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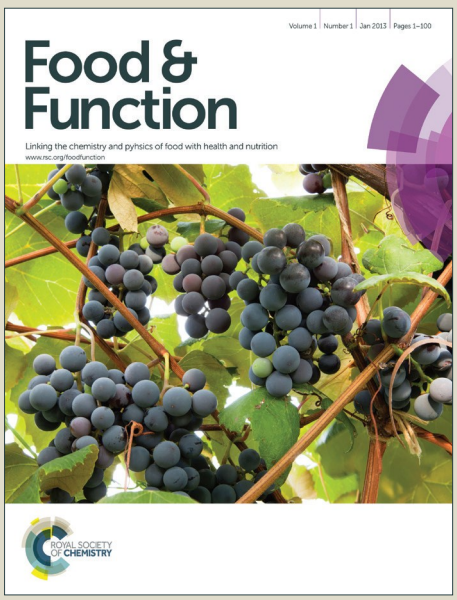

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KETO ANALOGUES AND AMINO ACID SUPPLEMENTATION AND ITS EFFECTS ON AMMONEMIA AND PERFORMANCE UNDER THERMONEUTRAL CONDITIONS

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Abstract

Alterations of cerebral function, fatigue and disturbance in cognitive-motor performance can be caused by hyperammonemia and/or hot environmental conditions during exercise. Exercise-induced hyperammonemia can be reduced through supplementation with either amino acids or combined keto analogues and amino acids (KAAA) to improve exercise tolerance. In the present study, we evaluated KAAA supplementation on ammonia metabolism and cognitive-motor performance after high-intensity exercise under a low heat stress environment. Sixteen male cyclists received a ketogenic diet for 2 d and were divided in two groups, KAAA (KEx) or placebo (CEx) supplementation. The athletes performed a 2 h cycling session followed by a maximum test (MAX), and blood samples were obtained at rest and during exercise. Cognitve-motor tasks were performed before and after the protocol, and exhaustion time was used to evaluate physical performance. Hydration status was also evaluated. The CEx group showed a significant increase (~ 70%) in ammonia concentration at MAX, which did not change in the KEx group. The non-supplemented group showed a significant increase in uremia. Both of the groups had a significant increase in blood urate concentrations at 120 min, and an early significant increase from 120 min was observed in the CEx group. There was no change in the glucose concentrations of the two groups. A significant increase in lactate was observed at the MAX moment in both groups. There was not a significant difference in the exhaustion times between the groups. No changes were observed in the cognitive-motor tasks after the protocol. We suggest that KAAA supplementation decreases ammonia concentration during high-intensity exercise but does not affect physical or cognitive-motor performances under a low heat stress environment.

Introduction

Ammonia is a base (NH_3) that forms a conjugated pair with the ammonium ion (NH_4^+). The pKa of the reversible reaction $NH_4^+ \leftrightarrow NH_3 + H^+$ is near 9.3. Thus, in the human body, most ammonia is protonated (ammonium). Henceforth, we will refer to ammonia as the sum of both its forms ($NH_3 + NH_4^+$) for clarity. Ammonia is a toxic metabolite, and increased concentrations of ammonia in the blood can lead to cerebral function impairment, altering glutamatergic neurotransmission [1-4]. Hyperammonemia affects intellectual function, personality, and conscious and neuromuscular coordination to various degrees [3-6].

We have been using Sportomics as an approach to study metabolism, specially ammonia metabolism [7, 8]. During exercise, ammonia production occurs via both amino acid deamination and other nitrogenated compound deamination (as AMP). These deamination pathways are activated in an intensity- and duration-dependent manner [9]. Furthermore, ammonemia during exercise depends on carbohydrate availability [10]. The consumption of a low-carbohydrate diet (herein referred to as a ketogenic diet) combined with exercise has been shown to reduce glycogen stores and induce hyperammonemia [11, 12].

It has been suggested that increased ammonemia during exercise may cause central fatigue by altering cerebral function, which manifest as ataxia, lethargy and stupor, similar to the symptoms of hepatic encephalopathy [13]. In addition, hyperammonemia may have a significant impact during exercise and can disturb cognitive performance (termed cognitive-motor performance) [14, 15]. It is believed that controlling the increase of ammonemia will improve exercise

performance [16, 17]. Previous studies performed by our group demonstrated that amino acids and ketoanalogues supplementation (KAAA) can reduce ammonemia concentration during moderate prolonged exercise [18, 19]. In addition, the use of the branched-chain keto acids (BCKA), 2-ketoisocaproate (KIC), 2-ketoisovalerate (KIV), 2-keto-3-methylvalerate (KMV) and 2-keto glutarate (AKG), which are keto acids of the branched-chain amino acids (BCAA) leucine, valine, isoleucine and glutamate, respectively, in our KAAA supplementation has been shown to improve blood ammonia concentrations and various exercise performance indicators and to alter the stress and recovery of the subjects [20, 21].

