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Effect of Acute L-Alanyl-L-Glutamine (Sustamine) and Electrolyte Ingestion on Cognitive Function, Multiple Object Tracking and Reaction Time Following Prolonged Exercise

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EFFECT OF ACUTE L-ALANYL-L-GLUTAMINE (SUSTAMINE^{TM}) AND ELECTROLYTE INGESTION ON COGNITIVE FUNCTION, MULTIPLE OBJECT TRACKING AND REACTION TIME FOLLOWING PROLONGED EXERCISE

by
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ABSTRACT

Changes in physiological function occurring during a body water deficit may result in significant decrements in performance, cognitive function and fine motor control during exercise. This may be due to the magnitude of the body water deficit. Rehydration strategies are important to prevent these deleterious effects in performance. The purpose of this study was to examine the changes before and after prolonged exercise of an alanine-glutamine dipeptide (AG) on cognitive function and reaction time.

Twelve male endurance-trained runners (age: 23.5 ± 3.7 y; height: 175.5 ± 5.4 cm; weight: 70.7 ± 7.6 kg) participated in this study. Participants were asked to run on a treadmill at 70% of their predetermined VO_2max for 1 h and then run at 90% of VO_2max until volitional exhaustion on four separate days (T1-T4). T1 was a dehydration trial and T2-T4 were all different hydration modalities (electrolyte drink, electrolyte drink with a low dose of AG, electrolyte drink with a high dose of AG, respectively) where the participants drank 250 mL every 15 min. Before and after each hour run, cognitive function and reaction tests were administered. Hopkins Magnitude Based Inferences were used to analyze cognitive function and reaction time data.

Results showed that physical reaction time was likely faster for the low dose trial than the high dose trial. Dehydration had a possible negative effect on the number of hits in 60-sec compared to both the low and high dose trials. Comparisons between only the electrolyte drink and the high dose ingestion appeared to be possibly negative. Analysis of lower body quickness indicates that performance in both the low and high dose trials were likely improved (decreased)

in comparison to the dehydration trial. Multiple object tracking analysis indicated a possible greater performance for dehydration and low dose compared to only the electrolyte drink, while there was a likely greater performance in multiple object tracking for the high dose trial compared to consumption of the electrolyte drink only. The serial subtraction test was possibly greater in the electrolyte drink trial compared to dehydration.

Rehydration with the alanine-glutamine dipeptide during an hour run at a submaximal intensity appears to maintain or enhance subsequent visual reaction time in both upper and lower body activities compared to a no hydration trial. The combination of the alanine-glutamine dipeptide may have enhanced fluid and electrolyte absorption from the gut and possibly into skeletal tissue to maintain neuromuscular performance.

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CHAPTER I

Introduction

Changes in physiological function occurring during a body water deficit may result in significant decrements in performance during exercise. These changes appear to be related to the magnitude of water deficit. During exercise in a temperate environment maximal aerobic power appears to be maintained when body weight loss does not exceed 3% (Goulet, 2012; 2013), however as body water deficits exceed 3% significant decreases in aerobic power and greater fatigue rates are reported (Casa et al., 2010; Goulet, 2012; 2013). During short duration anaerobic events (e.g., high intensity activity of 40 sec or less), the effect of a body water deficit on strength, power and anaerobic capacity does not appear to impede performance, even when the magnitude of dehydration reaches 5% body weight loss (Jacobs, 1980). This is relevant for sports that involve high intensity, short duration events. However, in sports that rely on intermittent bouts of high intensity activity, such as basketball or football, dehydration often occurs as a result of inadequate fluid intake. Although power performance has been shown to be maintained in such events (Dougherty, Baker, Chow & Kenney, 2006; Hoffman et al., 1995; 2012), levels of hypohydration of approximately 2% (ranging from 1.9% – 2.3%) have been shown to result in significant performance decrements (e.g., 8% - 12.5% difference in shooting percentages and a significant slower response in visual reaction time) (Hoffman et al., 1995; 2012). This may potentially impact game outcomes as a thirst response doesn't appear to occur until a body water deficit of approximately 2% is reached (Rothstein, Adolph, & Wells, 1947).

In addition to decrements in fine motor control and reaction time during mild levels of hypohydration, previous studies have also indicated that a body water deficit of this magnitude can also impair cognitive performance (Ganio et al., 2011; Lieberman et al., 2005; Tomporowski, Beasman, Ganio, & Cureton, 2007). Ganio and colleagues (2011) indicated that a combination of diuretic and exercise induced -1.59% loss in body weight resulted in a decrease in cognitive performance with specific decrements in visual vigilance and visual working memory. Others have demonstrated that slightly greater levels of dehydration (2% - 3% body weight loss) induced by exercise only, resulted in no detrimental effect in short term memory, but a significant decrement in executive functioning (i.e., ability to move through problem sets) (Tomporowski, Beasman, Ganio, & Cureton, 2007). However, when dehydration (2.6% body weight loss) is induced by water restriction only, cognitive-motor performance may not be affected (Szinnai et al., 2005). It appears that the combination of fatigue and fluid deprivation during exercise has a more profound effect on cognitive function than dehydration only.

To reduce potential performance decrements during exercise the concept of developing a rehydration strategy becomes imperative. Rehydrating with electrolyte drinks has been suggested to be a potential alternative to water only rehydration. The benefit of this rehydration strategy is that the flavored drink may induce greater hydration (Hubbard et al., 1990), but of even more importance is that electrolyte drinks may prevent hyponatremia that becomes a concern with water only rehydration (Almond et al., 2005). However, this does not appear to be an issue in exercise durations that are less than 3 – 4 hours in duration. Although electrolyte loss may affect motor unit recruitment and muscle contractile capabilities (Sjogaard, 1986), there is little to no research that has examined the efficacy of electrolyte supplementation on high

intensity activity. Recently, a rehydration strategy using an alanine-glutamine dipeptide was demonstrated to enhance fluid uptake and reduce the magnitude of performance decrements during exercise to exhaustion more than water alone in dehydrated subjects (Hoffman et al., 2010). A subsequent study examined the effect of this dipeptide during a competitive basketball game (Hoffman et al., 2012). Participants consuming the dipeptide were able to maintain shooting accuracy and respond to a visual stimulus significantly quicker than when they consumed water only. The alanine-glutamine dipeptide is thought to enhance fluid and electrolyte uptake from the gut (Lima et al., 2002). Interestingly, the previous investigations examining the ergogenic effects of this dipeptide have used water as the fluid medium that it is delivered. Whether these affects can be exacerbated when combined with an electrolyte drink has not been examined. Thus, the purpose of this study was to examine the efficacy of two different doses of the alanine-glutamine dipeptide in a commercially available electrolyte drink to the electrolyte drink only on multiple object tracking, reaction time and cognitive function following endurance activity.

Assumptions (Theoretical)

1. Subjects accurately answered the medical history and activity questionnaire.
2. All subjects gave maximal effort when performing the VO_2max 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. The consumption of any caffeine did not impact reaction, cognitive or strength testing measures.
6. Participants were well rested prior to each experimental testing session.
7. 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.
8. The weight loss during T1 was approximately the sweat rate for that participant, with no consideration to the loss of the metabolic fuel used during the run.
9. The absorption and effect of Sustamine™ was the same across individuals.

