Hanon, Thépaut-Mathieu, & Vandewalle, 2005). The VL is typically activated to a higher degree than the RF (Hanon et al., 2005).

The physiological responses during hydration and no hydration exercise protocols were typical. Plasma osmolality increased from PRE in a similar manner to that seen in other studies (Hoffman et al., 2010). There was a treatment effect in the present study which was not seen in the work of Hoffman and colleagues (2010). As expected fluid ingestion (ET, LD and HD trials) resulted in a lowering of plasma osmolality. No effect from the alanine-glutamine dipeptide was noted in changes in plasma osmolality compared to ET. As might be expected, plasma glucose concentrations increased at the outset of exercise and then remained at a constant level during the

1 hr run. During the ET trial, plasma glucose concentrations at 60 min significantly decreased compared to the 30 and 45 min measures. In comparison, glucose concentrations in the trials in which the alanine-glutamine peptide was consumed (LD and HD) did not decrease at 60 min. It is possible that this may have been indicative of the gluconeogenic effect of alanine. In a rat model, Sumida and Donovan (1995) reported a 27% increase in gluconeogenesis from alanine following endurance training. The participants in the present study were endurance trained and therefore may have benefited from this adaptation, especially with the delivery of exogenous alanine during the LD and HD trials. Hoffman et al. (2010) reported similar results and suggested that the lack of any change in plasma glucose during the trials in which the peptide was consumed may have been related to the gluconeogenic effect of alanine, and might have contributed to the delay in fatigue by sparing muscle glycogen.

There were no differences in VO_2 measures across the trials or between time points throughout the 1 hr run. This was not surprising considering that the participants were experienced runners who were running below their lactate threshold; therefore the physiologic strain was minimal during the 1 hr run. This does however contrast to the results reported by Fallowfield et al. (1996). They reported a significant VO_2 drift during a run to exhaustion at 70% of VO_2max in active adults, with the fluid replacement trial showing an even greater VO_2 drift. The greater drift was attributed to enhanced fat metabolism with the ingestion of water. As noted above, with experienced endurance athletes who had been performing 1 hr training sessions, VO_2 drift would not be expected (Ganio, Wingo, Carroll, Thomas, & Cureton, 2006). In the present study, even during the NHY trial, there was no significant change, supporting the evidence that this exercise protocol did not result in a significant physiological strain. This is

also supported by the RQ and heart rate measures seen in the present study. The cardiovascular strain experienced during the NHY trial compared to the ET and HD trials reflects the body water deficit experienced during the no hydration trial and is consistent the physiological effects of dehydration (Hoffman, 2014).

Conclusions

The results from this study indicated that ingestion of the alanine-glutamine dipeptide at either the low dose (300 mg·500 ml^{-1}) or high dose (1 g·500 ml^{-1}) during a moderate intensity run resulted in a significant performance improvement during a subsequent run to exhaustion at 90% of VO_2peak. The results of the study were unable to elucidate the precise mechanism that supported this ergogenic effect, but it may be related to an enhanced electrolyte uptake by skeletal muscle and the possible gluconeogenic effect of alanine.

1. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink would significantly increase absorption as measured by plasma glucose, electrolytes, and glutamine during prolonged running by endurance-trained males.

This hypothesis is accepted. The sodium measure at 60 min during the HD trial was significantly lower than the NHY and LD trials; plasma glutamine concentration during the LD and HD trials was significantly elevated at 45 min, with the HD trial remaining elevated at 60 min; and at 60 min, glucose dropped significantly during the ET trial and did not during the LD or HD trials.

2. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink will significantly reduce the cardiovascular strain as measured by oxygen consumption, heart rate, blood pressure, and respiratory quotient during prolonged running by endurance-trained males.

This hypothesis must be rejected, there were no differences in physiological measures between the ET trial and the LD and HD trials.

3. It was hypothesized that adding the dipeptide L-Alanyl-L-Glutamine to a sport drink will significantly improve muscle activation patterns and neuromuscular fatigue as measured by electromyography root mean square signals from the vastus lateralis and rectus femoris muscles during prolonged running by endurance-trained males.

