

ACUTE L-ALANYL-L-GLUTAMINE INGESTION DURING SHORT DURATION, HIGH INTENSITY EXERCISE AND A MILD HYDRATION STRESS

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Original scientific paper

UDC: 612:796.015:577-055.1

Abstract:

The effect of acute L-alanyl-L-glutamine (AG) ingestion on selected hormonal and electrolyte measures was examined during repetitive, short duration, high intensity exercise with mild hypohydration. Subjects (20.3±1.1 yrs; 180.3±10.4 cm; 83.1±14.0 kg; 11.6±3.6% body fat) reported to the Human Performance Laboratory on four occasions. During each trial subjects were hypohydrated to -2.5% of their baseline body mass. During one trial (DHY) subjects rested in a recumbent position for 45 minutes before commencing the exercise session. During the other three trials subjects were rehydrated to 1.5% of their baseline body mass, before exercise, by drinking water only (W), or with two different doses of AG – a low dose (LDAG: 0.05 g·kg⁻¹) and a high dose (HDAG: 0.2 g·kg⁻¹). The exercise protocol consisted of ten 10-second sprints on a cycle ergometer with a 1-min rest between each sprint. Blood draws were collected once the subject achieved the desired level of hypohydration, immediately pre-exercise, immediately post-exercise, and 24 hrs post-exercise. Blood samples were analyzed for glutamine, potassium, sodium, aldosterone, arginine vasopressin, C-reactive protein, interleukin-6, malondialdehyde, testosterone, cortisol, ACTH, and growth hormone. The area under the curve (AUC) analysis demonstrated significantly greater sodium concentrations for DHY compared to all other trials. The AUC analysis for aldosterone showed significantly lower concentrations at LDAG compared to DHY. No other differences between trials were observed in any other hormonal or biochemical responses. AG ingestion during a short duration, anaerobic exercise and mild hypohydration stress had a limited effect on selected hormonal and biochemical measures.

Key words: endocrine response, immune response, fluid regulatory hormones, electrolytes, anabolic hormones

Introduction

The effect of hydration stress on the hormonal, immunological and inflammatory response to exercise has been studied in a number of investigations (Hoffman, et al., 1994; 2010; Judelson, et al., 2008; Maresh, et al., 2004; 2006; Penkman, Field, Sellar, Harber, & Bell, 2008). Mild levels of hypohydration (between 2.0% – 3.0% reduction in body mass) have been shown to alter the fluid regulatory hormone response (Maresh, et al., 2004), increase cortisol concentrations, attenuate the response of testosterone response to exercise (Judelson, et al., 2008; Maresh, et al., 2006), and alter the immune response following exercise (Penkman, et al., 2008). This could have potential implications for recovery

from exercise, especially during situations where mild levels of hypohydration may not stimulate a thirst response, and result in an athlete remaining in a hypohydrated state (Rothstein, Adolph, & Wells, 1947). Recent investigations have suggested that an alanine-glutamine (AG) dipeptide may enhance fluid and electrolyte absorption (Hoffman, et al., 2010; Lima, et al., 2002). This may have important implications regarding exercise performance and recovery.

Glutamine is a nonessential amino acid suggested to be effective for antioxidant defense during situations of severe illness (Abilés, et al., 2008; Déchelotte, et al., 2006; Kumar & Anadan, 2007), and as a modulator of the immune response to exercise (Castell & Newsholme, 1998). Glutamine by

itself has also been reported to increase electrolyte and water absorption during dehydration resulting from intestinal infections (Lima, et al., 2002; Nath, et al., 1992; Silva, et al., 1998; van Loon, et al., 1996). However, it has been suggested that glutamine may not be as effective in enhancing absorption when provided as a single amino acid (Li, Xu, Liu, Tan, & Li, 2006). At a low pH, glutamine is not very stable, but its stability is improved when it is combined with another amino acid such as alanine (Fürst, 2001; Lima, et al., 2002).

In consideration of the physiological role that the AG has on fluid absorption, an interesting question arises concerning the benefit of ingesting this glutamine derivative during exercise or sport. This is especially relevant during exercise when athletes are dehydrated and lactic acid concentrations are elevated. Recently, Hoffman and colleagues (2010) demonstrated that ingestion of AG during exhaustive endurance exercise with a mild hydration stress (-2.5% dehydration) can provide a significant ergogenic benefit by increasing the time to exhaustion. In addition, differences in electrolyte and fluid regulatory hormone concentrations were suggestive that the ergogenic effect was mediated by an enhanced fluid and electrolyte uptake. However, AG ingestion did not have any effect on immune, inflammatory, oxidative stress or any other endocrine markers of stress. This may be attributed to the submaximal exercise protocol employed, but whether a similar effect is apparent during short-duration, high intensity exercise has not been examined. Thus, the purpose of this study was to examine the efficacy of rehydrating with AG on the endocrine, immune and inflammatory markers of recovery and the fluid regulatory response during a repetitive, short duration, high intensity exercise protocol following a mild (-2.5%) hypohydration stress.

Methods

Subjects

Ten college-aged males (20.3 ± 1.1 y; 180.3 ± 10.4 cm; 83.1 ± 14.0 kg; $11.6 \pm 3.6\%$ body fat) volunteered to participate in this study. Following an explanation of all procedures, risks and benefits, each subject gave his informed consent to participate in this study. The Institutional Review Board approved the research protocol. Subjects were not permitted to use any nutritional supplements, other than the AG used in this study, for at least four weeks prior to or during the study. Screening for supplement use was accomplished via a health history questionnaire completed during subject recruitment.