It is known that hot environmental conditions, hyperthermia or dehydration may impair cognitive-motor performance, such as motor coordination, reaction time and memory [22-25]. Likewise, an increase in body temperature can promote metabolic alterations, including high-energy demand and protein catabolism, which impair exercise performance and appear to exacerbate exercise-induced hyperammonemia [9, 26, 27]. However, it is important to highlight that the magnitude of this response is dependent on body temperature changes and that factors such as exercise itself (including thermoneutral conditions, termed a low heat stress environment) and acclimation status can affect the body temperature responses [28, 29].

To the best of our knowledge, there is no information available about the effect of short-term KAAA supplementation on ammonia metabolism and cognitive-motor performance after high-intensity exercise, under low heat stress conditions controlled in acclimatized athletes. We hypothesized that when

exercising in low heat stress conditions, KAAA supplementation reduces ammonia, but not improves physical and cognitive-motor performance.

However, in the present study, we evaluated the acute effect of KAAA supplementation on ammonia metabolism and cognitive-motor performance after high-intensity exercise under low body temperature and heat stress conditions.

Methods

Subjects

Sixteen male endurance-trained cyclists were divided into two groups of eight athletes each as follows: the KAAA experimental group – KEx, n = 8 (33.2 ± 2.6 years; 67.1 ± 2.3 kg; 1.71 ± 0.03 m; and 10.3 ± 0.8% of body fat); and the control group – CEx, n = 8 (33.1 ± 2.4 years; 66.53 ± 3.72 kg; 1.70 ± 0.02 m; 9.5 ± 2.0% body fat). The groups showed similar levels of maximal oxygen consumption (VO_2max) (60.3 ± 2.3 $mL.kg^{-1}.min^{-1}$ and 57.8 ± 2.5 $mL.kg^{-1}.min^{-1}$, KEx and CEx, respectively). All subjects had a mean of at least three years of training and often participated in competitions. Diseases or the use of ergogenic aids were exclusion criteria. The athletes were acclimatized to training in a hot environment and participated as volunteers. The subjects were informed previously about the study, and written informed consent was obtained. All the procedures were performed in accordance with the ethical standards of the Ethics Committee for Human Research at the Federal University of Alagoas, Brazil.

Study design and protocols

A week before the experimental protocol, the athletes came to the laboratory for a first visit and anthropometric variables and body fat percentages were determined [30]. After familiarization with the cycle ergometer and the determination of the VO_2max, a maximum incremental test (MIT) was performed at the same thermal conditions as those of the experimental protocol (Wet Bulb Globe Temperature (WBGT) Index ~ 19,5 º C). The MIT consisted of a three-minute warm up with an initial power output of 25 W with free cadence. The power output was set to 50 W at a cadence of 80 rpm immediately after the warm up. The power output was increased at 25 $W.min^{-1}$ until the subject reported voluntary exhaustion or the inability to maintain the pace established for more than five consecutive seconds. The VO_2max was determined using an automatic gas analyzer (Cosmed® Quark CPET's, Rome, Italy) and was calibrated before each test, and the VO_2max was determined when two or more of the following criteria were met: an increase in the VO_2 of less than 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ on two consecutive stages, a respiratory exchange ratio greater than 1.1 [31] and rated perceived exertion (RPE) values $\geq$ 19 [32].

After MIT, the athletes received an individualized ketogenic diet plan as described previously [19]. Briefly, 35% of the recommended energy intake was from protein, 55% of the recommended energy intake was from lipids, and 10% of the recommended energy intake was from carbohydrates. The association of both normal training and a ketogenic diet were used to reduce muscle glycogen stores and to induce a higher increase in ammonemia. The ketogenic diet was started two days before and maintained during the experimental protocol. The subjects were also asked to maintain their normal training schedule (~ 60 $km.d^{-}$

^{1}) until 24 h before the experimental protocol day and to maintain a fluid intake of ~ 3 L.d^{-1}, avoiding the use of caffeinated beverages.