Limitations

1. The participants were male only this could have impacted generalizability. Furthermore, the participants were endurance-trained males, which could have further impacted generalizability.
2. The main recruiting mechanism was in-class announcements through the College of Education courses, which made subject selection not truly random.
3. The sample was made up of volunteers, therefore not meeting the underlying assumptions of random selection.
4. The study involves a participant commitment of approximately 15 hours and includes repeated blood draws and 4 x 1-hour long runs with a trial to exhaustion at the end. Participant withdrawal may impact the sample size.

5. Participants may be unable to ingest 1 liter of sports drink during the 1-hour run at 75% of VO_2max. This will impact the amount of Sustamine™ ingested and could affect the results.

CHAPTER II

Review of Literature

Glutamine

Glutamine is a nonessential amino acid. Glutamine is the most abundant amino acid in the body and it is found in all tissues in the body including the plasma, but the largest storage area resides in skeletal muscle (Felig, 1975). The resting level of plasma glutamine has been reported between 550 and 750 $\mu mol \cdot L^{-1}$ with skeletal muscle glutamine concentrations of approximately 20 $mmol \cdot kg^{-1}$ wet weight (Jonnalagadda, 2007, from Gleeson, 2008). Physiologically, glutamine's functions include cellular proliferation, acid-base balance, transport of ammonia between tissues, and antioxidant synthesis (Curi et al., 2005; Newsholme, et al., 2003; Rutten, Engelen, Schols, & Deutz, 2005). Glutamine has also shown it can lead to an improvement in performance (Hoffman, et al., 2010). It enhances the absorption of fluid and electrolytes in both animals and humans (Silva et al., 1998; Lima et al., 2002; van Loon et al., 1996).

During times of severe catabolic stress, glutamine requirements are increased (Ziegler 1993). The different types of stress include starvation, sepsis, and extended time of physical activity (Parry-Billings, Leighton, Dimitriadis, Vasconcelos, & Newsholme, 1989; Santos, Caperuto, & Costa Rosa, 2007; Castell, Newsholme, & Poortmans, 1996; Hankard, Haymond, & Darmaun, 1997). Skeletal muscle catabolism occurs when internal stores cannot meet physical

requirements (Ziegler, 1993). Intravenous supplementation of glutamine has been shown to decrease mortality and morbidity (Novak, 2002).

Alanine

The addition of alanine to form a dipeptide (such as L-alanyl-L-glutamine) increases the stability of glutamine, especially at low pH as seen in the gut (Fürst, 2001). 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, Hoffman, Allsopp, & Routledge, 2012). Harris and colleagues (2012) had eight human male participants supplement 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 higher than the elevation in plasma glutamine following only glutamine supplementation.

Alanine is as a major gluconeogenic precursor in extended exercise (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974). Carraro, Naldini, Weber, and Wolfe (1994) examined the alanine flux during exercise in five healthy males utilizing labeled alanine. The participants walked on a treadmill at 45% of their VO_2max for two hours and during a second visit were also measured during a two-hour rest period following ingestion of the labeled alanine. Plasma alanine was measured every 5 minutes from 95 minutes to 120 minutes. The results showed a nearly 50% increase of plasma alanine during the exercise trial compared to the rest trial.

Dehydration, Fatigue and Cognitive Function

During endurance exercise, there is a need for fluid ingestion to decrease the effects of dehydration (Coyle, 2004). These effects include cardiovascular strain, hyperthermia and impaired muscle metabolism. 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} (Coyle and Montain, 1992a, b; Sawka and Coyle, 1999; Cheuvront & Haymes, 2001; Cheuvront & Haymes, 2001; Sawka, Montain, & Latzka, 2001). 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). As one becomes dehydrated, water is lost from both intracellular and extracellular spaces. As exercise duration increases a larger loss of water occurs intracellularly, partly due to the breakdown of intracellular glycogen (Costill et al., 1981). When a body water deficit becomes very low, water is redistributed to ensure vital organs remain functioning (Nose, Morimoto, & Ogura, 1983).

Dehydration causes significant changes to the physiological systems in the body, primarily impacting cardiovascular and thermoregulatory function. If the magnitude of hypohydration (e.g., body fluid loss) exceeds 2% of one's body mass, heart rate increases, and if exercise is being performed in a hyperthermic environment it may not fully compensate for a lowered stroke volume, thus reducing cardiac output (Nadel, Fotney, & Wenger, 1980; Sawka, Knowlton, & Critz, 1979). Core temperature is increased relative to the degree of dehydration (Sawka, Young, Francesconi, Muza, & Pandolf, 1985), which leads to a reduced ability to

dissipate heat. An increase in core temperature also leads to a decrease in sweat rate and blood flow to the skin (Sawka & Pandolf, 1990).

Endurance exercise performance in a temperate environment can be maintained when body weight loss does not exceed 3% (Goulet, 2012; 2013). Endurance performance begins to decline when a body water deficit exceeds 3% (Casa et al., 2010; Goulet, 2012; 2013). However, anaerobic power performance may be maintained at body water deficits of 2%, 4% and 5% (Jacobs, 1980).

Hoffman and colleagues (2010) studied the effects of hydration on endurance performance. Ten physically active males participated by exercising at 75% of their VO_2max on a cycle ergometer. The results showed that participant's time to exhaustion was increased with hydration trials when comparing them to the dehydration trial.

Not only does exercise that leads to dehydration affect performance, but fine motor skills and cognitive function are affected as well. According to Szinnai and colleagues (2005), dehydration alone seems to have no effect on cognitive-motor performance. This indicates that the combination of fatigue and fluid deprivation during exercise affects cognitive function rather than just dehydration alone. Dehydration is sometimes thought of as a competitive advantage in some sports. In wrestlers, athletes who dehydrated themselves to cut weight had impaired short-term memory (Choma, Sforzo, & Keller, 1998). This is potentially harmful when a competition comes around because their mindset is not at maximum working capacity. Cognitive function is not only an important skill related to sports or every day fitness, but military personnel are affected by this as well. Before, during and after 53 hours of intense exercise training in the heat, Lieberman and colleagues (2005) found that cognitive function is severely impaired due to

dehydration, fatigue and heat. Studies examining simulated, sustained combat situations have reported that the deleterious effects of dehydration on reaction time and vigilance, along with memory and logical reasoning are severely impaired; (Lieberman et al., 2005).

Research done by Ganio and colleagues (2011) used a combination of a diuretic along with exercise leading to dehydration or exercise leading to dehydration with a placebo or euhydrated exercise with a placebo. A 1.59% loss in body weight led to impairments in cognitive function tests like visual vigilance ($p = 0.048$) and visual working memory ($p = 0.021$). Tomporowski and colleagues (2007) studied eleven men who cycled at 60% of their VO_2max and assessed executive processing and short-term memory before and after the exercise. Short-term memory was not affected, but the response errors in the executive functioning test increased following exercise.

Rehydration Strategies

To prevent performance decrements it becomes imperative that a rehydration strategy is planned in order to reduce the effects of dehydration. Benefer and colleagues (2013) examined the effect of fluid hydration and cognitive performance in 22 males and 13 females was and reported a non-significant, but positive correlation trend between a water intake score and a word recall test score ($r= 0.564$, $p = 0.090$).