Protocol

Prior to the onset of the study subjects reported to the Human Performance Laboratory (HPL)

on two separate occasions in the morning to determine a baseline body mass. Subjects then reported back to the HPL on four separate occasions for participation in the study protocol. Prior to each trial subjects underwent hypohydration to -2.5% of their baseline body mass. In one trial (DHY) subjects achieved their goal weight (-2.5% of baseline body weight) and rested in a recumbent position for 45 minutes before commencing the exercise session. In the other three trials subjects reached their goal weight (-2.5% of baseline body mass) and then rehydrated back to -1.5% of their baseline body mass by drinking either water (W) or water mixed with either a low dose (LDAG) of the AG supplement ($0.05 \text{ g}\cdot\text{kg}^{-1}$) or a high dose (HDAG) of the AG supplement ($0.2 \text{ g}\cdot\text{kg}^{-1}$) prior to exercise. During the hydration trials the exercise protocol began 45 minutes following reaching their goal weight. The trial order was randomly determined.

Hypohydration protocol

Prior to the onset of the study subjects reported to the HPL for the determination of baseline body mass. These measures were performed on nonconsecutive days and occurred approximately one week before the start of experimental testing. Subjects were weighed during these visits in a postabsorptive, euhydrated state to establish a baseline body weight. A urine sample was analyzed for osmolality (U_{osm}) by freezing point depression and urine specific gravity (U_{sg}) by refractometry was used to document euhydration on all preliminary days; $U_{\text{sg}} \leq 1.020$ was defined as euhydration (Armstrong, et al., 1994).

On the night before testing (17:00 hrs) subjects reported to the HPL for body weight and urine specific gravity measures to ascertain that subjects were euhydrated. Subjects were then instructed not to consume any food or water until the next day when they reported back to the HPL (07:00 hrs). This resulted in an average body mass change of $-1.6 \pm 2.1\%$. On the morning of each trial subjects reported to the HPL where they were weighed and then began the active dehydration protocol to achieve the desired weight loss. This required subjects to walk on a motorized treadmill at $3.4 \text{ mi}\cdot\text{h}^{-1}$ and at a 2% incline. Subjects were fully clothed in a tracksuit (long cotton heavy weight fleece sweat pants and top). Nude body weight, heart rate, and rating of perceived exertion (Borg, 1982) were monitored at 20-minute increments. The subjects continued to walk until they (a) had lost 2.5% of their body mass, (b) heart rate value of $\geq 180 \text{ b}\cdot\text{min}^{-1}$, (c) displayed signs or symptoms of an exercise-induced heat illness, or (d) requested to stop due to exercise fatigue. At the end of the hypohydration period, a urine specimen and blood samples were obtained. Dehydration was verified by measuring urine specific gravity and both urine and plasma osmola-

lity. The time necessary per subject to reach the goal body mass (-2.5% weight loss) was 42.8 ± 30.9 min. There were no significant differences in time to reach goal body mass between trials.

Subjects consumed water or water with AG following the dehydration protocol as part of their rehydration to -1.5% of their mass. The AG supplement (either LDAG or HDAG) was mixed with water and was indistinguishable in appearance and taste from the water ingestion trial.

Anaerobic exercise protocol

To quantify anaerobic power performance all subjects performed ten 10 s Wingate anaerobic power tests (WAnT) (Lode Excalibur, Groningen, The Netherlands). After a warm-up period of 5-min pedaling at 60 rpm interspersed with four all-out sprints lasting 5 s, the subject pedaled for 10 s at maximal speed against a constant force. The force setting was $1.2 \text{ N} \cdot \text{kg body mass}^{-1}$. Following a 1-min active rest (pedaling at 60 rpm) subjects performed an additional nine 10 s WAnT using a 6:1 rest:work ratio. The onset of each sprint was from a rolling start that was performed at 75 rpm. Peak power, mean power and a fatigue rate was determined for each sprint. Peak power was defined as the highest mechanical power output elicited during each exercise bout. Mean power was defined as the average mechanical power during each exercise bout, and the rate of fatigue was determined by dividing the highest power output from the lowest power output for each set. The average peak power, average mean power and the average rate of fatigue were determined for each exercise set (10 sprints) per trial.

Blood measures

Prior to the study, subjects reported to the HPL at the same time as all subsequent trials for a baseline (BL) blood draw. During all trials blood draws were taken once the goal body mass was achieved (HHY), immediately prior to the exercise stress (RHY) and immediately following the exercise protocol (IP). Subjects returned to the laboratory 24 hours post exercise for an additional blood draw (24P). All day of trial blood samples (HHY, RHY and IP) were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male luer lock adapter. The cannula was maintained patent that is, open and unobstructed using an isotonic saline solution (with 10% heparin). IP blood samples were taken within 15 seconds of exercise cessation. All BL and 24P blood samples were drawn with a plastic syringe while the subject was in a seated position. These blood samples were obtained from an antecubital arm vein using a 20-gauge disposable needle equipped with a Vacutainer® tube holder (Becton Dickinson, Franklin Lakes, NJ). Each subjects' blood samples were obtained at the same time of day during each

session. Blood samples were collected into two Vacutainer® tubes, one containing SST® Gel and Clot Activator and the second containing EDTA. A small aliquot or part of whole blood was removed from the second tube and used for microcapillary determination of hematocrit. The remaining blood in that tube was used for hemoglobin and several of the hormonal and biochemical analyses. Hemoglobin measures were performed without freezing, but the remaining plasma was placed into separate 1.8-ml microcentrifuge tubes and frozen at -80°C for later analysis. The blood in the first tube was allowed to clot at room temperature and subsequently centrifuged at $1500 \times g$ for 15 minutes. The resulting serum was placed into separate 1.8-ml microcentrifuge tubes and frozen at -80°C for later analysis.