One week later, the subjects reported to the laboratory for a second visit in a fasting state and received a ketogenic breakfast with ~ 200 mL of water. One hour after breakfast, the KEx group received five tablets of a KAAA mixture (Ketosteril®; Fresenius, Bad Homburg, Germany), and the CEx group five 200 mg tablets of lactose, which served as a placebo (Farmaderm®, Alagoas, Brazil) in a randomized double-blind manner. The composition of the KAAA tablet was as follows: a-keto analogues of isoleucine (335 mg), leucine (505 mg), phenylalanine (430 mg) and valine (340 mg); a-hydroxy analogue of methionine (295 mg); L-lysine acetate (75 mg L-lysine); L-threonine (265 mg); Ltryptophan (115 mg); L-histidine (190 mg); and L-tyrosine (150 mg). Both of the supplements were provided as indistinguishable tablets with ~ 300 mL of water. We rigorously controlled the liquid intake to avoid bias from temperature differences caused by water intake.

The athletes were tested to evaluate their cognitive-motor performances using measures of immediate memory, motor coordination and simple reaction time 30 minutes after supplementation, as follows:

Immediate memory was evaluated as described by McCrory *et al* [33]. A list of five words (a word per second) was given, and the athlete was asked to repeat as many words as possible in any order. The same list was repeated three times in ten second intervals.

Motor coordination was evaluated using the finger-to-nose test adapted from McCrory *et al* [33]. Briefly, while seated and facing the examiner (having their dominant arm laterally extended at a 90° angle in relation to the body and their

eyes open), the athletes were asked to touch the tip of their nose with their extended index finger and return to the initial position. They were asked to repeat this procedure as quickly and as accurately possible five times. The cognitive tests were filmed for analysis at a later time by five independent evaluators. Reaction time was measured within a 0.01 sec accuracy.

The simple reaction time, as described by Eckner *et al* [34], was obtained immediately after the finger-to-nose test. While seated, the athletes had their dominant arm flexed and abducted to 90°, rested on a flat surface with their outstretched hand in a neutral position, also forming the same angle perpendicular to the thumb. The zero mark of the apparatus was positioned inside the open hand of the athlete. Thus, the examiner (standing) vertically suspends the apparatus, allowing the spacer portion of the device to rest inside the open hand of the test participant. When the examiner drops the apparatus, the test participant catches it as quickly as possible. The athletes were tested in 8 trials and the test average was determined. The fall distance was measured from the superior surface of the weighted disk to the most superior aspect of the athlete's hand and was converted into a reaction time using the formula for a body falling under the influence of gravity ($d = ½gt^2$), where d (cm) is distance, g is acceleration due to gravity and t is time (in milliseconds – ms).

Immediately after the cognitive-motor performances tests, hydration status was assessed by evaluating a change in body weight (Δ %), urine color and specific gravity (USG). Urine color was evaluated according to Armstrong *et al* [35], and USG was measured using a manual refractometer (Biobrix®, São Paulo, Brazil). Hydration status was classified according with Casa *et al* [36]. Urine samples were also used for the ketonuria analysis by qualitative reagent

strips for urinalysis (Biocolor/Bioeasy®, Belo Horizonte, Minas Gerais). We considered the presence of ketonuria to be a positive test for ketosis.

One hour after supplementation, the athletes stretched, followed by a 10 min warm up at 50% maximum heart rate. The athletes started a 120 min cycling session at 80 rpm, following a power output from 75% to 85% of the estimated maximum heart rate. Heart rate was recorded throughout the exercise protocol using a heat rate monitor (Polar® FT1, Kempele, Finland). Before the cycling session, a catheter was also placed into the median cubital vein. Blood samples were obtained at rest (0 min) and throughout the exercise period (30; 60; 90 and 120 min). The athletes did not receive fluids during the trial.

The blood samples were analyzed in duplicate after collection. The blood samples were immediately centrifuged (3000 x *g*) to avoid the loss of volatile compounds. The serum was aliquot and stored at 4 ºC. To prevent loss, seric ammonia was measured immediately, and the other biochemical analyses were performed within a 24 h period. Glucose, urea and urate were measured using commercial spectrophotometric assays (Labtest®, Minas Gerais, Brazil). Lactate and ammonia were measured using an enzymatic UV method (Randox, Crumlin UK) on a Dade Model Dimension RXL Automated Chemistry Analyzer (Dade Behring®, Eschborn, Germany).