A popular hydration strategy utilizes electrolyte drinks and their potentially greater effectiveness in rehydrating an individual (Hubbard et al., 1990). After the heat exposure, subjects were given a glucose drink or nothing. After the battery of cognitive and reaction tests

was given, the researchers found that dehydration in a passive heat environment inhibited reaction time. Almond and colleagues (2005) looked at Boston Marathon runners' electrolytes in their blood and fluid consumption throughout the race. Of the 488 qualified subjects, 13% had hyponatremia and 0.6% of those had critical hyponatremia. Cian, Barraud, Melin & Raphel (2001) showed how cognitive function is impaired with a loss of electrolytes. Seven male subjects were placed in a passive heat environment or in an exercise setting in a heated environment as well. Free recall was significantly higher under the fluid ingestion trials than under the dehydration or control trials ($t > 2$, $P < 0.05$). Electrolyte drinks could help solve that problem in order to reduce any potential fatal issues.

An alanine-glutamine dipeptide (L-alanyl-L-glutamine) has been shown to enhance fluid absorption in animals and humans. Silva and colleagues (1998) showed an oral rehydration solution with added glutamine increases the rate of fluid absorption than just water alone in rabbits. Lima et al. (2002) showed that glutamine in an oral nutritional rehydration solution enhances electrolyte and water absorption in rats. Van Loon and colleagues (1996) demonstrated an increase in water absorption with a glutamine supplement mixed in an oral hydration solution in humans. Hoffman and colleagues (2012) studied the effects of an alanine-glutamine dipeptide and performance during a competitive basketball game. Ten NCAA women's basketball players were recruited for this study. They participated in four 40-min basketball games with timeouts as their rehydration time. One of the trials provided no water for the athletes, another provided only water and the other two provided a low dose and a high dose of water with the alanine-glutamine dipeptide. The low dose of the dipeptide trial showed a significantly better visual reaction time ($p = 0.014$) than the dehydration trial.

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 examined cognitive function, reaction time and multiple object tracking in euhydrated endurance-trained males. The potential outcomes of this research could contribute to expand the knowledge of exercise science students and researchers. Specifically, whether the changes from different trials (dehydration, hydration, or hydration with alanine-glutamine dipeptide) maintain cognitive function and fine motor control following prolonged endurance exercise.

CHAPTER III

Methods

Participants

Twelve male endurance-trained runners (age: 23.5 ± 3.7 y; height: 175.5 ± 5.4 cm; weight: 70.7 ± 7.6 kg) were recruited for this study. All participants were recruited by flyer or word of mouth throughout the university and the local running community. Participants needed to be free of any physical limitations or injuries by completing a Confidential Medical and Activity questionnaire and/or Physical Activity Readiness Questionnaire (PAR-Q). Following an explanation of all procedures, risks, and benefits, each participant gave his informed consent prior to participation in this study. The Institutional Review Board of the University approved the research protocol. Participants were not permitted to use any additional nutritional supplements or medications while enrolled in the study. Screening for nutritional supplements and performance enhancing drug use was accomplished via a health history questionnaire completed during participant recruitment.

Research Design

The design of this research was a double-blind, randomized, placebo-controlled, cross-over study (Figure 1). Participants were asked to report to the University Human Performance Lab (HPL) on 6 separate visits. 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, PAR-Q, and informed consent. After the paperwork was completed, a urine sample was collected from each subject. Participants were provided with a specimen cup to use for urine collection. Each urine sample was analyzed for osmolality and specific gravity. These measures were used to document euhydration on all testing days. Participants were considered euhydrated if urine specific gravity ≤ 1.020. During PV1 and PV2, participants were weighed in a postabsorptive, euhydrated state to establish a baseline body weight. During PV1 and PV2, familiarization trials were conducted with the reaction and cognitive function tests. Familiarization sessions on the cognitive and reaction tests occurred twice during each visit day. Before PV2, participants were asked to complete a 24-hour food log, which was considered their pre-testing diet and participants were asked to repeat this diet prior to the experimental trials.

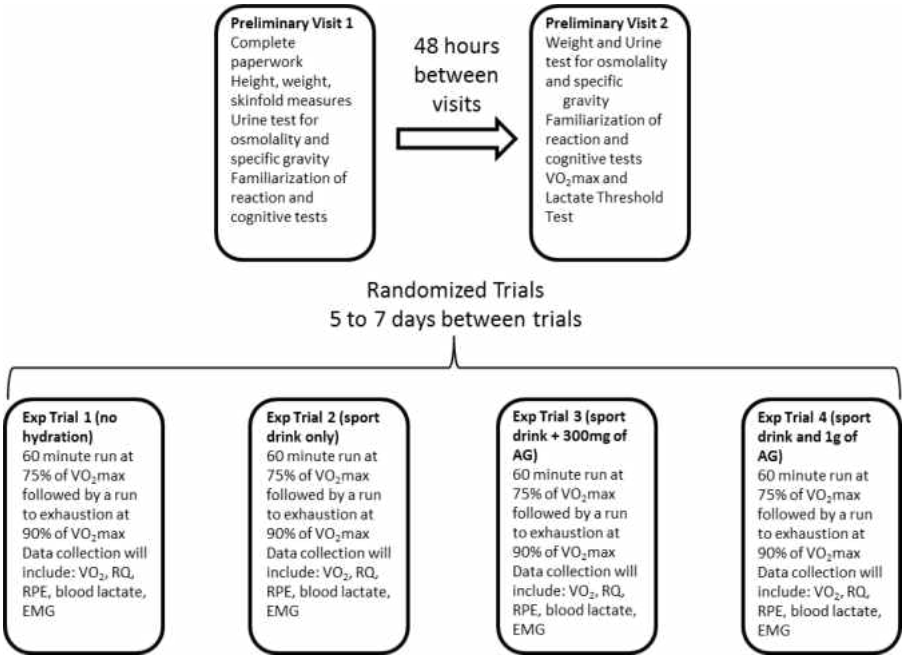

Figure 1. Study Protocol

There was a minimum of 48 hours between PV1 and PV2. During PV2 participants performed a VO_2peak and lactate threshold test, which determined the treadmill speed for the experimental trials.

The experimental testing protocol occurred on four occasions separated by 5 to 7 days. Each session required participants to perform a 1-h run at 75% of their previously measured VO_2peak from PV1. At the 60-min mark, the treadmill speed was adjusted so that all participants ran at 90% of their VO_2peak until volitional exhaustion. All participants performed the first trial without any rehydration (DHY). During this session the total weight lost during the run was determined. Participants were weighed in their running shorts using a Health-O-Meter Professional scale (Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Once the trial to exhaustion was finished, participants changed into another pair of dry running shorts and weight was measured again. The amount of fluid lost during this session determined 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 $L \cdot hr^{-1}$. During the next three trials, participants had to drink 250 ml of fluid every 15 minutes. During one of these trials participants consumed only a flavored sports drink (ED), while during the other trials participants consumed the alanine-glutamine supplement (Sustamine™). This was mixed in the same flavored sports drink at either a 300mg/500ml (LD) or 1g/500ml (HD). Trials T2, T3, and T4 were performed in a randomized order to follow the double-blind, randomized study design. Prior to and following each experimental trial, participants performed the reaction, cognitive function, and serial subtraction tests.