Biochemical and hormonal analyses

Serum testosterone, cortisol and growth hormone concentrations were determined using enzyme immunoassays (EIA) and enzyme-linked immunosorbent assays (ELISA) (Diagnostic Systems Laboratory, Webster, TX). Serum aldosterone and IL-6 concentrations were determined using EIA assays (ALPCO Diagnostics, Salem, NH). Plasma arginine vasopressin (AVP) concentrations were also determined using an EIA assay (Cayman Chemical Co., Ann Arbor, MI). Plasma adrenocorticotrophic hormone (ACTH) concentrations were determined using an ELISA assay (ALPCO Diagnostics, Salem, NH). Plasma C-reactive protein concentrations were determined using an ELISA assay (Diagnostic Systems Laboratory, Webster, TX), and plasma malondialdehyde (MDA) concentrations were determined using an ELISA assay (Cell Biolabs Inc., San Diego, CA). Determination of serum immunoreactivity values was made using a SpectraMax340 Spectrophotometer (Molecular Devices, Sunnyvale, CA). To eliminate inter-assay variance, all samples for a particular assay were thawed once and analyzed in the same assay run. All samples were run in duplicate with a mean intra-assay variance of $<10\%$. Serum creatine kinase concentrations were analyzed with the use of a spectrophotometer and a commercially available enzymatic kit (Pointe Scientific, Inc., Canton, MI).

Hemoglobin was analyzed in triplicate from whole blood using the cyanmethemoglobin method (Sigma Diagnostics, St. Louis, MO). Hematocrit was analyzed in triplicate from whole blood via microcentrifugation (IEC micro-MB centrifuge, Needham, MA) and microcapillary technique. Plasma volume shifts following the workout were calculated using the formula of Dill & Costill (1974); however, blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue receptor level. Plasma glutamine, glucose and lactate concentrations

were determined in duplicate with an automated analyzer (Analox GM7 enzymatic metabolite analyzer, Analox Instruments USA, Lunenburg, MA). Plasma sodium and potassium concentrations were assessed via ion-selective electrodes (Model984-S; AVL Scientific Corporation, Roswell, GA). Plasma and urine osmolality were determined without freezing via freezing point depression osmometer (Model 3320; Micro-Sample Osmometer, Advanced Instruments, Inc., Norwood, MA).

Statistical analysis

Statistical evaluation of performance, hormonal and biochemical changes were accomplished using a 2 x 2 (time x trial) repeated measures analysis of variance (ANOVA). In the event of a significant F-ratio, Scheffe *post-hoc* tests were used for pairwise comparisons. Prior to the ANOVA, all data were assessed and met assumptions for normal distribution, homogeneity of variance, and sample independence. Plasma volume shifts and performance comparisons were analyzed using a one-way ANOVA. One-way ANOVAs were also used to analyze the area under the curve (AUC), which was calculated by using a standard trapezoidal technique. Significance was accepted at an alpha level of $p < .05$. All data are reported as mean \pm SD.

Results

Urine specific gravity (1.027 ± 0.003), urine osmolality (712 ± 288 mOsm) and plasma osmolality (297.3 ± 7.0 mOsm) at HHY were similar for all trials. These results reflected the overnight fasting and exercise-induced hypohydration performed during and prior to each trial. Glutamine concentrations for HDAG were significantly higher at RHY and IP than the other trials (see Figure 1a), and AUC analysis showed a significantly greater glutamine concen-

tration for HDAG at all time points compared to the other experimental trials (see Figure 1b).

No significant performance differences were noted between trials in peak power (890 ± 130 W), mean power (702 ± 81 W), total work (7005 ± 836 J) or fatigue rate (18.9 ± 5.4 W \cdot s $^{-1}$). Significant main effects were seen in both plasma lactate and glucose responses to the exercise protocol. Significant elevations were seen at IP in both of these variables (14.1 ± 1.8 mmol \cdot L $^{-1}$ and 7.4 ± 1.5 mmol \cdot L $^{-1}$ for plasma lactate and glucose, respectively) compared to all other time points. However, no significant differences were seen between trials. Plasma osmolality at IP (308 ± 17 mOsm) was significantly elevated compared to BL (295 ± 4 mOsm), HHY (297 ± 7 mOsm) and RHY (293 ± 6 mOsm) across all time measures. However, no between trial differences in plasma osmolality were observed. Changes in plasma potassium and sodium concentrations can be seen in Table 1. Plasma potassium was significantly lower at HHY compared to BL, RHY and IP. No other differences were noted and no between trial effects were observed. Plasma sodium concentrations at IP were significantly greater than that observed at BL, HHY and RHY, and plasma sodium concentrations at HHY were significantly greater than that observed at BL and RHY. When collapsed across time, plasma sodium concentrations were significantly greater at DHY than compared to all other trials. AUC analysis demonstrated a significantly greater sodium concentration for DHY compared to all other trials.