Two hours after the cycling session, new MIT (without gas analysis), blood evaluations, cognitive-motor performance and hydration status tests were performed. The voluntary exhaustion time or the inability to maintain the pace established for more than five consecutive seconds in both groups obtained after the new MIT was used to evaluate physical performance. Moreover, sweat

rate (SR) was obtained and calculated according to Casa *et al* [36], as described below:

$$SR = ((BMb - BMa) + Flt - VUt)/T$$

where *BM*b is the body mass (kg) before the cycling session, *BM*a is the body mass (kg) after the new MIT, *Fl*t is the total fluid intake consumed in the experimental protocol (zero because there was no fluid intake), *VU*t is the total urine volume (L) after the new TIM, and *T* is time (h).

During the experimental protocol, we used the ambient temperature, the relative humidity, the air movement and the solar radiation to calculate the WBGT Index (Instrutemp®, São Paulo, Brazil). We considered a WBGT up to 22.2º C to be a low heat stress environment [37].

Body temperature was measured using a tympanic thermometer (GeniusTM 2®, Minnesota, USA). The tympanic temperature values were used to calculate an equivalent rectal temperature [38]. Furthermore, thermal and comfort sensations were evaluated during the experimental protocol [39].

Statistical analyses

After testing for normality (Kolmogorov–Smirnov) and equality test variance (Levene median), the changes in ambient temperature and body temperature and the biochemistry between the time points were analyzed by a one-way ANOVA (treatments), and the group changes were evaluated by a two way ANOVA (treatments x time) for repeated measures. Tukey's test was used as a *post hoc* analysis.

Physical and cognitive-motor performances and markers of hydration status were analyzed by an unpaired and/or paired Student's *t* test. The area under the curve (AUC) for the blood ammonia data from each individual in each treatment was determined using the following equation:

$$AUC = Ai(Ti + 1 - Ti) + 0.5(Ai + 1 - Ai)(Ti + 1 - Ti)$$

Assuming that the ammonia level at baseline corresponds to the resting ammonia level, and the AUC is the area under the curve, *A* is ammonia (µmol/L) and *T* is time (min). When the sample showed a non-normal distribution, nonparametric test correspondents were used. For all of the measurement differences, $P < 0.05$ was considered to be statistically significant. The data are expressed as the means ± SE.

Results

We used a 120 min cycling session followed by an MIT to drive the athletes to voluntary exhaustion and to evaluate the acute effects of KAAA supplementation on both physical and cognitive-motor performances. The ketonuria analyses revealed that the subjects in both groups were under ketosis before the cycling session. There was no difference in the hydration status between the groups according to the pre and post-cycling session. In addition, urine color and USG values were elevated prior to the start of the cycling session and showed that the athletes were dehydrated to a degree that was classified between minimal and significant (table 1).

There was no significant difference in the rectal temperature-equivalent body temperature values with regard to time or supplement condition (figure 1A). The thermal stress measurement by WBGT increased above baseline levels in response to the similar low heat stress exercise conditions in both groups (figure 1B). The perception investigation obtained from the thermal sensation (figure 1C) and the comfort sensation (figure 1D) showed no differences between the groups. However, the thermal and comfort sensations increased from 0 min within the groups.

To investigate the effect of KAAA on metabolism, we measured the concentration of urea, urate (serum) and ammonia (plasma). The blood ammonia concentrations were not significantly different between the groups (figure 2A). On the other hand, there was significant increase at MAX in the CEx group compared with the resting values within the group. In contrast, the ammonia concentration was not increased in the KEx group at any time within the group. Ammonemia changes in response to exercise were dramatically changed by the use of KAAA. Ammonia in the blood increased with exercise in the first 2 h in the CEx group (~ 16 μmol/L per hour), and there were no changes or decreases in the supplemented group (KEx). In the final minutes of exercise, the slopes of both groups were the same. Analyzing the whole exercise as a whole, the KAAA supplemented group had an ammonemia velocity increase of 30% compared to the control group.

In addition, when measured by the percentage change, we observed reductions from 30, 60, 90, and 120 min (~ 8%, 14%, 22%, 16%, respectively) or delay at MAX (~ 19%) in the ammonia concentrations in the KEx group compared with the baseline values within the group. The CEx group showed an

increase in ammonia concentrations at 60, 90, 120 min and MAX (~ 18%, 20%, 37% and 73%, respectively) compared with the baseline values within the group (figure 2C). Furthermore, the AUC of ammonia for the KEx group was ~20% lower than that for the CEx group.