This hypothesis must be rejected, there were no differences in EMG measures of the vastus lateralis or rectus femoris during the run to exhaustion.

APPENDIX A: UCF IRB APPROVAL LETTER

University of Central Florida Institutional Review Board
Office of Research & Commercialization
12201 Research Parkway, Suite 501
Orlando, Florida 32826-3246
Telephone: 407-823-2901, 407-882-2901 or 407-882-2276
www.research.ucf.edu/compliance/irb.html

Notice that UCF will Rely Upon Other IRB for Review and Approval

From : **UCF Institutional Review Board**
FWA00000351, IRB00001138

To : **William P. McCormack**

Date : **August 02, 2013**

IRB Number: **SBE-13-09396**

Study Title: **Effect of Acute L-Alanyl-L-Glutamine (SustamineTM) and Electrolyte Ingestion on Reaction, Tracking, Cognitive Function, and Neuromuscular Fatigue during Endurance Exercise**

Dear Researcher:

The research protocol noted above was reviewed by the University of Central Florida IRB Chair designated Reviewer on August 02, 2013. The UCF IRB accepts the New England Institutional Review Board's review and approval of this study for the protection of human subjects in research. **The expiration date will be the date assigned by the New England Institutional Review Board and the consent process will be the process approved by that IRB.**

This project may move forward as described in the protocol. It is understood that the New England IRB is the IRB of Record for this study, but local issues involving the UCF population should be brought to the attention of the UCF IRB as well for local oversight, if needed.

All data, including signed consent forms if applicable, must be retained in a locked file cabinet for a minimum of five years (six if HIPAA applies) past the completion of this research. Additional requirements may be imposed by your funding agency, your department, or other entities. Access to data is limited to authorized individuals listed as key study personnel.

Failure to provide a continuing review report for renewal of the study to the New England IRB could lead to study suspension, a loss of funding and/or publication possibilities, or a report of noncompliance to sponsors or funding agencies. If this study is funded by any branch of the Department of Health and Human Services (DHHS), an Office for Human Research Protections (OHRP) IRB Authorization form must be signed by the signatory officials of both institutions, and a copy of the form must be kept on file at the IRB office of both institutions.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Patria Davis on 08/02/2013 11:20:36 AM EDT

IRB Coordinator

APPENDIX B: NEW ENGLEND IRB APPROVAL LETTER

NEIRB
New England Institutional Review Board

August 1, 2013

William P. McCormack
University of Central Florida
12494 University Boulevard
Orlando, FL 32816

Re: (IRB# 13-254): SBE-13-09475: "Effect of Acute L-Alanyl-L Glutamine (Sustamine ™) and Electrolyte Ingestion on Reaction, Tracking, Cognitive Function, and Neuromuscular Fatigue During Endurance Exercise"

This is to inform you that New England Institutional Review Board (NEIRB), via expedited review (Thursday Board), has approved the above-referenced research protocol and the participation of the above-referenced investigative site in the research. The approval period is **8/1/2013 to 7/25/2014. Your study number is 13-254. Please be sure to reference either this number or the name of the principal investigator in any correspondence with NEIRB.**

Continued approval is conditional upon your compliance with the following requirements:

- A copy of the **Informed Consent Document**, NEIRB version 1.0, approved on 8/1/2013 is enclosed. Only NEIRB-approved informed consent documents should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.
- The following must be promptly reported to NEIRB: changes to the study site, and all unanticipated problems that may involve risks or affect the safety or welfare of subjects or others, or that may affect the integrity of the research.
- Approval is valid for enrollment of the number of subjects indicated on your submission form. If you anticipate enrolling more than this number of subjects, NEIRB approval must be obtained prior to exceeding the approved enrollment number.
- All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB except where necessary to eliminate apparent immediate hazards to the study subjects.
- Compliance with all federal and state laws pertaining to this research, and with NEIRB's SOPs.
- The enclosed subject materials (*Flyer, Medical and Activity History Questionnaire, and PAR-Q and You Questionnaire*) have been approved. Advertisements, letters, internet postings and any other media for subject recruitment must be submitted to NEIRB and approved prior to use. Please refer to *NEIRB Guidelines for Recruitment and Advertising*, available at www.neirb.com
- All deaths, life-threatening problems or serious or unexpected adverse events, *whether related to the study article or not*, must be reported to the IRB. The Serious Adverse Event Form is available at www.neirb.com.
- Any and all necessary FDA approvals must be received prior to your initiation of the trial. If this study is being conducted under an IDE, a copy of the FDA IDE approval letter must be submitted to NEIRB.
- The study cannot continue after 7/25/2014 until re-approved by NEIRB. A Study Renewal Report must be completed and returned to NEIRB prior to the expiration of the approval period.
- When the study is completed, terminated, or if it is not being renewed - complete and submit a Study Completion Report to NEIRB. The Study Completion Report can be accessed via the NEIRB website at www.neirb.com.