The serum aldosterone response to the experimental trials can be seen in Figure 2. Aldosterone concentrations during all trials were significantly lower at RHY than BL and HHY, while aldosterone concentrations at HHY were significantly higher than IP. AUC analysis showed that the aldosterone response at LDAG was significantly less than that observed during DHY.

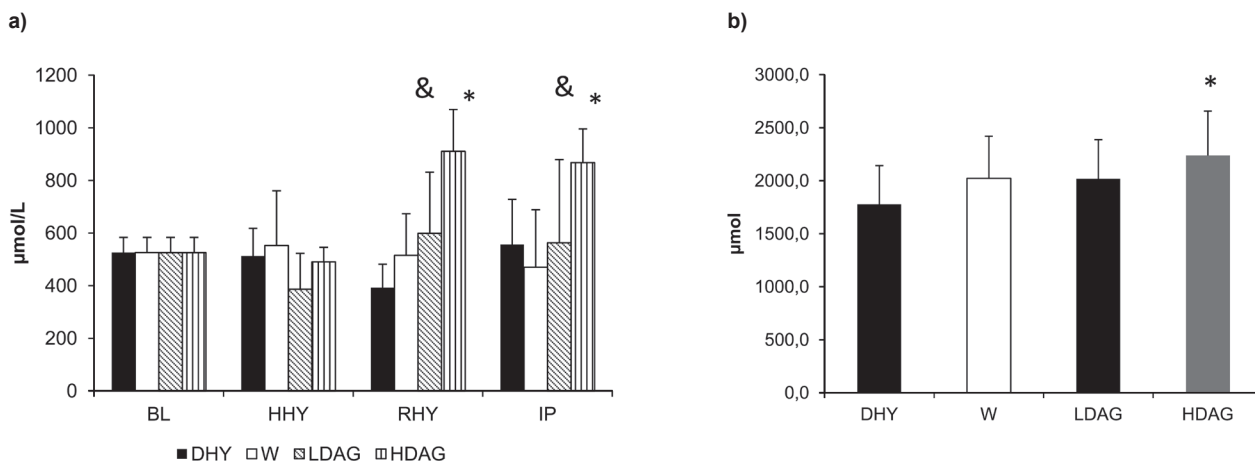


Figure 1. a) Plasma glutamine concentrations: & = significant main effect for time versus BL and HHY; * = significantly different from other trials. b) AUC glutamine: * = significantly different from other trials.

The plasma AVP responses are shown in Figure 3. AVP was significantly elevated at HHY, RHY and IP compared to BL measures. In addition, AVP concentrations at HHY were significantly higher

than IP across all trials. No significant differences between trials were seen and no significant interactions between time and trial were observed.

Table 1. Plasma electrolyte response to exercise

Variable	Time Point	DHY	W	LDAG	HDAG	Main Effect Time
		Potassium (mmol·L ⁻¹)	BL	4.18±0.43	4.18±0.43	
	HHY	3.61±0.68	3.88±0.31	4.01±0.47	3.92±0.30	3.85±0.47§
	RHY	4.32±0.56	4.39±1.09	4.29±0.97	4.05±0.42	4.26±0.79
	IP	4.50±0.58	4.54±1.06	4.34±0.51	4.07±0.33	4.36±0.68
Main Effect Trial		4.15±0.64	4.25±0.81	4.21±0.63	4.06±0.37	
Sodium (mmol·L ⁻¹)	BL	140.0±1.8	140.0±1.8	140.0±1.8	140.0±1.8	140.0±1.8
	HHY	141.4±1.6	140.9±1.4	141.7±2.6	141.2±1.8	141.3±1.9ϕ
	RHY	141.4±2.0	139.3±3.0	138.2±3.1	139.0±2.0	139.5±2.8
	IP	146.9±1.7	143.2±2.4	144.3±2.1	144.4±2.0	144.7±2.4**
Main Effect Trial		142.4±3.2*	140.8±2.6	141.0±3.3	141.4±3.2	

Legend: § = significant main effect for time versus BL, RHY and IP; * = significantly different from all other trials; ** = significantly different from BL, HHY and RHY; ϕ = significant difference between BL and RHY.

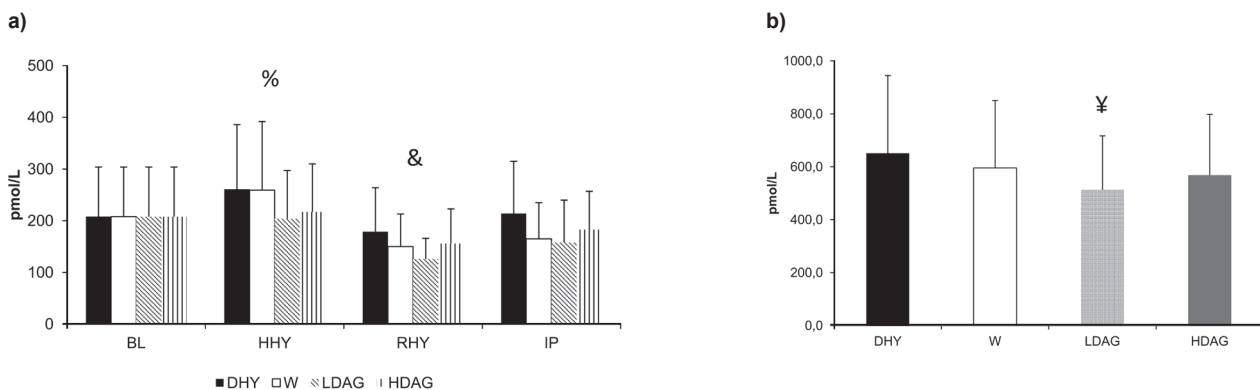


Figure 2. a) Serum aldosterone response: & = significant main effect for time versus BL and HHY; % = significant main effect for time versus RHY and IP. b) AUC aldosterone: ¥ = significant difference between DHY and LDAG.