To discriminate the ammonia produced by amino acid deamination from that produced by AMP deamination, we measured blood urea and urate concentrations. There was no significant difference in the blood concentrations of urea (figure 2B) or urate (figure 2D) between groups. However, the CEx group showed an early significant increase in urea concentrations from 90 min compared with the baseline values within the group. The KEx group showed maintenance of urea concentrations at 120 min. In the first 120 min of exercise, the urea level increased ~ 35% in the CEx group (~ 1030 μmol/L per hour); the level increased 23% in the supplemented group (~ 660 μmol/L per hour). During the final exercise, both groups exhibited a decrease in the total uremia. Considering the entire exercise as one, uremia increased in the CEx group at ~ 835 μmol/L per hour, whereas the velocity was ~ 540 μmol/L per hour in the KEx group.

The urate concentrations also showed an early significant increase from 120 min in the CEx group, whereas the KEx group showed a significant increase only at 120 min compared with the baseline values within the group. The urate velocity increase in the KEx group was ~ 40% lower than that in the control group (both at the first 120 minutes and final 5 minutes of maximal exercise). The urate increased at a rate of approximately 22 μmol/L per hour in the CEx group compared with ~14 μmol/L per hour in the KEx group. In the final minutes, the uratemia velocity slope increased nearly 11-fold in both groups.

To understand the role of KAAA in gluconeogenesis, we measured blood glucose concentrations during the cycling session and the MIT. No significant difference in blood glucose concentrations was found during the trial time between the groups. However, the KEx group showed a significant increase at MAX from 0 min and 120 min (Figure 3A). In addition, we evaluated the blood lactate concentrations, an indicator of consistent glucose metabolism during exercise. No difference in blood lactate concentrations was observed between the groups. However, there was a significant increase at the MAX moment in both of the groups compared with all of the exercise moments (figure 3B).

After the 120 min cycling session, the new MIT was employed to induce voluntary exhaustion in both groups and to evaluate the participants' physical (exhaustion time) and cognitive-motor performances. Our assumption that this test generated a similar intensity in the two studied groups was confirmed by measuring the blood lactate concentration at the MAX moment. The groups showed similar exhaustion times at the end of the new MIT (KEx 5.13 ± 0.34 min versus CEx 6.23 ± 0.72 min). In addition, we were not able to measure any significant KAAA-induced difference in the groups' cognitive-motor performances. No significant differences in the finger-to-nose test, simple reaction time or immediate memory were found before and/or after the new MIT (data not shown).

Discussion

The aim of this study was to evaluate the acute effects of KAAA supplementation on ammonia metabolism after high-intensity exercise and

physical and cognitive-motor performance under low heat stress conditions. The acute use of a mixture of KAAA supplementation reduced the increase in ammonemia caused by high-intensity exercise, but we were not able to measure any significant KAAA supplementation-induced difference in the athletes' physical or cognitive-motor performances under the low heat stress conditions.

It has been suggested that alterations in cerebral function that result from liver failure, such as HE, lead to different cerebral and neurological alterations, and under these conditions, ammonia accumulates and is suggested as a main contributor to these cerebral alterations [15]. Patients with clinical HE show various neuropsychiatric symptoms, including impairment in cognitive and intellectual function and motor activity and coordination, as well as alterations [3, 4, 40].

Exercise-induced hyperammonemia can not only impair physical performance but also affect cognitive-motor functions [41]. Ammonemia accumulation during exercise leads to impairment in both neuronal and astrocyte mechanisms with consequent damage to the activities of neurotransmitters, such as glutamate and gamma-aminobutyric acid, that are involved in cognitive-motor disturbances [6, 8, 13].

In the present study, we used a ketogenic diet plus a prolonged exercise followed by high-intensity exercise to drive the athletes to exhaustion, to decrease the availability of glycogen in the liver and muscle and to increase the availability of amino acids to supply energy [12, 42, 43]. Prolonged exercise followed by high-intensity exercise increases both amino acid catabolism and AMP deamination, leading to the release of more ammonia into the bloodstream [9].

Even under a ketogenic diet and previous training, both studied groups were normo-ammonemic at the beginning of the protocol (~ 80 μmol/L) [44].

