

ORIGINAL ARTICLE

Effect of acute L-Alanyl-L-Glutamine and electrolyte ingestion on cognitive function and reaction time following endurance exercise

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Abstract

The purpose of this study was to examine the effect of the L-Alanyl-L-Glutamine dipeptide (AG) on cognitive function and reaction time (RT) following endurance exercise. Twelve male endurance athletes (23.5 ± 3.7 y; 175.5 ± 5.4 cm; 70.7 ± 7.6 kg) performed four trials, each consisting of running on a treadmill at 70% of VO_2max for 1h, then at 90% of VO_2max until exhaustion. One trial consisted of no hydration (DHY), another required ingestion of only a sports electrolyte drink (ED) and two trials required ingestion of a low dose (LD; $300 \text{ mg} \cdot 500 \text{ ml}^{-1}$) and high dose (HD) of AG ($1 \text{ g} \cdot 500 \text{ ml}^{-1}$) added to the ED. Cognitive function and reaction tests were administered pre- and post-exercise. Magnitude based inferences were used to analyze Δ cognitive function and Δ reaction test data. Results indicated that DHY had a possible negative effect on number of hits in a 60-sec reaction test compared to LD and HD, while ED appeared to have a negative effect compared to HD. Analysis of lower body quickness indicated that LD and HD were likely improved in comparison to DHY. Performance on the serial subtraction test appeared to be possibly better in ED than DHY, while other comparisons between groups regarding cognitive function were unclear. In conclusion, rehydrating with AG during submaximal exercise may maintain or enhance subsequent RT in upper and lower body activities compared to DHY. These same effects were not apparent when participants consumed ED.

Keywords: *Dietary supplement, hydration, dehydration, rehydration, sport drink*

Introduction

Dehydration during exercise will result in changes in physiological function that can result in significant decrements in performance. These changes appear to be related to the magnitude of the 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 may occur 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, Stavsky, & Falk, 1995; Hoffman et al., 2012), levels of hypohydration of approximately 2% (ranging from 1.9% to 2.3%) have been shown to result in significant decrements in activities involving fine motor control (e.g., 8–12.5% difference in shooting percentages) and reaction time (RT) (Hoffman et al., 1995, 2012). This may potentially impact game outcomes as a thirst response does not appear to occur until a body water

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deficit of approximately 2% is reached (Rothstein, Adolph, & Wills, 1947).

Mild levels of hypohydration can not only result in decrements in fine motor control and RT, but may also impair cognitive performance (Ganio et al., 2011; Lieberman et al., 2005; Tomporowski, Beasman, Ganio, & Cureton, 2007). Ganio and colleagues (2011) reported that a combination of diuretic ingestion and exercise induced a -1.59% loss in body weight and a significant decrease in 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 et al., 2007). However, when dehydration (2.6% body weight loss) is induced by water restriction only, cognitive-motor performance may not be effected (Szinnai, Schachinger, Arnaud, Linder, & Keller, 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 a commercially available flavoured sport electrolyte drink (ED) is a potential alternative to water only rehydration. The benefit of this rehydration strategy is that it may induce greater hydration (Hubbard, Szlyk, & Armstrong, 1990). It may also have greater importance in preventing hyponatremia, which is a risk associated with drinking large volumes of water only, but does not appear to be an issue in exercise durations less than 3–4 hours in duration (Almond et al., 2005). 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). Previous investigations examining the ergogenic benefits of this dipeptide have provided the supplement in water, whether these effects can be

enhanced when combined with an ED 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 ED to the ED only on RT and cognitive function following prolonged exercise.