Reaction Testing

Both upper and lower body reaction testing took place prior to and following each experimental trial. The upper body reaction testing consisted of three separate testing protocols utilizing the Dynavision D2 Visuomotor Training Device (D2; Dynavision International LLC, West Chester, OH). The D2 is a light-training reaction device, developed to train sensory motor integration through the visual system. It consists of a light board measuring 1.22 m x 1.22 m. The light board contains 64 light (target) buttons arranged in five concentric circles surrounding a center LCD screen that can be illuminated to serve as a stimulus for the participant (Figure 1). Participants were instructed to assume a comfortable athletic stance in front of the light board and stand at a distance from the board where they were able to easily reach all of the lights. The light board was raised or lowered relative to the height of the participant. The light board height was adjusted so the LCD screen is located just below eye level.

The first assessment measured the participant's visual, motor, and physical reaction times to a light with the dominant hand. Participants were told to stand in a comfortable athletic stance centered on the row of five lights that illuminated during the test. The test initiated when the participant placed and held his hand on an illuminated "home" button. At this point, a light was presented randomly in one of five locations in the row either to the left of the LCD screen for right handed participants or to the right of the LCD screen for left handed participants. Visual reaction time was measured as the amount of time it takes to identify the light and initiate a reaction by leaving the home button. Motor response time was measured as the amount of time it took to physically strike the illuminated light following the initial visual reaction and was

measured as the amount of time between the hand leaving the home button and striking the light. Physical reaction time is a measurement of the total elapsed time from the introduction of the target light to the physical completion of the task (returning to the home button after striking the light). All measures were to the 1/100's of a second. This was repeated ten times per assessment. (Visual Reaction Time = ICC: 0.835; SEM: 0.033s; Motor Reaction Time = ICC: 0.632; SEM: 0.035s; Wells et al., 2014).

The second assessment measured the participant's ability to react to a light as it randomly changes position on the board. An initial light was presented on the light board in a random location. The light remained lit until it was struck by the participant. The light then appeared at another random location. The participant was instructed to successfully identify and strike as many lights as possible within 60 s. The number of hits and the average time per hit were recorded for each participant. The third assessment was similar to the previous measure in that participants were required to react to a visual light as it randomly changed position on the board. However, during this trial the stimulus only remained lit for 1 s before it changed to another random location. Every 5 seconds a 5-digit number appeared on the LCD screen. The participant had to verbally recite the five digit number as they continued to strike the lights. The appearance of the digits placed an additional demand on the information processing resources of the participant. The participants were instructed to successfully identify and strike each stimulus before it changed position and score as many strikes as possible within 60 s. The number of successful hits was recorded for each participant. During these 2 reaction tests, participants were instructed to focus their gaze on the center of the light board and utilize their peripheral vision to acquire the lights. (MODE A Hits = ICC: 0.747; SEM: 5.44s; MODE A Average Reaction Time

= ICC: 0.675; SEM: 0.043s; MODE B Hits = ICC: 0.734; SEM: 8.57s; MODE B Average Reaction Time = ICC: 0.717; SEM: 0.03s: Wells et al., 2014).

The lower body reaction testing consisted of a 20-second reaction test on the Quick Board™ (The Quick Board, LLC, Memphis, TN) reaction timer. Participants stood on a board of five circles, in a 2 x 1 x 2 pattern. Participants straddled the middle circle and reacted to a visual stimulus located on a display box that depicts one of five potential lights that corresponds with the circles on the board. Upon illumination of a light, the participant attempted to strike the corresponding circle on the board with their foot. Upon a successful "hit" with the foot, the next light appeared. The total number of successful attempts during the 20-second test and the average time between the activation of the light and the response to the corresponding circle were recorded.

Cognitive Function Measurements

Cognitive function was assessed using a Cave Automatic Virtual Environment (CAVE) system (NeuroTracker, CogniSens, Montreal, Quebec). The CAVE consists of a 2.4 m × 2.4 m × 2.4 m room that includes a projection screen on the front wall which serves as the surface for image projection. A three-dimensional image of 8 yellow balls was projected onto the screen. Four of the balls turned to a grey color for 3 seconds then returned to their normal color. Participants were instructed to track the 4 balls that were grey. The balls moved in three-dimensions for 8 seconds. If the participant correctly identified the four balls at the end of the 8 seconds the speed increased for the next trial. If the participant incorrectly identified any of the

balls the speed of ball movement decreased for the next trial. Each participant performed 20 trials per session. During each trial participants wore three-dimensional glasses. The velocity of movement that was most successful was recorded as the score.

A modified version of the original Serial Sevens Test (Smith, 1967) was the second cognitive function test. This test consists of a two-minute timed oral test in which participants were required to subtract the number 7 from a random computer generated four digit number, in order to measure how quickly and accurately they could compute a simple mathematical problem. The computer generated numbers were written onto standard note cards. Participants were given a randomized stack of note cards and asked to complete as many calculations as possible in the two minute period. Participant and scorer sat opposite each other during testing. The answers to the calculations were written on the back of the note cards in pencil for the scorer to see. Participants were not able to see the correct answer. Once the participant released the note card, their answer was considered unchangeable. The number of correct answers and the average time per correct answer was recorded.

Statistical Analysis

All data is reported as mean ± standard deviation. All reaction and cognitive data was analyzed utilizing a two-way (time x treatment) repeated measures analysis of variance (ANOVA). If no significance was found using the ANOVA, then Hopkin's Magnitude Based Inferences were used. To make inferences on true effects of the different treatment modalities on cognitive function and reaction time, an analysis based on the magnitude of differences,

calculated from 90% confidence intervals, as described by Batterham and Hopkins (2006) were used in this study. Changes between the different trials were analyzed to assess differences between groups (DHY, ED, LD and HD). These values were then analyzed via a published spreadsheet (Hopkins, 2007), with the smallest non-trivial change set at 20% of the grand standard deviation (Batterham & Hopkins, 2006). All data is expressed with percent chances of a positive, trivial and negative outcome. Qualitative inferences, based on quantitative chances were assessed as: <1% almost certainly not, 1-5% very unlikely, 5-25% unlikely, 25-75% possibly, 75-95% likely, 95-99% very likely and >99% almost certainly (Hopkins, 2002).

CHAPTER IV

Results

The temperature and relative humidity for all trials were consistent (22.9 ± 0.3º C, and 44.2 ± 1.3%, respectively). During the DHY trial subjects lost 1.7 ± 0.23 kg of body mass during the 60 min run. This represented 2.4% body weight loss. This was significantly more than that seen during all other trials (Figure 2). No other significant differences between trials were noted. Fluid intake was the same for all trials (1 L).