Elevated blood ammonia concentrations can be reduced through the use of amino acids or carbohydrate supplementation because they interfere with ammonia metabolism [45]. Previous studies have shown that KAAA supplementation can effectively decrease blood ammonia, urea and urate concentrations during exercise [18, 19]. Additionally, KAAA supplementation has been widely accept and used as a therapeutic agent for liver failure and hepatic encephalopathy [46-48]. Ammonemia increased in response to high-intensity exercise in the CEx group, and this effect was reduced by the administration of KAAA in the KEx group. In the present study, acute KAAA supplementation delayed the increase in blood urea and urate concentrations during prolonged exercise and high-intensity exercise, respectively. Our data suggest that the effect of KAAA during exercise is primarily due to ammonia removal via the synthesis of urea during the final phase of prolonged exercise, whereas AMP deamination appears to be inhibited during high-intensity exercise with decreased blood urate concentrations in the KEx group. KAAA supplementation increased glucose availability via gluconeogenesis, especially in the KEx group after high-intensity exercise compared with that in the CEx group. On the other hand, the blood lactate concentration fluctuated similarly in both groups. These results are consistent with our previous findings [19].

When athletes used a standard dietary protocol (possibly a low-carbohydrate diet, such as a ketogenic diet), glucose increased during exercise. These data suggest that amino acids were being used by the muscles as an energy source and by the liver to produce glucose, which is a typical

gluconeogenic response. On the other hand, after nutritional interventions, based on a careful analysis of his diet (20% from proteins, 60% from carbohydrates), there were decreases in the glycemic variability [49].

Mourtzakis and Graham [50] showed that the alanine concentrations changed nearly twofold in a 20-min exercise (300-350 to 600-750 μmol/L), and there was a seven-fold increase in the area under the curve. Wilkinson et al [51] studied an exercise protocol that involved a 1.5 h cycle ergometer ride at 65% VO2 peak. The authors showed an increase in the alanine and glutamine levels after exercise. Muscle exports alanine and glutamine so that the carbon skeletons can be used as a gluconeogenic and energy source as well as to export ammonia from the muscle, preventing this metabolite from traveling free in the blood (for reviews see [9, 52, 53]). Our group previously showed an increase of ~ 480 μmol/L (Ala + Gln) during 90 min of intermittent exercise [54]. In our study, the ammonia transport in the form of Ala and Gln was 270 μmol/L per hour (considering one and two moles of NH_3 for Ala or Gln, respectively). It is important to emphasize that intermittent exercise produces a lower level of ammonia than high intensity exercise.

Performing stoichiometry at the whole-body level is tricky because there are multiple players involved in the production and clearance of metabolites. Increases in ammonia are mainly due to the deamination of purines and amino acids (we are not considering some changes, at the gut level, of ammonia production, absorption or metabolism). During high intensity exercise, ammonia is mainly produced by purine deamination. Because urate is the final metabolite of inosine monophosphate (IMP), it makes sense that KAAAs supplementation could decrease the need for energy derived from the myokinase reaction. This

is supported by the decrease in both the urate and free ammonia inhibition with KAAA supplementation.

Urea synthesis does not discriminate between the ammonia sources; as a result, the increase in urea is because ammonia comes from multiple sources (gut microbes, amino acids and purine deamination). Considering this equation and assuming that the level and content of gut microbes is constant, we can assume that urea reflects the total ammonia production as well as that urate measurement can indirectly demonstrate the ammonia production due to AMP deamination.

KAAA is a mixture of both ketoanalogues and amino acids. As previously described by us, the use of KAAA decreased the increases in the ammonia and urea levels caused by exercise in both human and animal models. This decrease can be caused by the following different possibilities: a) the use of amino acids as an energy source; b) augmentation of glucose synthesis and c) possibility for keto analogues to buffer free ammonia.

A recent study showed that the supplementation with α-keto acids in healthy, untrained subjects significantly improved exercise tolerance, training effects, and stress-recovery state [21]. In our study, KAAA supplementation reduced the exercise-induced increase in blood ammonia concentrations by ~ 20% compared with those of the non-supplemented group, but we did not identify changes in the tested cognitive-motor tasks or exhaustion time between the groups.