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, VO_{2max} : 55.9 ± 5.9 ml·kg·min⁻¹) were recruited for this study. All participants were recruited by flyer or word of mouth throughout the university and the local running community. To be considered for enrolment into the study participants were required to have a history of running at least one-hour in duration, be free of any physical limitations as determined by the Confidential Medical and Activity Questionnaire and Physical Activity Readiness Questionnaire (PAR-Q), be between the ages of 18 and 35, and have a sweat rate that was at or exceeded 1.3 L·hr⁻¹. 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 investigation was a double-blind, randomised, placebo-controlled, crossover study. Participants were asked to report to the University Human Performance Lab (HPL) on six separate visits. The first two visits were preliminary visits (PV1 and PV2) followed by four experimental trial visits. During PV1 participants completed the Confidential Medical and Activity questionnaire, PAR-Q and informed consent. Participants were then 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, familiarisation trials were conducted with the reaction and cognitive function tests. Familiarisation

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 all experimental trials. There was a minimum of 48 hours between PV1 and PV2. During PV2 participants performed a VO_2 peak and lactate threshold test, which determined the treadmill speed for the experimental trials.

The experimental testing protocol occurred on four occasions separated by a minimum of 7 days with a mean of 8.4 ± 3.3 days between each trial. Prior to each trial, participants were weighed in running shorts. During each trial, participants completed a 60 min run at 75% of their previously measured VO_2 peak, followed by a run to volitional exhaustion at 90% of their VO_2 peak. Following the run, participants were towel dried, put on dry running shorts and were weighed again. Participants performed the first trial without any rehydration (DHY). The fluid loss during this session was used to determine the participant's sweat rate ($\text{L}\cdot\text{hr}^{-1}$). The mean sweat rate was $1.68 \pm 0.22 \text{ L}\cdot\text{hr}^{-1}$. This trial was performed first to determine whether the participant fulfilled the sweat rate criteria for participant enrolment. The remaining three trials were performed in a randomised fashion. During each of these trials, participants were provided 250 ml of sports drink every 15 min. The sports drink was a commercial product containing 21 calories, 4.9 g of carbohydrate, 113 mg of sodium and 32 mg of potassium per 250 ml (Gatorade G2, PepsiCo, Purchase, NY). During one of these trials participants consumed a flavoured sports drink (ED) only, while during the other trials participants consumed the alanine-glutamine supplement marketed as Sustamine® (Kyowa Hakko USA, New York, NY) mixed in the same flavoured sport drink at either a low ($300 \text{ mg}\cdot 500 \text{ ml}^{-1}$) or high dose (HD; $1 \text{ g}\cdot 500 \text{ ml}^{-1}$; low dose (LD) and HD, respectively). 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 utilising 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 $1.22 \text{ m} \times 1.22 \text{ m}$. The light board contains 64 light (target)

buttons arranged in five concentric circles surrounding a centre LCD screen that can be illuminated to serve as a stimulus for the participant. 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 was located just below eye level.

The first assessment measured the participant's visual, motor and physical RTs to a light with the dominant hand. Participants were told to stand in a comfortable athletic stance centred 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 RT was measured as the amount of time it took 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 RT is a measurement of the total elapsed time from the introduction of the target light to the physical completion of the task (visual + motor RT). All measures were to the 1/100's of a second. This was repeated ten times per assessment. (visual RT = ICC: 0.835; SEM: 0.033s; motor RT = ICC: 0.632; SEM: 0.035s) [18].

The second assessment measured the participant's ability to react to a light as it randomly changed position on the board (MODE A). 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 sec. The number of hits was 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 (MODE B). However, during this trial the stimulus only remained lit for 1 sec before it changed to another random location. Every 5 sec 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 sec. The number of successful hits was recorded for each participant. During these two reaction tests, participants were instructed to focus their gaze on the centre of the light board and utilise their peripheral vision to acquire the lights. (MODE A Hits = ICC: 0.747; SEM: 5.44s; MODE B Hits = ICC: 0.734; SEM: 8.57s (Wells et al., 2014).

Lower body reaction testing consisted of a 20-second reaction test on the Quick Board™ reaction timer (The Quick Board, LLC, Memphis, TN). Participants stood on a board of five circles, in a $2 \times 1 \times 2$ pattern. Participants straddled the middle circle and reacted to a visual stimulus located on a display box that depicted one of five potential lights that corresponded 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 hits during the 20-second test was recorded.