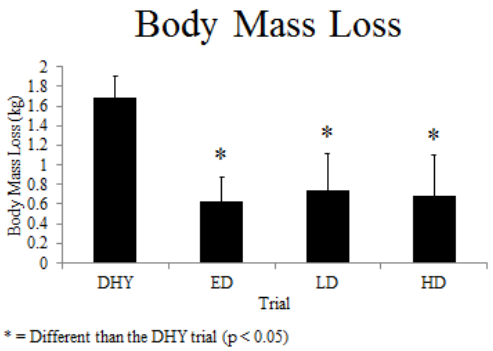

Figure 2. Body weight losses for all four run trials.

Changes in visual, motor and physical reaction times to a visual stimulus can be observed in Figures 3a-c, respectively. Inferential analysis indicated that physical reaction time was likely faster for LD than HD (see Table 1) No other differences were noted between trials in reaction performance.

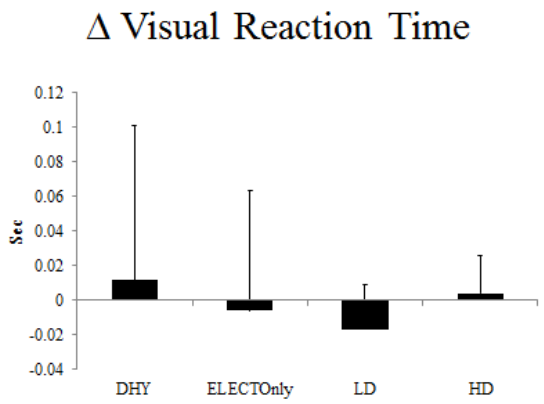

Figure 3a. Changes in Visual Reaction Time between dehydration and drinking trials.

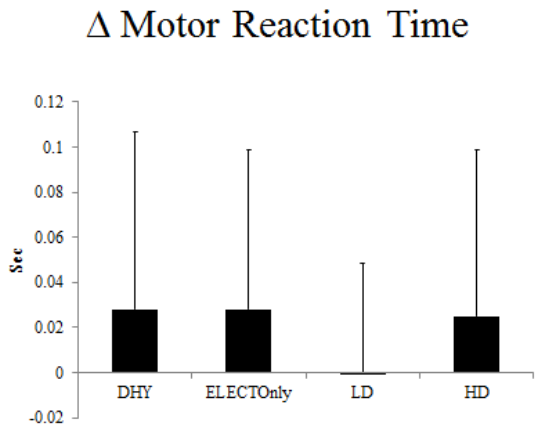

Figure 3b. Changes in Motor Reaction Time between dehydration and drinking trials.

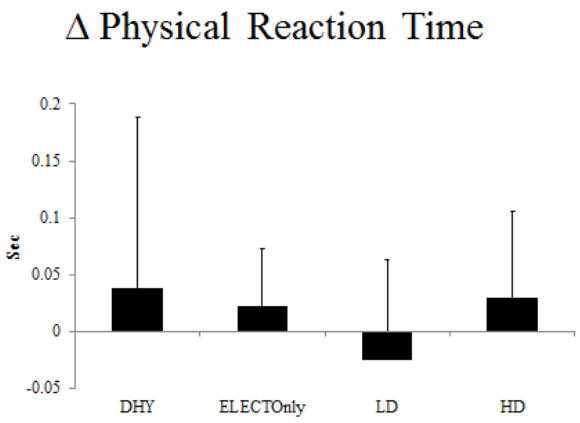

Figure 3c. Changes in Physical Reaction Time between dehydration and drinking trials.

Table 1. Hopkins Magnitude Based Inferences of the reaction time test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
RT Visual Avg Δ	*Dehydration vs Low Dose*	0.01 ± 0.09	-0.02 ± 0.03	0.305	0.01	76.89	13.64	9.47	0.03 ± 0.049	Unclear
RT Visual Avg Δ	*Dehydration vs Gatorade*	0.01 ± 0.09	-0.01 ± 0.07	0.666	0.01	59.69	13.42	26.89	0.02 ± 0.078	Unclear
RT Visual Avg Δ	*Dehydration vs High Dose*	0.01 ± 0.09	0 ± 0.02	0.779	0.01	51.52	18.38	30.09	0.01 ± 0.06	Unclear
RT Visual Avg Δ	*Low Dose vs Gatorade*	-0.02 ± 0.03	-0.01 ± 0.07	0.666	0.01	21.20	26.46	52.34	-0.01 ± 0.039	Unclear
RT Visual Avg Δ	*Low Dose vs High Dose*	-0.02 ± 0.03	0 ± 0.02	0.57	0.01	20.91	16.42	62.67	-0.02 ± 0.06	Unclear
RT Visual Avg Δ	*Gatorade vs High Dose*	-0.01 ± 0.07	0 ± 0.02	0.622	0.01	18.09	29.23	52.68	-0.01 ± 0.034	Unclear
RT Motor Avg Δ	*Dehydration vs Low Dose*	0.02833 ± 0.07895	-0.00083 ± 0.04889	0.338	0.01	73.24	16.89	9.87	0.029 ± 0.051	Unclear
RT Motor Avg Δ	*Dehydration vs Gatorade*	0.02833 ± 0.07895	0.0275 ± 0.07098	0.978	0.01	37.38	27.34	35.29	0.00083 ± 0.05	Unclear
RT Motor Avg Δ	*Dehydration vs High Dose*	0.02833 ± 0.07895	0.025 ± 0.07379	0.879	0.01	37.39	35.94	26.67	0.0033 ± 0.037	Unclear
RT Motor Avg Δ	*Low Dose vs Gatorade*	-0.00083 ± 0.04889	0.0275 ± 0.07098	0.978	0.01	48.48	0.82	50.71	-0.028 ± 1.7	Unclear
RT Motor Avg Δ	*Low Dose vs High Dose*	-0.00083 ± 0.04889	0.025 ± 0.07379	0.286	0.01	6.97	19.07	73.96	-0.026 ± 0.041	Unclear
RT Motor Avg Δ	*Gatorade vs High Dose*	0.0275 ± 0.07098	0.025 ± 0.07379	0.901	0.01	34.67	39.29	26.04	0.0025 ± 0.034	Unclear
RT Physical Avg Δ	*Dehydration vs Low Dose*	0.04 ± 0.15	-0.03 ± 0.05	0.212	0.01	84.60	8.36	7.04	0.07 ± 0.093	Unclear
RT Physical Avg Δ	*Dehydration vs Gatorade*	0.04 ± 0.15	0.02 ± 0.09	0.779	0.01	53.82	14.04	32.14	0.02 ± 0.12	Unclear
RT Physical Avg Δ	*Dehydration vs High Dose*	0.04 ± 0.15	0.03 ± 0.08	0.788	0.01	46.61	26.68	26.71	0.01 ± 0.063	Unclear
RT Physical Avg Δ	*Low Dose vs Gatorade*	-0.03 ± 0.05	0.02 ± 0.09	0.779	0.01	36.18	5.64	58.18	-0.05 ± 0.3	Unclear
RT Physical Avg Δ	*Low Dose vs High Dose*	-0.03 ± 0.05	0.03 ± 0.08	0.097	0.01	2.31	7.20	90.49	-0.06 ± 0.059	Likely Negative
RT Physical Avg Δ	*Gatorade vs High Dose*	0.02 ± 0.09	0.03 ± 0.08	0.839	0.01	31.96	20.60	47.44	-0.01 ± 0.084	Unclear

Differences in number of successful hits during the MODE A assessment are depicted in Figure 4. Inferential analysis (see Table 2) indicated that DHY had a possible negative effect on the number of hits in 60-sec compared to both LD and HD. Results between DHY and ED were unclear. Similarly, comparisons between ED and HD ingestion appeared to be possibly negative, suggesting that high dose glutamine and alanine ingestion provide a possible advantage in

number of successful hits in a 60-sec reaction test. Differences in number of successful hits during the MODE B assessment can be observed in Figure 5. Inferential analysis of the differences between trials on MODE B hits (see Table 3) indicated that performance differences between the trials were unclear. Differences in lower body reaction time can be observed in Figure 6. Inferential analysis (see Table 4) indicates that performance in both LD and HD were likely improved in comparison to DHY. All other comparisons for changes in lower body quickness appeared to be unclear.