There is evidence that hyperthermia, *per se*, promotes a central fatigue state from disturbances in the brain's ability to sustain the activation of skeletal muscles, thus impairing cognitive-motor performance [16]. Walters *et al* [55]

indicated reduced running performance at rectal temperatures between 39.7 and 40.3 °C. This negative effect can be enhanced by environmental conditions under which the exercise is performed, and when the ambient temperature and humidity are high, the capacity to sustain prolonged exercise is reduced [23]. It is possible to postulate that the controlled environmental conditions at the laboratory ameliorate the negative effect to the central nervous system, which is a consequence of a synergetic increase in both temperature and ammonemia. This hypothesis needs to be further studied.

In this study, the subjects were in a state of dehydration (loss of ~ 2.5% of body mass), but there was no change in either physical or cognitive-motor performance. It is recognized that cognitive-motor performance is impaired when there is excessive loss of 1% - 3% of body weight during exercise in the heat [36]. Cian *et al* [56], reported a reduction in cognitive function in dehydrated subjects after a 2% loss of body mass due to heat and exercise. Our study can point to different metabolic and cognitive effects in either physical or cognitive-motor performances. This may occur due to the low thermal stress caused by the controlled laboratory conditions, where it was maintained at a WBGT of ~ 19.5 ° C and equivalent rectal temperatures of ~ 38 º C, during the final phase of exercise.

In our study, we measured a low increase of ammonemia even after a ketogenic diet. It is also possible that dehydration in athletes who are acclimated to exercise in the heat with no significant increase in body temperature does not induce increased blood ammonia sufficient to impair the athletes' cognitive-motor performance. It has been suggested that a hot environment associated with elevated body temperatures contributes to

exacerbated exercise-induced hyperammonemia [41]. Exacerbated exercise-induced hyperammonemia was demonstrated in individuals undergoing prolonged exercise in the heat (40 ° C) compared those undergoing prolonged exercise at 20 ° C [57]. Ammonemia was higher in the hot trials (30 °C, 55% and 35 °C, 62%) than in the control trials (22 °C; 65%), where the participants of both had rectal temperatures of ~ 37.2 °C after a 30-s sprint cycle exercise [58]. Similar to hyperammonemia, hyperthermia can also induce CNS dysfunction by causing an imbalance of neurotransmitters associated with cognitive impairments [24]. Both hyperammonemia and hyperthermia can act synergistically to impair CNS function. In future studies, we will evaluate this possibility.

Conclusion

To our knowledge, this study is the first investigation to evaluate the acute effect of KAAA supplementation on ammonia metabolism after high-intensity exercise and cognitive-motor performance under low heat stress conditions. Our data suggest that KAAA supplementation might decrease ammonemia after high-intensity exercise. On the other hand, no affects were observed in physical or cognitive-motor performances because of KAAA supplementation under low heat stress conditions. We were not able to find differences in cognitive function due to KAAA supplementation under our conditions.

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Table 1. Hydration status did not change during the protocol. Hydration markers from the groups KEx and CEx were measured before (Pre) and after (Post) the cycling session.