Cognitive function

A modified version of the original Serial Sevens Test (Smith, 1967) was used to assess cognitive function. 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 randomised 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 was recorded.

Statistical analysis

Data were analyzed using magnitude based inferences, calculated from 90% confidence intervals, as previously described (Batterham & Hopkins, 2006). Change scores were analyzed using the p -value from dependent t -test to determine a mechanistic inference utilising a published spreadsheet (Hopkins, 2007). Qualitative inferences were based upon the chances that the true magnitude of the effect at POST were substantially greater or smaller

than baseline values (PRE), and were assessed as: <1% almost certainly smaller, 1–5% very likely smaller, 5–25% likely smaller, 25–75% possibly greater, 75–95% likely greater, 95–99% very likely greater and >99% almost certainly greater (Hopkins, 2002). If there was a greater than 5% chance that the true value was both greater and smaller, the effect was considered mechanistically unclear. The smallest non-trivial change, or smallest worthwhile change, was set at 20% of the grand standard deviation for all PRE-values (Batterham & Hopkins, 2006). Using previously described procedures (Gravetter & Wallnau, 1996) for estimating sample sizes for repeated measures designs, a sample size of 12 of each group resulted in a statistical power ($1-\beta$) of > 0.90 based on the changes in reaction performance from alanine-glutamine ingestion previously reported (Hoffman et al., 2012).

Results

The temperature and relative humidity for all trials was consistent ($22.9 \pm 0.3^\circ\text{C}$, and $44.2 \pm 1.3\%$, respectively). No adverse events were reported by any participant during the study. During the no hydration (DHY) trial subjects lost 1.7 ± 0.23 kg (2.4% body weight) of body mass during the 60 min run. Body weight loss during the DHY trial was significantly greater ($p < 0.05$) than that seen during ED (0.7 ± 0.4 kg), LD (0.6 ± 0.2 kg) and HD (0.7 ± 0.4 kg). No other significant differences in body weight loss between trials were noted. Fluid intake was the same (1 L) for all trials in which fluid was consumed (ED, LD and HD). Time to exhaustion during the 90% of VO_2max run was significantly longer during the LD and HD trials compared to DHY. These results though are reported elsewhere (McCormack et al., *in press*).

Changes in visual, motor and physical RTs to a visual stimulus can be observed in Figures 1a–c, respectively. Inferential analysis indicated that physical RT was likely faster for LD than HD (mean difference: -0.06 ± 0.059 sec) No other differences were noted between trials in reaction performance.

Differences in the number of successful hits during the MODE A assessment are depicted in Figure 2. Inferential analysis indicated that DHY had a possible negative effect on the number of hits in 60-sec compared to both LD (-2.2 ± 3.6 hits) and HD (-0.25 ± 4.2 hits). Results between DHY and ED were unclear. Similarly, comparisons between ED and HD ingestion appeared to be possibly negative (-2.7 ± 3.8 hits), suggesting that HD alanine-glutamine ingestion provided a possible advantage in the number of successful hits in a 60-sec reaction test.