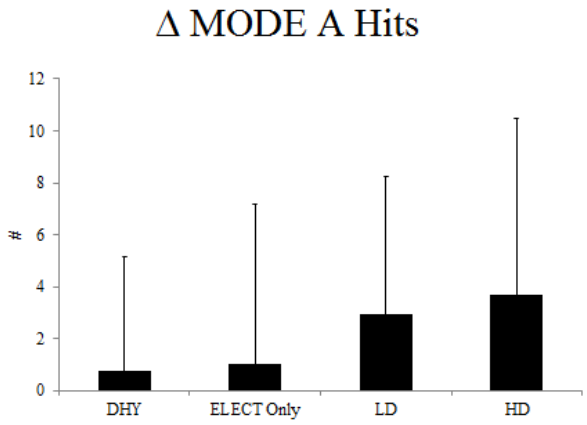

Figure 4. Changes in number of hits in 60 sec between dehydration and drinking trials.

Table 2. Hopkins Magnitude Based Inferences of the MODE A test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
Mode A Hits Δ	*Dehydration vs Low Dose*	0.75 ± 4.39	2.92 ± 5.26	0.318	1.75	3.88	38.43	57.68	-2.2 ± 3.6	Possibly Negative
Mode A Hits Δ	*Dehydration vs Gatorade*	0.75 ± 4.39	1 ± 6.22	0.919	1.75	20.83	52.12	27.04	-0.25 ± 4.2	Unclear
Mode A Hits Δ	*Dehydration vs High Dose*	0.75 ± 4.39	3.67 ± 6.79	0.191	1.75	2.09	27.70	70.21	-2.9 ± 3.7	Possibly Negative
Mode A Hits Δ	*Low Dose vs Gatorade*	2.92 ± 5.26	1 ± 6.22	0.919	1.75	50.35	7.39	42.26	1.9 ± 32	Unclear
Mode A Hits Δ	*Low Dose vs High Dose*	2.92 ± 5.26	3.67 ± 6.79	0.315	1.75	0.11	90.77	9.11	-0.75 ± 1.3	Likely Trivial
Mode A Hits Δ	*Gatorade vs High Dose*	1 ± 6.22	3.67 ± 6.79	0.239	1.75	2.87	31.25	65.88	-2.7 ± 3.8	Possibly Negative
Mode A Avg Δ	*Dehydration vs Low Dose*	-0.003 ± 0.037	-0.023 ± 0.044	0.251	0.01	61.93	35.45	2.61	0.02 ± 0.029	Possibly Positive
Mode A Avg Δ	*Dehydration vs Gatorade*	-0.003 ± 0.037	-0.005 ± 0.063	0.938	0.01	31.14	42.91	25.95	0.002 ± 0.044	Unclear
Mode A Avg Δ	*Dehydration vs High Dose*	-0.003 ± 0.037	-0.028 ± 0.061	0.22	0.01	69.46	27.71	2.83	0.025 ± 0.034	Possibly Positive
Mode A Avg Δ	*Low Dose vs Gatorade*	-0.023 ± 0.044	-0.005 ± 0.063	0.938	0.01	44.41	5.04	50.55	-0.018 ± 0.4	Unclear
Mode A Avg Δ	*Low Dose vs High Dose*	-0.023 ± 0.044	-0.028 ± 0.061	0.302	0.01	2.51	97.47	0.02	0.005 ± 0.0081	Very Likely Trivial
Mode A Avg Δ	*Gatorade vs High Dose*	-0.005 ± 0.063	-0.028 ± 0.061	0.206	0.01	67.69	30.14	2.17	0.023 ± 0.03	Possibly Positive

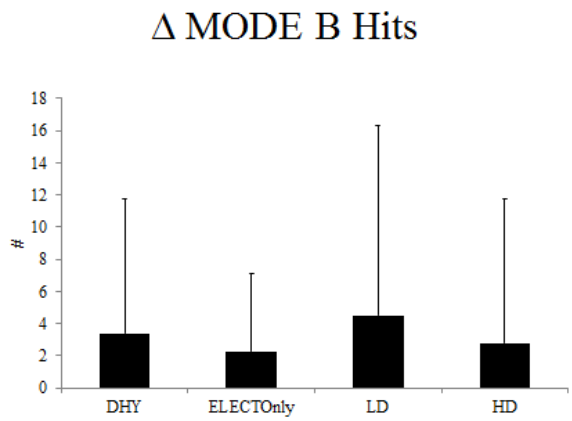

Figure 5. Changes in hits with a cognitive stimulus between dehydration and drinking trials.

Table 3. Hopkins Magnitude Based Inferences of the MODE B test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
Mode B Hits Δ	*Dehydration vs Low Dose*	3.42 ± 8.43	4.5 ± 4.81	0.7	2.46	10.62	58.32	31.06	-1.1 ± 4.7	Unclear
Mode B Hits Δ	*Dehydration vs Gatorade*	3.42 ± 8.43	2.25 ± 11.82	0.747	2.46	36.06	47.88	16.05	1.2 ± 6.1	Unclear
Mode B Hits Δ	*Dehydration vs High Dose*	3.42 ± 8.43	2.75 ± 8.97	0.858	2.46	31.57	48.20	20.22	0.67 ± 6.3	Unclear
Mode B Hits Δ	*Low Dose vs Gatorade*	4.5 ± 4.81	2.25 ± 11.82	0.747	2.46	48.77	26.19	25.04	2.3 ± 12	Unclear
Mode B Hits Δ	*Low Dose vs High Dose*	4.5 ± 4.81	2.75 ± 8.97	0.544	2.46	40.19	52.22	7.59	1.8 ± 4.9	Unclear
Mode B Hits Δ	*Gatorade vs High Dose*	2.25 ± 11.82	2.75 ± 8.97	0.913	2.46	25.96	40.61	33.43	-0.5 ± 7.8	Unclear
Mode B Avg Δ	*Dehydration vs Low Dose*	-0.0233 ± 0.0398	-0.0158 ± 0.032	0.543	0.01	8.13	49.99	41.89	-0.0075 ± 0.021	Unclear
Mode B Avg Δ	*Dehydration vs Gatorade*	-0.0233 ± 0.0398	0 ± 0.0369	0.15	0.01	2.22	18.03	79.75	-0.023 ± 0.027	Likely Negative
Mode B Avg Δ	*Dehydration vs High Dose*	-0.0233 ± 0.0398	0 ± 0.0226	0.113	0.01	1.37	16.50	82.12	-0.023 ± 0.024	Likely Negative
Mode B Avg Δ	*Low Dose vs Gatorade*	-0.0158 ± 0.032	0 ± 0.0369	0.15	0.01	1.16	28.39	70.44	-0.016 ± 0.018	Possibly Negative
Mode B Avg Δ	*Low Dose vs High Dose*	-0.0158 ± 0.032	0 ± 0.0226	0.306	0.01	5.03	30.22	64.75	-0.016 ± 0.026	Unclear
Mode B Avg Δ	*Gatorade vs High Dose*	0 ± 0.0369	0 ± 0.0226	1	0.01	N/A	N/A	N/A	N/A	No Difference