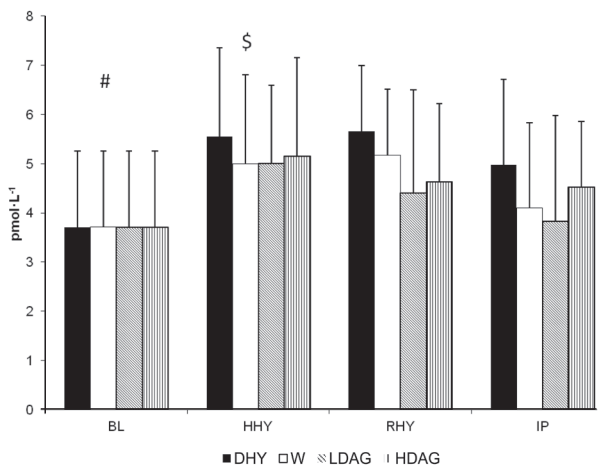


Figure 3. Arginine vasopressin: # = significant main effect for time BL versus HHY, RHY and IP; \$ = significant main effect between HHY and IP.

No significant differences were observed between trials in changes to C-reactive protein, IL-6, and MDA concentrations (see Figures 4 – 6). In addition, no significant interactions were noted either. AUC analysis for C-reactive protein, IL-6 and MDA did not reveal any significant differences between trials.

A significant main effect for time was seen in testosterone concentrations (Figure 7). Testosterone concentrations at HHY and IP were significantly greater than that seen at BL, RHY and 24P. However, no significant interactions were observed. A significant main effect for time was seen in both the ACTH and cortisol response to the exercise and hypohydration protocol (Figure 8 and 9, respectively). When collapsed across trials, ACTH and cortisol concentrations were significantly elevated at

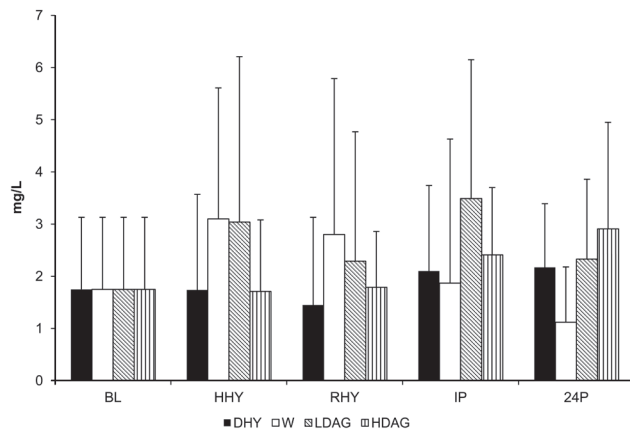


Figure 4. C-reactive protein response.

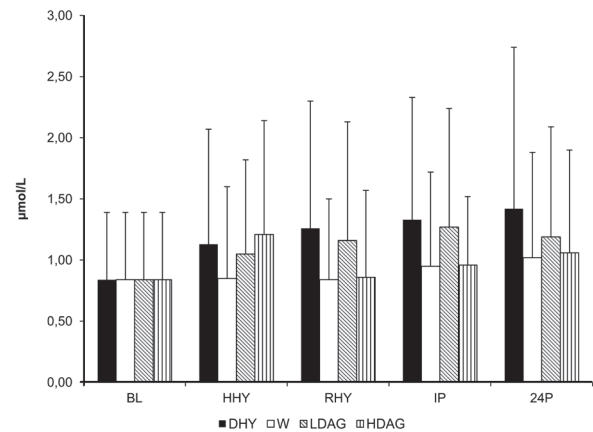


Figure 6. MDA response.

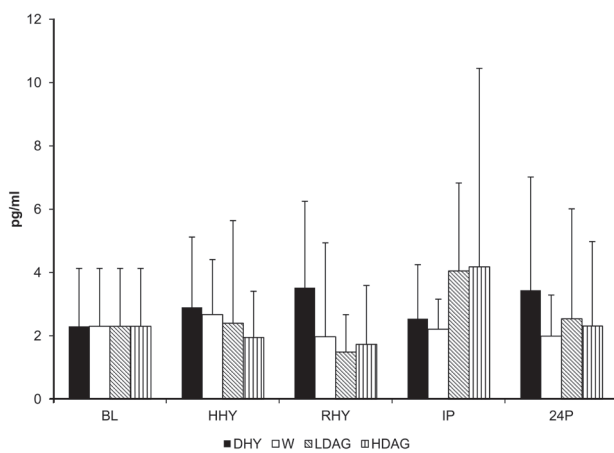


Figure 5. IL-6 response.