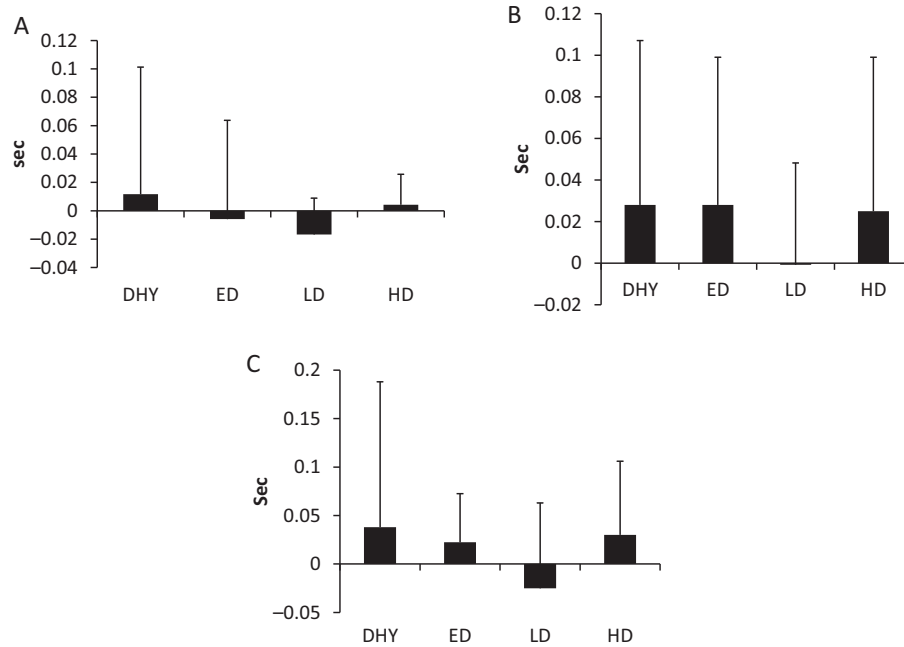


Figure 1. Visual (A), Motor (B) and Physical (C) RT. DHY, dehydration trial; ED, electrolyte sports drink trial; LD, low dose alanine-glutamine; HD, high dose alanine-glutamine.

Differences in number of successful hits during the MODE B assessment can be observed in Figure 3. No decreases in MODE B performance were noted in any trial. Inferential analysis of the differences between trials on MODE B hits indicated that performance differences between the trials were unclear. Differences in lower body RT can be observed in Figure 4. Inferential analysis indicated that performance in both LD (2.2 ± 2.2 hits) and HD (2.6 ± 2.5 hits) were likely improved in comparison to DHY. All other comparisons for changes in lower body quickness appeared to be unclear.

Cognitive performance as determined by the serial subtraction was maintained during all trials. During DHY participants increased the number of correct answers by 1.25 ± 5.02 , while during ED the number of correct answers increased by 3.92 ± 4.74 . In the LD and HD trials the number of correct answers increased by 3.08 ± 5.96 and 3.98 ± 7.3 , respectively. Inferential comparisons on the serial subtraction test indicated that performance in the serial subtraction test was possibly greater in the ED trial compared to DHY (2.7 ± 3.4 correct answers). No other differences between trials were noted.

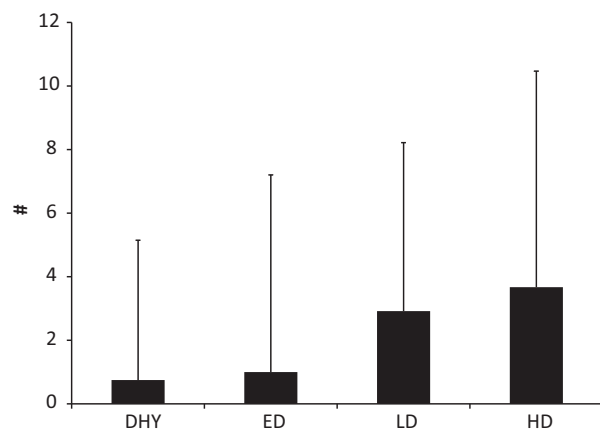


Figure 2. Mode A reaction test. DHY, dehydration trial; ED, electrolyte sports drink trial; LD, low dose alanine-glutamine; HD, high dose alanine-glutamine.

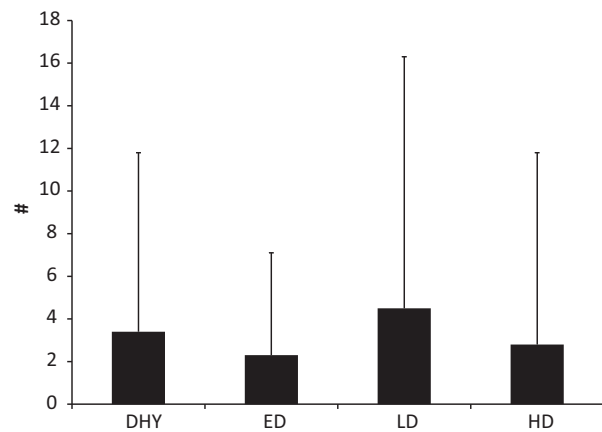


Figure 3. Mode B reaction test. DHY, dehydration trial; ED, electrolyte sports drink trial; LD, low dose alanine-glutamine; HD, high dose alanine-glutamine.