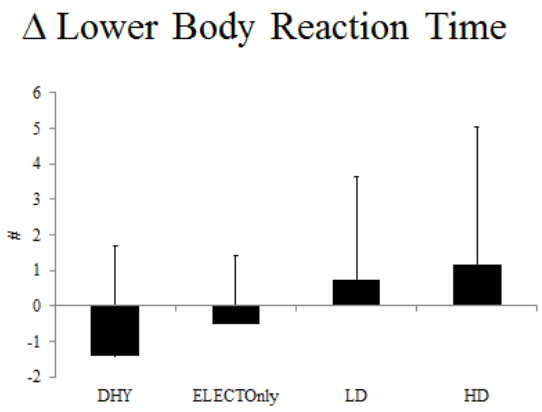

Figure 6. Changes in lower body quickness between dehydration and drinking trials.

Table 4. Hopkins Magnitude Based Inferences of the lower body reaction time test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
QuickBoard Hits Δ	*Dehydration vs Low Dose*	-1.42 ± 3.12	0.75 ± 1.91	0.098	0.59	1.93	9.16	88.90	-2.2 ± 2.2	Likely Negative
QuickBoard Hits Δ	*Dehydration vs Gatorade*	-1.42 ± 3.12	-0.5 ± 2.88	0.528	0.59	15.18	25.83	58.99	-0.92 ± 2.5	Unclear
QuickBoard Hits Δ	*Dehydration vs High Dose*	-1.42 ± 3.12	1.17 ± 3.88	0.087	0.59	1.92	7.10	90.99	-2.6 ± 2.5	Likely Negative
QuickBoard Hits Δ	*Low Dose vs Gatorade*	0.75 ± 1.91	-0.5 ± 2.88	0.528	0.59	63.09	19.15	17.75	1.3 ± 3.3	Unclear
QuickBoard Hits Δ	*Low Dose vs High Dose*	0.75 ± 1.91	1.17 ± 3.88	0.195	0.59	0.19	70.12	29.69	-0.42 ± 0.54	Possibly Trivial
QuickBoard Hits Δ	*Gatorade vs High Dose*	-0.5 ± 2.88	1.17 ± 3.88	0.334	0.59	9.72	16.75	73.53	-1.7 ± 2.9	Unclear

Figure 7 compares differences between trials in multiple object tracking. Inferential analysis indicated a possible greater performance for DHY and LD compared to ED., while there was a likely greater performance in multiple object tracking for HD compared to consumption of

the electrolyte drink only. All other comparisons appeared to be unclear (Table 5)

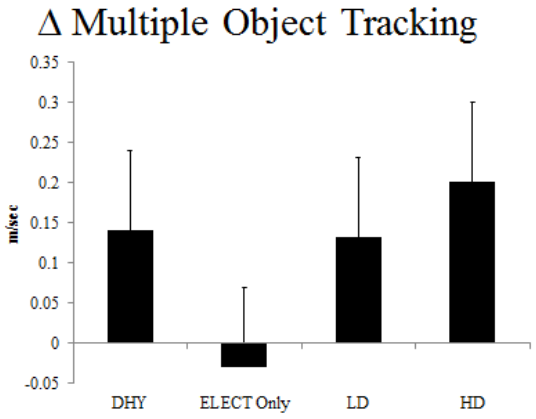

Figure 7. Changes in multiple object tracking between dehydration and drinking trials.

Table 5. Hopkins Magnitude Based Inferences of the multiple object tracking test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
NeuroTracker Δ	*Dehydration vs Low Dose*	0.14 ± 0.257	0.132 ± 0.314	0.956	0.11	24.48	54.33	21.19	0.008 ± 0.24	Unclear
NeuroTracker Δ	*Dehydration vs Gatorade*	0.14 ± 0.257	-0.033 ± 0.33	0.292	0.11	65.59	29.72	4.70	0.17 ± 0.28	Possibly Positive
NeuroTracker Δ	*Dehydration vs High Dose*	0.14 ± 0.257	0.201 ± 0.402	0.671	0.11	12.32	50.47	37.20	-0.061 ± 0.24	Unclear
NeuroTracker Δ	*Low Dose vs Gatorade*	0.132 ± 0.314	-0.033 ± 0.33	0.292	0.11	64.40	31.18	4.42	0.17 ± 0.26	Possibly Positive
NeuroTracker Δ	*Low Dose vs High Dose*	0.132 ± 0.314	0.201 ± 0.402	0.275	0.11	0.45	72.83	26.72	-0.069 ± 0.11	Possibly Trivial
NeuroTracker Δ	*Gatorade vs High Dose*	-0.033 ± 0.33	0.201 ± 0.402	0.061	0.11	0.44	14.46	85.10	-0.23 ± 0.2	Likely Negative

Inferential comparisons on the serial subtraction test can be observed in Table 6. Results indicated that performance in the serial subtraction test was possibly greater in the ED trial compared to DHY. No other differences were noted between any of the other comparisons.

Table 6. Hopkins Magnitude Based Inferences of the serial subtraction test.