Shana R. Ross

Shana R. Ross, MCJ, CIM, CIP
Lead Administrator

Copy: NEIRB Chair
Enclosures

85 Wells Avenue . Suite 107 . Newton, MA 02459 Phone: 617-243-3924 . Fax: 617-969-1310 www.neirb.com

APPENDIX C: INFORMED CONSENT

University of Central Florida

Effect of Acute L-Alanyl-L-Glutamine (Sustamine™) and Electrolyte Ingestion on Reaction, Tracking, Cognitive Function, and Neuromuscular Fatigue during Endurance Exercise

Informed Consent

Principal Investigator(s):	William P. McCormack, M.A. Jay R. Hoffman, Ph.D.
Sub-Investigators:	Jeffrey R. Stout, Ph.D. Maren S. Fragala, Ph.D.
Study Clinician:	Leonardo P. Oliveira, MD
Sponsor:	KYOWA HAKKO BIO CO., LTD., Japan
Investigational Site(s):	University of Central Florida College of Education and Human Performance Sport and Exercise Science

Introduction: Researchers at the University of Central Florida (UCF) study many topics. To do this we need the help of people who agree to take part in a research study. You are being asked to take part in a research study which will include 12 men at UCF. You have been asked to take part in this research study because you are an active young adult who routinely participates in endurance running. You must be between 18 and 35 years of age to be included in this research study.

The principal investigators conducting the research are William P. McCormack and Dr. Jay R. Hoffman (Sport and Exercise Science in the College of Education and Human Performance). They will be supported by Dr. Jeffrey R. Stout, Dr. Maren S. Fragala (Sport and Exercise Science in the College of Education and Human Performance), and Dr. Leonardo Oliveira (Sports Medicine Physician at UCF and medical monitor of the study).

Approved by NEIRB on 8/1/2013
NEIRB ICF Version 1.0

What you should know about a research study:

- Someone will explain this research study to you.
- A research study is something you volunteer for, whether or not you take part is up to you.
- You should take part in this study only because you want to.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

Background

There has been research preformed recently that has shown by adding two proteins, alanine and glutamine to water, will help the absorption of the fluid. This was performed in a study with basketball players and the results showed that their shooting skills and visual reaction time following a competitive game were maintained. What has not been researched yet is adding alanine and glutamine to a sport drink (i.e. Gatorade or Powerade). We will be examining whether the two proteins will help the sugar and electrolytes (sodium and potassium) absorb more quickly and therefore help running performance. We know that as we sweat there is a loss of electrolytes, sodium being to most abundant electrolyte in sweat, and a loss of sodium can affect running performance.

Purpose of the research study: There are four objectives to this study: 1) To examine the efficacy of the dipeptide L-Alanyl-L-Glutamine (Sustamine™) on upper and lower body reaction, multiple object tracking, and cognitive function following prolonged endurance activity; 2) To examine the efficacy of Sustamine™ ingestion on changes in plasma concentrations of glucose, lactate, glutamine, sodium and potassium compared to a flavored sports drink alone; 3) To examine effects of Sustamine™ on oxygen consumption, heart rate, blood pressure, and respiratory quotient during prolonged endurance exercise; and 4) To examine the effects of Sustamine™ on muscle activation patterns and fatigue during prolonged endurance exercise.

Testing location and time requirements:

All testing will be conducted in the Human Performance Lab (HPL) in the College of Education and Human Performance building at the University of Central Florida. All measures and tests are conducted for research purposes only. The results will not be used to diagnose any illness or disease, and will not provide any meaningful information to your physician.