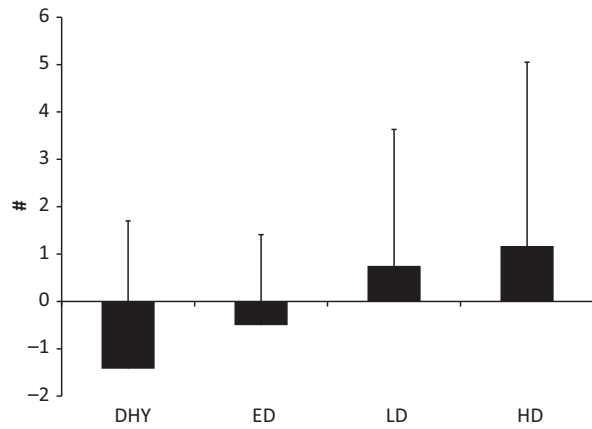


Figure 4. Lower body reaction test. DHY, dehydration trial; ED, electrolyte sports drink trial; LD, low dose alanine-glutamine; HD, high dose alanine-glutamine.

Discussion

The results of this study indicated that rehydrating with the alanine-glutamine dipeptide in a commercially available sports drink (both LD and HD) may possibly enhance reactive ability to multiple visual stimuli in a 60-sec test (MODE A) compared to DHY. In addition, ingestion of the higher dose of the alanine-glutamine dipeptide also appeared to enhance performance in the MODE A measure following exercise to a greater extent than ingestion of the commercial sports drink (ED) only. In addition, lower body RT to a visual stimulus was likely better during LD and HD compared to DHY. This magnitude of dehydration though did not appear to impact cognitive performance (as seen in MODE B and the serial subtraction test). These results are similar to a previous investigation that reported a significantly greater reaction scores in athletes ingesting the alanine-glutamine dipeptide mixed in water compared to when they were dehydrated (Hoffman et al., 2012). The magnitude of the body water deficit between this present study and the previous study were similar (2.4% versus 2.3%, respectively). The major differences between these studies were the mode of exercise and the medium in which the supplement was delivered. The study by Hoffman and colleagues (2012) 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 et al., 2007). However, decrements in cognitive performance at the lower magnitudes of dehydration appear to occur only 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% and 3% of body weight loss (Cian et al., 2001; Lieberman et al., 2005; Tomporowski et al., 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 RT (Baker, Dougherty, Chow, & Kenney, 2007; Hoffman et al., 1995, 2012).

The findings from this study support previous investigations demonstrating that the alanine-glutamine dipeptide mixed in water is more effective than water only in maintaining fine motor control and RT 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 from the gut (Lima et al., 2002). These findings have also been confirmed by others (Harris, Hoffman, Allsopp, & Routledge, 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 previously unknown if consuming the dipeptide combined with an electrolyte containing commercial sports drink would provide a greater benefit than an ED by itself. The results of this present study indicate that when the alanine-glutamine dipeptide is combined with a commercial sports drink the ergogenic benefits are greater than that seen with the commercial sports drink only. The mechanisms of the ergogenic benefit are well-understood. However, although speculative, consumption of the dipeptide with electrolytes may have increased electrolyte absorption by skeletal muscle, which may have maintained or enhanced motor unit recruitment patterns and muscle contractility (Sjogaard, 1986). Activities that require fine motor control 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 seen 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 RT 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 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. Future studies appear warranted in examining the effect of the alanine-glutamine dipeptide on electrolyte absorption into skeletal tissue and its role in enhancing RT. 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 regarding cognitive performance.

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