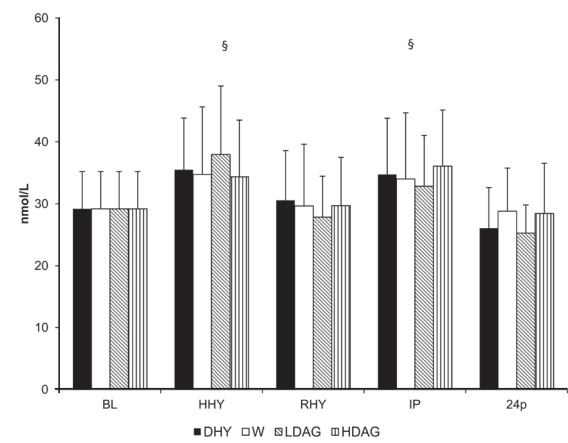


Figure 7. Serum testosterone response: § = significant main effect for time versus BL, RHY and 24P.

HHY, RHY, IP and 24P compared to BL. In addition, ACTH and cortisol concentrations at HHY were significantly greater than IP and 24P. No other significant differences were observed and no significant interactions were noted.

A significant main effect for time was also seen for growth hormone. When collapsed across trials, growth hormone concentrations were significantly elevated at IP compared to all other time points (Figure 10). No other differences were observed. AUC analyses for testosterone, ACTH, cortisol and growth hormone did not result in any significant differences between trials.

No significant change from rest was observed in creatine kinase concentrations (55.0 ± 19.8 IU) between trials or time, and no significant interactions were noted either.

Plasma volumes decreased $-8.94 \pm 14.01\%$ at HHY for all experimental trials, plasma volumes were decreased at RHY ($-9.02 \pm 15.04\%$) for all experimental trials and continued to decrease at IP

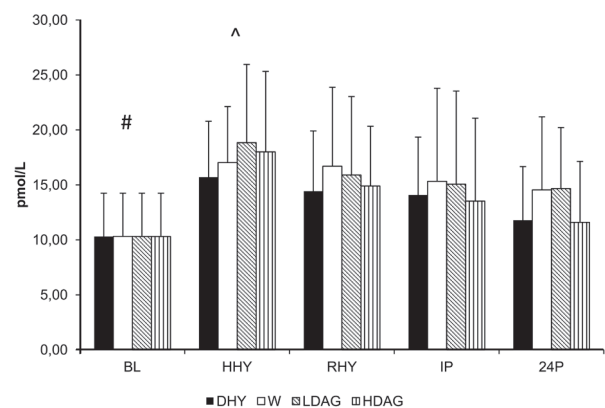


Figure 8. Plasma ACTH response: # = significant main effect for time versus DHY, RHY, IP and 24P; ^ significant main effect for time versus IP and 24P.

($-27.01 \pm 11.72\%$). However, the difference between trials was not significant.

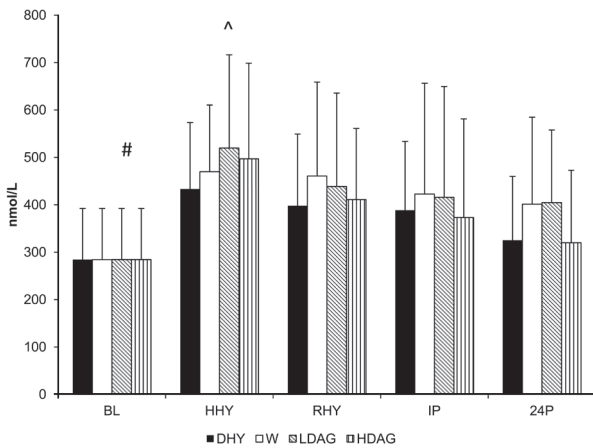


Figure 9. Serum cortisol response: # = significant main effect for time versus DHY, RHY, IP and 24P; ^ significant main effect for time versus IP and 24P.

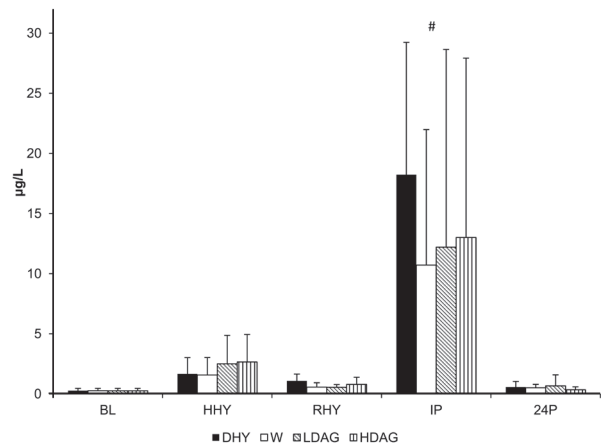


Figure 10. Plasma growth hormone response: # = significant main effect for time versus BL DHY, RHY, and 24P.

Discussion and conclusions

The results of this study indicated that a mild hypohydration stress (-2.5% of body mass) did not result in any performance decrement during repetitive high intensity exercise. This is consistent with recent research that reported no performance decrements during intermittent sprint performance at similar hypohydration levels (Maxwell, McKenzie, & Bishop, 2009). Rehydration with AG (both low and high doses) did not provide any ergogenic benefit during the mild hydration stress. As expected, the higher dose of AG produced a greater increase in plasma glutamine concentrations. The time course of glutamine appearance in the plasma was similar to that previously reported by our laboratory (Hoffman, et al., 2010) and others (Klassen, Mazariegos, Solomons, & Furst, 2000). In the latter study, a 20 g oral feeding (approximate to the high dose used in this study) resulted in a peak increase occurring at 49 ± 8 min (range 30 – 120 min) following dosing, which would correspond to the RHY and IP blood draws. Although dosing patterns of $0.1 \text{ g} \cdot \text{kg} \cdot \text{BM}^{-1}$ has been shown to increase plasma glutamine concentration by approximately 50% (Ziegler, et al., 1990), the ability to increase plasma glutamine concentrations with lower concentration doses is not clear. Based on the present findings, an AG dose of $0.05 \text{ g} \cdot \text{kg} \cdot \text{BM}^{-1}$ was unable to cause a significant elevation in plasma glutamine concentrations.