Time requirements: We expect that you will be in this research study for approximately 5 weeks and will consist of 6 visits to the HPL. The first visit will last approximately one hour, the second visit about an hour and a half, and the final four visits may last up to three hours.

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What you will be asked to do in the study:
Preliminary Visits (2):

Visit 1: During this first visit, the following will be done:

- Complete the Physical Activity Readiness Questionnaire (PAR-Q).
- Complete the self-reported Confidential Medical and Activity questionnaire.
- Read and sign the study informed consent form.
- Your age, race and gender will be collected.
- Your body measurements (height, weight, body composition) will be measured.
- You will be asked to provide a urine sample to check for urine osmolality (which is the concentration of electrolytes, sodium and potassium) and specific gravity (how much more dense urine is when compared to water, which is a test for dehydration). You will be given a specimen cup and asked to proceed to the male restroom in the Education Complex Building, fill the specimen cup and return to the lab. The sample will only be checked for osmolality and specific gravity and when these tests are complete and results recorded, any remaining sample will be discarded.
- You will be given familiarization trials on the reaction and cognitive function tasks.
    - Reaction time will be measured for both the upper and lower body. Upper body reaction time will be tested on the Dynavision D2 Visuomotor Training Device, which is a 4 foot by 4 foot board with 64 lights in 5 concentric circles. The height of the board is adjusted to each individual so they are able to reach every light on the board. Three separate tests will be performed with the Dynavision:
        - 1) The first assessment will measure your visual, motor, and physical reaction time with the dominant hand. The test will be initiated when you place and hold your hand on the illuminated "home" button. At this point, a stimulus (light) will present in one of five locations, parallel to the home button. Visual reaction time will be measured as the amount of time it takes to identify the stimulus (light) and initiate a reaction by taking your hand off the home button. Motor response time will be measured as the amount of time it takes to physically touch the stimulus (light) with your hand following the initial visual reaction, and physical reaction time is a measurement of the total elapsed time from the introduction of the target stimulus to the physical completion of the task (returning to the home button after touching the stimulus with your hand). This will be repeated ten times.

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- 2) The second assessment will measure your ability to react to a stimulus (light) as it changes positions on the board. An initial stimulus (light) will present on the D2 in a random location. The stimulus will remain lit until you touch it. The stimulus (light) will then appear at another random location. You will be instructed to identify and touch as many stimuli as possible within 60 s. The number of "hits" and the average time per hit will be recorded as your score.

- 3) The third assessment will be similar to the previous measure in that you will be required to react to a visual stimulus (light) as it changes positions on the board. However, during this test you will be asked to verbally recite a 5-digit number that is presented on the center screen of the D2. A new 5-digit number will appear on the screen every 5 seconds. You will be asked to touch each stimulus before it changes position and verbally repeat the five digit numbers as they appear on the screen. Your score will be the number of successful hits during the 60 s trial.

- Lower body reaction time will also be measured using a 20-second reaction test on the Quick Board™. You will stand on a board of five circles, in a 2 x 1 x 2 pattern straddling the middle circle. You will be asked to react to a visual stimulus located on a display box that depicts one of five potential lights that correspond with the circles on the board. Upon activation of the light, you will attempt to move the foot closest to the circle that corresponded to the visual stimulus. Upon a successful "hit" with the foot the next stimulus will appear. The total number of successful "hits" during the 20-second test and the average time between the activation of the light and the response to the corresponding circle will be recorded.

- Cognitive function will be measured utilizing a Cave Automatic Virtual Environment (CAVE) system. The CAVE consists of a 7 ft × 7 ft × 7 ft room that includes a canvas projection screen on the front wall which will serve as the surface for image projection. A three-dimensional image of 8 tennis balls will be projected onto the front screen. You will be instructed to track 4 of the 8 balls that will move in three-dimensions. At the beginning of test, the balls appear frozen on the screen for 2 seconds, half of them will be grey, these are the balls you will track. After the 2 seconds, the balls will begin to move in three dimensions. At the conclusion of the trial (8 seconds), the balls will freeze and a number will appear on each ball. You will call out the numbers of the four balls you were supposed to be tracking. Velocity of movement will begin at a slow tracking speed and will increase or decrease depending on your correct responses. The test will consist of 20 trials. You will wear three dimensional glasses during the trials. Your score will be the velocity of movement that was most successful.