Mechanistic Interpretations		Paired T-test; CI = 90%				Percent				
		Group 1	Group 2	P - Value	Ind. SE Diff./Thresh.	Positive	Trivial	Negative	Mean Difference	Interpretation
Serial Sub Correct Δ	*Dehydration vs Low Dose*	1.25 ± 5.029	3.083 ± 5.961	0.428	1.58	7.33	38.23	54.45	-1.8 ± 3.9	Unclear
Serial Sub Correct Δ	*Dehydration vs Gatorade*	1.25 ± 5.029	3.917 ± 4.738	0.197	1.58	2.27	27.33	70.40	-2.7 ± 3.4	Possibly Negative
Serial Sub Correct Δ	*Dehydration vs High Dose*	1.25 ± 5.029	3.917 ± 7.305	0.405	1.58	9.52	27.08	63.40	-2.7 ± 5.4	Unclear
Serial Sub Correct Δ	*Low Dose vs Gatorade*	3.083 ± 5.961	3.917 ± 4.738	0.197	1.58	0.04	87.54	12.41	-0.83 ± 1.1	Likely Trivial
Serial Sub Correct Δ	*Low Dose vs High Dose*	3.083 ± 5.961	3.917 ± 7.305	0.763	1.58	19.32	41.27	39.40	-0.83 ± 4.7	Unclear
Serial Sub Correct Δ	*Gatorade vs High Dose*	3.917 ± 4.738	3.917 ± 7.305	1	1.58	N/A	N/A	N/A	N/A	No Difference
Serial Sub Sec/Corr Δ	*Dehydration vs Low Dose*	-0.173 ± 0.774	-0.26 ± 0.468	0.751	0.17	38.15	44.20	17.66	0.087 ± 0.46	Unclear
Serial Sub Sec/Corr Δ	*Dehydration vs Gatorade*	-0.173 ± 0.774	-0.406 ± 0.427	0.374	0.17	59.62	33.84	6.54	0.23 ± 0.44	Unclear
Serial Sub Sec/Corr Δ	*Dehydration vs High Dose*	-0.173 ± 0.774	-0.24 ± 0.628	0.858	0.17	39.23	34.27	26.50	0.067 ± 0.64	Unclear
Serial Sub Sec/Corr Δ	*Low Dose vs Gatorade*	-0.26 ± 0.468	-0.406 ± 0.427	0.374	0.17	44.22	52.67	3.11	0.15 ± 0.28	Possibly Trivial
Serial Sub Sec/Corr Δ	*Low Dose vs High Dose*	-0.26 ± 0.468	-0.24 ± 0.628	0.516	0.17	0.00	100.00	0.00	-0.02 ± 0.052	Most Likely Trivial
Serial Sub Sec/Corr Δ	*Gatorade vs High Dose*	-0.406 ± 0.427	-0.24 ± 0.628	0.497	0.17	8.83	41.78	49.40	-0.17 ± 0.41	Unclear

CHAPTER V

Discussion

Results of this study indicated that participants performing the exercise protocol and not rehydrating lost approximately 2.4% of their body mass, which was significantly greater than that observed during the other trials. Participants consuming the alanine-glutamine dipeptide (both LD and HD) appeared to possibly enhance their performance to successfully react to multiple visual stimuli in 60-sec (MODE A assessment) following the exercise protocol more so than in DHY. In addition, ingestion of a high dose of the alanine-glutamine dipeptide (HD trial) also appeared to enhance performance in the MODE A measure following exercise to a greater extent than the commercial sports drink (ED trial) only. In addition, lower body reaction time to a visual stimulus was likely better during LD and HD compared to DHY. Although this magnitude of dehydration did not appear to impact cognitive performance (as seen in MODE B and the serial subtraction tests), there did appear to be a likely benefit for greater performance in tracking multiple objects with ingestion of HD compared to ED only. These results are similar to previous research by Hoffman and colleagues (2012) that reported that alanine-glutamine ingestion was able to enhance visual reaction time significantly greater than when subjects were dehydrated. The magnitude of the body water deficit between this present study and the previous study by Hoffman et al. (2012) were similar (2.4% versus 2.3%, respectively). The major differences between these studies were the mode of exercise and the medium that the supplement was delivered in. The former study examined reaction performance following a competitive

basketball game, while this present study examined performance following prolonged endurance exercise and a bout of high intensity exercise performed until exhaustion. In addition, in the former study participants consumed the dipeptide dissolved in water, whereas in the present study a commercial sport drink containing electrolytes was used.

Previous studies have indicated that body water deficits of 1.6% - 3% have been shown to decrease cognitive performance (Cian, Barraud, Melin, & Raphel, 2001; Ganio et al., 2011; Lieberman et al., 2005; Tomporowski, Beasman, Ganio, & Cureton, 2007). However, decrements in cognitive performance at the lower magnitudes of dehydration appear to occur when dehydration occurs from the combination of a diuretic and exercise (Ganio et al., 2011). When dehydration occurs through exercise only, it appears that loss of cognitive ability is only seen when dehydration is between 2% - 3% of body weight loss (Cian et al., 2001; Lieberman et al., 2005; Tomporowski, Beasman, Ganio, & Cureton, 2007). Considering that the magnitude of body water deficit in this study was at 2.4%, this may not have reached the threshold level necessary to cause cognitive function loss. Our results though do support the deleterious effects associated with low to moderate levels of dehydration on fine motor control and reaction time (Baker, Dougherty, Chow, & Kenney, 2007; Hoffman et al., 1995; 2012).

The results of this study do support our previous work that demonstrated that the alanine-glutamine dipeptide mixed in water is more effective than water only in maintaining fine motor control and reaction time in competitive and recreational athletes (Hoffman et al., 2010; 2012). The mechanism suggested for these effects is focused on the ability of the alanine-glutamine dipeptide to enhance both fluid and electrolyte absorption in the gut (Lima et al., 2002). These

findings have also been confirmed by others (Harris et al., 2012), and suggest that during activity lasting for at least an hour the ability to enhance fluid and/or electrolyte uptake may allow athletes to maintain fine motor control and reaction ability. Interestingly, these studies have used water only as the ingestion medium. Considering that the alanine-glutamine dipeptide can enhance electrolyte absorption as well, it was interesting to explore the potential benefits of consuming the dipeptide combined with an electrolyte containing commercial sports drink and determine whether it would provide a greater benefit than an electrolyte drink by itself. The results of this present study indicate that when the alanine-glutamine dipeptide is combined with a commercial sports electrolyte drink the ergogenic benefits are greater than that seen with a commercial sports electrolyte drink only. Therefore, it appears that consumption of a commercial sports drink with the alanine-glutamine dipeptide enhances fluid and electrolyte absorption greater than that seen from an electrolyte drink only. The benefits of a greater electrolyte absorption by skeletal muscle may be related to enhanced motor unit recruitment patterns and muscle contractility (Sjogaard, 1986). During an activity requiring fine motor control, these performance decrements may become more sensitive to a dehydration stress. Thus, the greater absorption capability seen during the alanine-glutamine ingestion trials likely contributed to the ergogenic effects noted in this study, and contributed to the likely benefit noted between ED and HD during the MODE A measure. It is possible that the higher concentration of the alanine-glutamine dipeptide in the HD trial was able to achieve a threshold effect that was not seen in the comparison between LD and HD.

In conclusion, rehydration with the alanine-glutamine dipeptide during an hour run at a submaximal intensity appears to maintain or enhance subsequent visual reaction time in both

upper and lower body activities compare to a no hydration trial. These same effects were not apparent when participants consumed the commercial sports electrolyte drink only, suggesting that the combination of the alanine-glutamine dipeptide enhanced fluid and electrolyte absorption from the gut and possibly into skeletal tissue to maintain neuromuscular performance. Differences between groups regarding cognitive function were unclear, indicating that at this low to mild level of body fluid deficit no advantage was noted between any of the hydration methods examined in this study.

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 ENGLAND 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
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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

B-254c

RECEIVED JUL 26 2013

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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1

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

Approved by NEIRB on 8/1/13
As Is ✓ As Revised ___ Initials

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

Approved by ~~NEIRB~~ on 8/1/13
As Is___ As Revised___ Initials

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

PAR-Q & YOU

13-254cc
RECEIVED JUL 26 2013

(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 and 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 any other reason 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 all 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.

Informed Use of the PAR-Q: 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

Approved by NEIRB on 8/1/13
As Is ___ As Revised ___ Initials

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.

Approved by NEIRB on 8/6/13 Initials
As Is ✓ As Revised ___

Please contact William McCormack
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