Glutamine is the most abundant amino acid and under normal conditions is considered to be nonessential. During illness or intense physical activity plasma glutamine concentrations may become reduced, and be considered to be conditionally essential (Lacey & Wilmore, 1990). Considering that the exercise and hypohydration protocol employed in this study did not result in any significant performance decrements or reduction in plasma glutamine concentrations, the results are not surprising. Under

these conditions, there is no support for AG to be ergogenic. Ingestion of AG may have greater relevance in maintaining performance during an exercise and hydration stress, or in enhancing recovery. This is supported from a previous study from our laboratory, which suggested that AG supplementation can minimize performance reductions to a greater extent than water alone during a time trial run under a similar hydration stress (Hoffman, et al., 2010). It was hypothesized that AG ingestion can increase fluid and electrolyte uptake in the gut, possibly through an enhanced signaling pathway within the intestinal mucosal cells (Lima, et al., 2002; Rhoads & Wu, 2009). In addition, AG ingestion has also been demonstrated to enhance muscle glutamine uptake (Rogero, et al., 2006). However, the results of the present study do not support a role for AG in maintaining performance or enhancing recovery during a short duration, high intensity exercise protocol, coupled with a mild hydration stress.

The exercise and mild hydration stress resulted in significant perturbations to serum electrolyte concentrations. However, AG ingestion provided no additional benefit versus water alone in maintaining serum electrolyte concentrations close to baseline levels. As expected, serum aldosterone responses were significantly higher at HHY than following RHY and IP, and significantly lower at RHY compared to IP. This response is consistent for the physiological role that aldosterone has in maintaining fluid homeostasis (Kenefick, et al., 2007; Verbalis, 2006). Although no significant differences at IP were observed between trials, differences between DHY and the rehydration trials (W, LDAG and HDAG) ranged from 16% - 35% ($p > .05$). Considering that aldosterone responds in a graded manner to levels of hypohydration (Francesconi, et al., 1985; Montain, Laird, Latzka, & Sawka, 1997), the magnitude of hypohydration in this study was likely not severe enough to tease out differences in aldos-

terone production in this mild hydration and short duration anaerobic exercise protocol. Interestingly, AUC analysis showed a significantly greater aldosterone response at DHY compared to LDAG, suggesting that AG ingestion may be advantageous in stimulating electrolyte uptake and minimizing the aldosterone response to the mild hypohydration perturbation. However, results showing no difference between HDAG and any of the other trials question that conclusion. Likely, the mild hydration stress during a short duration bout of anaerobic exercise contributed to the inconsistent results.

The response of AVP to this mild dehydration and exercise protocol was not significantly different between trials. Changes in AVP concentrations are dependent upon exercise intensity and changes in plasma osmolality and blood volume (Brandenberger, Candas, Follenius, Libert, & Kahn, 1986; Convertino, Keil, & Greenleaf, 1983; Montain, et al., 1997). Considering no differences were seen between trials in exercise performance, and no differences were noted in plasma osmolality or plasma volume changes between trials, these results are not surprising. Previous studies examining the effect of hypohydration levels have typically examined body water deficits of greater magnitudes (~5%) than that used in this present study (Kenefick, et al., 2007; Montain, et al., 1997).

Several investigations have suggested that AG may be effective in modulating the immune, inflammatory and antioxidant defense during exercise (Abiles, et al., 2008; Dechelotte, et al., 2006; Hiscock, et al., 2003; Kumar & Anandan, 2007; Castell & Newsholme, 1998). C-reactive protein is often used as a marker of inflammation and muscle damage (Castell, et al., 1997; Miles, et al., 2008), while IL-6 and MDA are markers of immune and oxidative stress responses, respectively. The results of the current study indicate that short duration, high intensity repetitive sprints performed on a cycle ergometer did not cause significant immune, inflammatory, or oxidative stress responses, even during a mild hypohydration stress. Lack of muscle damage was confirmed by the response of CK during each trial (no change from baseline concentrations). These results contrast with previous research that have reported that exhaustive exercise results in significant elevations in IL-6 (Meckel, et al., 2009) and MDA (Paik, et al., 2009), but are supported by others that have also indicated that a repeat sprint protocol (6 x 10 s cycle ergometer sprints) had no effect on plasma MDA concentrations (Bloomer, et al., 2006).

The anabolic and catabolic response to the study protocol did not differ between trials suggesting that AG was unable to provide any significant benefit regarding enhanced recovery from the exercise and hypohydration stress. The elevated testosterone response at HHY for all trials likely reflects the

~42 minute active hypohydration protocol used to achieve goal body weights. Previous studies have indicated that exercise duration exceeding 30 minutes in duration can significantly elevate plasma testosterone concentrations (Hughes, Johnson, Housh, Weir, & Kinder, 1996; Wilkerson, Horvath, & Gutin, 1980). The significant increase in testosterone seen at IP is consistent with other studies reporting significant elevations in anabolic hormones following high intensity exercise (Kindermann, et al., 1982; Kuoppasalmi, Naveri, Harkonen, & Adlercreutz, 1980). The similar response of testosterone between trials is also consistent with investigations suggesting that mild hydration perturbations in fit individuals do not impact testosterone concentrations (Hoffman, et al., 1994).