- The second measure of cognitive function is a modified version of the original Serial Sevens Test. This test consists of a two minute timed oral test in which you will subtract the number 7 from a random computer generated four digit number in order to measure how quickly and accurately you can compute a simple mathematical problem. The computer generated numbers will be written onto standard note cards. You will be

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given a randomized stack of note cards and asked to complete as many calculations as possible in the two minute period. A scorer will sit opposite of you during testing. Once you release the note card, your answer will be considered unchangeable. The number of correct answers and the average time per correct answer will be recorded.

- You will be given a 24-hour food log to complete prior to visit 2. The dietary intake on this food log will be considered your pre-testing diet and you will be asked to replicate this diet during all experimental trials. This recall is not looking for any specific food types or quantity, the goal is to have you consume the same foods prior to each visit in the same pattern as you would prior to an hour run, as if it were a normal training evolution, so that dietary intake is not a confounding factor in the investigation.

Visit 2: The second visit will take place no sooner than 48 hours following visit 1. You will be asked to repeat several things from visit 1, including:

- Your height and weight will be measured
- Provide a urine sample to check for urine osmolality and specific gravity (as explained above).
- Repeat the familiarization trials on the reaction time and cognitive function tasks in the same order and on the same devices as visit 1. This is done to eliminate any learning effect with the tests, so that you are completely familiar by the time the experimental trials begin.
- You will be outfitted with surface electrodes over two of the front thigh muscles (vastus lateralis and rectus femoris) in your right leg to measure electromyography (EMG). EMG is measuring the electrical activity of the muscle. It is completely painless. You will also be asked to perform a maximal leg extension to record a maximal EMG signal. The EMG signal will also be collected during the maximal aerobic test (VO_2max).
- You will also be asked to perform a VO_2max test, which will include running on the treadmill at increasing speed until you can no longer continue. Expired gases will be collected via a mask to determine oxygen uptake, respiratory quotient, and energy expenditure. As a part of the VO_2max test, we will conduct a lactate threshold test to ensure you can comfortably run at 75% of your VO_2max for an hour during the experimental trials. This will involve running 4 minute discontinuous stages on the treadmill at increasing speed. The speed increases will be 10 meters·min^{-1} starting at a slow training pace. At the end of each stage a finger prick will be performed to collect 50μL (a small capillary tube) of blood that will be analyzed for blood lactate. Once the concentration of 4mmol·L^{-1} has been achieved and there has been a 2mmol·L^{-1} increase above baseline, the VO_2max test will begin. Each stage of this portion of the test will be 1 minute in duration. The stages will be continuous, meaning you will not rest between stages. The speed will increase until you can no longer complete a 1 minute stage.

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Experimental Trial Visits (4):

You will be asked to report to the Human Performance Laboratory (HPL) on four additional occasions to conduct the experimental trials. The first of these trials will be no sooner than 48 hours following PV2. Each session will require you to perform a 60-min run at 75% of your previously measured VO_2max speed. At the 60-min mark, the treadmill speed will be adjusted so that you will then run at 90% of your VO_2max speed until volitional exhaustion. At the beginning of each session you will be asked provide a urine sample to check for urine osmolality and specific gravity to ensure proper hydration status. You will be asked to perform the first trial without any rehydration (T1). During this session your total weight lost during the run will be determined. The fluid loss occurring during this session will determine your sweat rate ($L \cdot hr^{-1}$). To continue in the study, your sweat rate will need to be or exceed 1.3 $L \cdot hr^{-1}$. During the next 3 trials (T2, T3, T4) you will be asked to perform the same running protocol as T1 and you will be provided 250 ml of fluid every 15 minutes. During one of these trials you will be asked to consume only a flavored sports drink (Gatorade G2), while during the other trials you will consume the alanine-glutamine supplement (Sustamine™) mixed in the same flavored sports drink at either a low (300 mg per 500 ml) or high dose (1 g per 500 ml). These trials (T2, T3, and T4) will be randomized and separated by 5 to 7 days. You will be asked to schedule the visits at approximately the same time of day and possibly on the same day of the week throughout the study to make it easier on your weekly schedule. Prior to and at the completion of each running trial, you will be asked to perform a series of upper and lower body reaction tests as well as 2 cognitive function tests as described in PV1.