The elevated cortisol response at HHY likely reflects the stress associated with the hypohydration protocol. This is consistent with the metabolic stress associated with sprint activity (Bussau, Ferreira, Jones, & Fournier, 2006) and mild hydration stresses (Brandenberger, Canda, Follenius, & Kahn, 1989; Maresh, et al., 2006). The elevated cortisol seen at 24P suggests that recovery was not complete following the exercise and hypohydration protocol, suggesting that the AG dipeptide was unable to influence recovery from the high intensity exercise and mild hydration stress. These findings also suggest that the pituitary-adrenal axis responded similarly to this high intensity exercise and hypohydration perturbation as ACTH responded in a similar pattern as cortisol.

Growth hormone secretion patterns have been shown to be quite responsive to changes in the acid-base balance of muscle (Gordon, Kraemer, Vos, Lynch, & Knuttgen, 1994). Considering that no differences were noted in the lactate response between the trials, it is not surprising to see no difference in the growth hormone response to the exercise and hypohydration stress. These results are in agreement with Judelson et al. (2008), but conflict with others that suggest that a hydration stress could blunt the growth hormone response to exercise (Peyreigne, Bouix, Fédou, & Mercier, 2001). Previous investigations have suggested that glutamine concentrations can elevate the growth hormone response at rest (Suminski, et al., 1997; Welbourne, 1995), but not during exercise (Suminski, et al., 1997). The findings of the current study confirm the latter results suggesting that glutamine has little effect on the growth hormone response to exercise. The most compelling stimulus for glutamine's role in stimulating growth hormone release appears to be during prolonged critical illness when plasma glutamine concentrations are below normal levels (Duska, et al., 2008). The high variability in the growth hormone response in this study could be partly attributed to the normal glutamine concentrations seen at rest.

In conclusion, the results of the present study demonstrate that AG ingestion was unable to provide any ergogenic benefit during short duration, high intensity exercise with a mild hypohydration stress in fit men. Ingestion of AG also had no effect on immune, inflammatory or oxidative stress responses, and it did not stimulate a more ana-

bolic response of the pituitary-adrenal-testicular axis during this exercise and mild hypohydration perturbation. Future research should examine the efficacy of AG during longer duration anaerobic exercise protocols with or without a greater hydration/thermal stress.

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Submitted: October 27, 2011

Accepted: November 9, 2011

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UNOS L-ALANIL-L-GLUTAMINA TIJEKOM KRATKOTRAJNE VISOKOINTENZIVNE VJEŽBE U UVJETIMA BLAGOGA HIDRACIJSKOGA STRESA

Učinci akutnoga, trenutačnoga uzimanja L-alanil-L-glutamina (AG) na odabrane hormonske i elektrolitne pokazatelje ispitani su tijekom ponavljajuće kratkotrajne visokointenzivne aktivnosti u uvjetima blage hipohidracije ispitanika. Ispitanici ($20,3 \pm 1,1$ godina; $180,3 \pm 10,4$ cm; $83,1 \pm 14,0$ kg; $11,6 \pm 3,6\%$ tjelesne masti) bili su testirani u Human Performance Laboratory u četiri navrata. Tijekom svakoga pojedinačnoga mjerenja ispitanici su bili hipohidrirani do $-2,5\%$ svoje početne, osnovne tjelesne mase. Tijekom prvoga testiranja (DHY) ispitanici su se odmarali ležeći 45 minuta prije no što su počeli provoditi protokol vježbanja. Tijekom sljedeća tri mjerenja ispitanici su rehidrirani do $1,5\%$ njihove početne tjelesne mase prije vježbanja, i to: pijenjem samo vode (W) te unosom dviju različitih doza AG - male doze (LDAG: $0,05 \text{ g} \cdot \text{kg}^{-1}$) i velike doze (HDAG: $0,2 \text{ g} \cdot \text{kg}^{-1}$). Protokol vježbanja sastojao se od po deset sprintova na bicikl-ergometru u trajanju od 10 sekunda s jednominutnim odmorom između svakoga sprinta. Uzorci krvi vađeni su odmah nakon što je ispitanik dosegao željenu razinu hipohidracije, nepo-

sredno prije početka vježbanja, neposredno nakon završetka vježbanja i 24 sata nakon vježbanja. U uzorcima krvi analizirana je koncentracija glutamina, kalija, natrija, aldosterona, arginin vazopresina, C-reaktivnoga proteina, interleukina-6, malondialdehida, testosterona, kortizola, ACTH-a i hormona rasta. Analiza površine ispod krivulje pokazala je statistički značajno veću razinu koncentracije natrija u ispitanika u prvom testu (DHY) u odnosu na sva ostala mjerenja. Analiza površine ispod krivulje za aldosteron je pokazala značajno nižu koncentraciju u testu LDAG u odnosu na test DHY. Nisu zapažene značajne razlike između pojedinih mjerenja ni u jednoj drugoj hormonskoj i biokemijskoj reakciji na protokol vježbanja. Uzimanje AG tijekom kratkotrajne anaerobne aktivnosti i u stanju blagog hipohidracijskoga stresa pokazalo je ograničene učinke na odabrane hormonske i biokemijske pokazatelje.

Ključne riječi: endokrina reakcija, imuna reakcija, hormonski regulatori tekućine, elektroliti, anabolički hormoni