Prior to exercise, surface electrodes will be placed over two of the front thigh muscles (vastus lateralis and rectus femoris). A reference electrode will be placed over your right anterior, superior iliac crest. The skin will be shaved, cleaned, and abraded in the area that the electrodes will be placed. Prior to each trial you will perform a maximal effort isometric contraction of the knee extensors using the knee extension machine. During each experimental trial EMG values will be recorded every 10 minutes and reported as a % of maximal value.

During each experimental session a baseline (BL) blood sample will be obtained at pre-exercise. Additional blood samples will be drawn at 30 min, 45 min and 60 min during the exercise session. The total amount of blood drawn during the trials will not exceed 24 ml (6 ml per blood draw). This is approximately the amount held in a single tablespoon. To put the volume of blood being drawn in proper perspective, one pint (475 ml) of blood is typically drawn when donating blood. All blood samples will be obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a 3-way stopcock with a male luer lock adapter. A cannula is a hollow tube, which can be inserted into the opening of a vein and serve as a channel for the transport of fluid. The cannula prevents the need for multiple needle pricks from being performed. The risks associated with the placement of the cannula are not any different than that experienced by a normal blood draw using a needle and syringe. Cannula placement and blood draws will be performed by personnel trained in phlebotomy with extensive experience in both research and clinical settings. The cannula will be maintained patent using an isotonic saline solution. BL blood samples will be drawn following a 15-min equilibration period (you will be lying down) prior to exercise. The discomforts associated with the blood drawing procedures are minimal, but sometimes bruising and infection may occur, and your arm might become sore. This soreness usually resolves in a few days. If it persists, contact your doctor. Blood samples

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obtained will only be used for this specific study and any leftover blood will be discarded following analysis.

Funding for this study: This research study is being funded by KYOWA HAKKO BIO CO., LTD., Japan. Even though funding is coming from an international company, no individual data will be sent to the company. They will receive a final copy of the compiled results, no individual data will leave the HPL.

Risks:

The risks involved with this study are minimal, but may include musculoskeletal injuries occurring during the running protocol. These injuries include muscle strains and pulls. However, the running portion of the study is similar to a hard training session that all experienced endurance runners have previously performed during training. The risks associated with the blood draw include some momentary pain at the time the cannula is inserted into the vein, but other discomfort should be minimal. It is also possible for a bruise to develop at the cannula site or for individuals to report dizziness and faint after the blood is drawn. It is also rare, but possible to develop minor infections and pain after the blood draw. To minimize the risks, the skin area where the cannula is to be inserted will be cleaned and prepared with a disinfectant wipe before the cannula is inserted. In addition, the cannula will be inserted while you are lying supine.

You should report any discomforts or injuries to one of the principal investigators William McCormack, 407-823-2367, william.mccormack@ucf.edu, Dr. Jay Hoffman, 407-823-2367, jay.hoffman@ucf.edu, or support investigators Dr. Leonardo Oliveira, 407-266-1055, Leonardo.Oliveira@ucf.edu; Dr. Maren Fragala, 407-823-2367, maren.fragala@ucf.edu, or Dr. Jeff Stout, 407-823-2367, jeffrey.stout@ucf.edu. If immediate assistance is needed it will be provided via the emergency medical system. For non-emergency injuries, you must seek treatment from your own physician. You will be responsible for payment of any treatment from your doctor.

Benefits

There are no direct benefits to participants.

Compensation or payment:

Upon completion of the study, you will receive a $150 payment for participation. However, if you are only able to complete certain parts of the study, you will only be compensated for what you complete. You will receive $30 for completing the initial testing and T1, and an additional $40 for each additional trial completed (T2 through T4). No compensation will be provided if you are only able to complete the preliminary testing.

Confidentiality: The results of this study will be published as a group as part of a scientific publication. No individual results will be published or shared with any person or party. All information attained from the medical and activity questionnaire or performance tests will be held in strict confidence. Individual results will remain confidential and only be relayed to the subject upon request. All medical and activity questionnaires, as well as data collection sheets will be kept in a locked cabinet during and following the study. All information will be destroyed 5 years from the end

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of the study and not used for other research purposes. Participant folders and blood storage tubes will be marked with an I.D. number to protect against a breach of confidentiality and the ID number will be removed upon disposal of the samples. Participant names and I.D. numbers will be stored apart from the blood samples; the identifiers will be removed from the samples and destroyed when the samples are disposed.

Records of your participation in this study will be held confidential so far as permitted by law. However, the study doctor, the sponsor or it's designee, and, under certain circumstances, the New England Institutional Review Board (IRB) will be able to inspect and have access to confidential data that identifies you by name.

Study contact for questions about the study or to report a problem: If you have questions, concerns, or complaints, or think the research has hurt you, talk to William McCormack or Dr. Jay Hoffman, Human Performance Laboratory, Sport and Exercise Science (407) 823-2367 or by email at william.mccormack@ucf.edu or jay.hoffman@ucf.edu.

IRB contact about your rights in the study or to report a complaint: This research is being carried out under the oversight of the New England Institutional Review Board (NEIRB). If you have questions about this study or about the rights of people who take part in research, please contact the NEIRB at: New England Institutional Review Board, 85 Wells Avenue, Suite 107, Newton, MA, 02459 or by phone at (617) 243-3924. You may also talk to them for any of the following:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You want to get information or provide input about this research.

Withdrawing from the study:
You have the right to discontinue participation without penalty, regardless of the status of the study. Your participation in the study may also be terminated at any time by the researchers in charge of the project. This could be based upon your refusal to follow study instructions or follow the study protocol or not meet the sweat rate requirement. Depending upon when you withdraw, you may be able to receive compensation for the time that you did participate. Please refer back to the "Compensation or Payment" section on the top of this page.

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VOLUNTEER'S STATEMENT:

I have been given a chance to ask questions about this research study. These questions have been answered to my satisfaction. I may contact Mr. William McCormack if I have any more questions about taking part in this study. Mr. William McCormack or the company he/she is employed by is being paid by the sponsor for my participation in this study.

I understand that my participation in this research project is voluntary. I know that I may quit the study at any time without harming my future medical care or losing any benefits to which I might be entitled. I also understand that the investigator in charge of this study may decide at any time that I should no longer participate in this study.

If I have any questions about my rights as a research subject in this study I may contact:

New England Institutional Review Board
Telephone: 1-800-232-9570

By signing this form, I have not waived any of my legal rights.

I have read and understand the above information. I agree to participate in this study. I understand that I will be given a copy of this signed and dated form for my own records.

_____ _____
Study Participant (signature) Date

_____
Print Participant's Name

_____ _____
Person who explained this study (signature) Date

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APPENDIX D: MEDICAL QUESTIONNAIRE AND PAR-Q

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Human Performance Laboratory
University of Central Florida

Confidential Medical and Activity History Questionnaire

Participant #_____

When was your last physical examination? _____

1. **List any medications, herbals or supplements you currently take or have taken the last month**:

Medication	Reason for medication

2. **Are you allergic to any medications? If yes, please list medications and reaction.**

3. **Please list any allergies, including food allergies that you may have?**

4. **Have you ever been hospitalized? If yes, please explain.**

Year of hospitalization	Reason

5. **Illnesses and other Health Issues**

List any chronic (long-term) illnesses that have caused you to seek medical care.

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Have you ever had (or do you have now) any of the following. Please circle questions that you do not know the answer to.

Sickle cell anemia	yes	no
Cystic fibrosis	yes	no
Water retention problems	yes	no
Heart pacemaker	yes	no
Epilepsy	yes	no
Convulsions	yes	no
Dizziness/fainting/unconsciousness	yes	no
Asthma	yes	no
Shortness of breath	yes	no
Chronic respiratory disorder	yes	no
Chronic headaches	yes	no
Chronic cough	yes	no
Chronic sinus problem	yes	no
High blood pressure	yes	no
Heart murmur	yes	no
Heart attack	yes	no
High cholesterol	yes	no
Diabetes mellitus or insipidus	yes	no
Rheumatic fever	yes	no
Emphysema	yes	no
Bronchitis	yes	no
Hepatitis	yes	no
Kidney disease	yes	no
Bladder problems	yes	no
Tuberculosis (positive skin test)	yes	no
Yellow jaundice	yes	no
Auto immune deficiency	yes	no
Anemia	yes	no
Endotoxemia	yes	no
Thyroid problems	yes	no
Hyperprolactinemia	yes	no
Anorexia nervosa	yes	no
Bulimia	yes	no
Stomach/intestinal problems	yes	no
Arthritis	yes	no
Back pain	yes	no
Gout	yes	no
Hepatic encephalopathy	yes	no
Mania	yes	no
Hypermania	yes	no
Monosodium glutamate hypersensitivity	yes	no
Seizure disorders	yes	no

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Any others (specify):_____
_____

Do you smoke cigarettes or use any other tobacco products?	yes	no
Do you have a history of drug or alcohol dependency?	yes	no
Do you ever have any pain in your chest?	yes	no
Are you ever bothered by racing of your heart?	yes	no
Do you ever notice abnormal or skipped heartbeats?	yes	no
Do you ever have any arm or jaw discomfort, nausea, Or vomiting associated with cardiac symptoms?	yes	no
Do you ever have difficulty breathing?	yes	no
Do you ever experience shortness of breath?	yes	no
Do you ever become dizzy during exercise?	yes	no
Are you pregnant?	yes	no
Is there a chance that you may be pregnant?	yes	no
Have you ever had any tingling or numbness in your arms or legs?	yes	no
Has a member of your family or close relative died of heart problems or sudden death before the age of 50?	yes	no
Has a health care practitioner ever denied or restricted your participation in sports for any problem	yes	no

If yes, please explain: _____
_____

Are you presently taking any nutritional supplements or ergogenic aids? (if yes, please detail. _____
_____
_____

Over the past 6 months, on average, how many miles per week have you been running?_____
_____

Over the past month, how long (in miles) has your longest run been?_____
_____

_____
Signature Date

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Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

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(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions →

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP SCPE

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APPENDIX E: FLYER

Looking for ENDURANCE-TRAINED MEN 18 TO 35 years of age interested in volunteering for a research study entitled:

Effect of Acute L-Alanyl-L-Glutamine (Sustamine™) and Electrolyte Ingestion on Reaction, Tracking, Cognitive Function, and Neuromuscular Fatigue during Endurance Exercise

The purpose of this study is:

1) To examine the efficacy of the dipeptide L-Alanyl-L-Glutamine (Sustamine™) on upper and lower body reaction, multiple object tracking, and cognitive function following prolonged endurance activity.
2) To examine the efficacy of Sustamine™ ingestion on changes in plasma concentrations of glutamine, sodium, and potassium compared to a flavored sports drink alone.
3) To examine effects of Sustamine™ on oxygen consumption, heart rate, blood pressure, and respiratory quotient during prolonged endurance exercise.
4) To examine the effects of Sustamine™ on muscle activation patterns and fatigue during prolonged endurance exercise.

To be included in the study you must be:

1) Endurance-trained male runner with a recent training history of at least one-hour run duration
2) Free of any physical limitations (determined by a Confidential Medical/Activity and PAR-Q questionnaires)
3) Between the ages of 18 and 35

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Your commitment:

1) 6 visits to the Human Performance Lab each lasting 1 to 3 hours.
2) Visit 1 is a familiarization visit.
3) Visit 2 is a VO_2max and lactate threshold test.
4) Visits 3 – 6 include a 1-hour run with a time trial to exhaustion at the end (#3 = no hydration; #4-6 drinking Gatorade every 15 minutes during 1-hour run).
5) Urine test for dehydration at beginning of all visits; blood sample every 15 minutes during 1-hour run.

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As Is ✓ As Revised ___

Please contact William McCormack
Human Performance Lab
Sport and Exercise Science, College of Education
(407) 823-2367, or via email at william.mccormack@ucf.edu

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