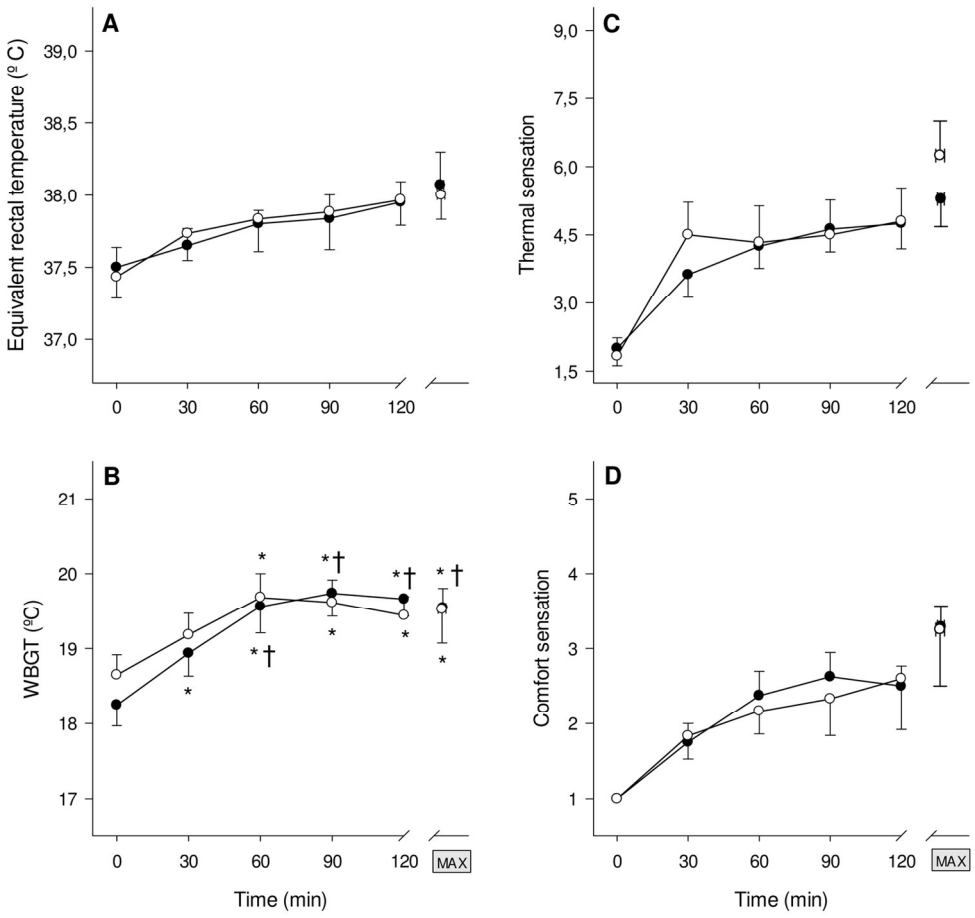
Groups	Moment	% Body Weight Change (Δ %)	Urine Color	Urine specific gravity (USG)	Sweat Rate ($L.h^{-1}$)
KEx	Pre	- 2.47 ± 0.30	4.0 ± 0.9	1019.4 ± 3.4	0.63 ± 0.08
	Post		4.4 ± 0.4	1024.4 ± 1.9	
CEx	Pre	- 2.59 ± 0.46	3.8 ± 0.8	1017.7 ± 3.1	0.84 ± 0.18
	Post		5.0 ± 0.5	1023.3 ± 2.0	

Figure 1. Environmental conditions, body temperature, and thermal and comfort sensations were similar throughout the experimental protocol. The athletes exercised for 2 h followed by a new maximum incremental test (new MIT) to drive the athletes to a reported voluntary exhaustion (MAX) after KAAA supplementation (experimental group - KEx, ●) or control supplementation (CEx, ○). The values are the means and the standard errors. (A) Equivalent rectal temperature: resting values were KEx 37.5 ± 0.1 ºC and CEx: 37.4 ± 0.1 ºC; (B) WBGT: resting values were KEx 18.2 ± 0.2 ºC and CEx 18.6 ± 0.2 ºC; (C) Thermal sensation: resting values were KEx 2.0 ± 0.3 and CEx 1.8 ± 0.4; (D) Comfort sensation: resting values were KEx 1.0 ± 0.0 and CEx 1.0 ± 0.0. The MAX moment varied for each athlete in a range from 124.1-128.4 min (125.6 ± 0.5). The error bars are not visible because they are inside the symbols. * The mean values were significantly different from 0 min within the group. † The mean values were significantly different from 30 min within the group ($P < 0.05$).

Figure 2. Acute KAAA supplementation affects ammonia, urea and urate metabolism. The athletes exercised for 2 h followed by a new maximum incremental test (new MIT) to drive the athletes to exhaustion (MAX) after KAAA supplementation (experimental group - KEx, ●) or control supplementation (CEx, ○). The values are the means and standard errors. (A) Ammonia: resting values were KEx 89.12 ± 8.59 µmol/L and CEx 83.90 ± 10.19 µmol/L; (B) urea: resting values were KEx 5.80 ± 0.71 mmol/L and CEx 6.10 ± 0.63 mmol/L; (C) Ammonemia normalized (∆%) to the resting values; (D) urate: resting values were KEx 308.83 ± 20.68 µmol/L and CEx 317.66 ± 16.56 µmol/L. MAX moment varied for each athlete in a range from 124.1-128.4 min (125.6 ± 0.5). The error bars are not visible because they are inside the symbols. * The mean values were significantly different from 0 min within the group; † The mean values were significantly different from 30 min within the group; ‡ The mean values were significantly different from 60 min within the group; $ The mean values were significantly different from 90 min within the group; # The mean values were significantly different from 120 min within the group ($P < 0.05$). The values of the three metabolites did not significantly differ between the treatments ($P > 0.05$).

Figure 3. KAAA supplementation affects glucose but does not change lactate metabolism. The athletes exercised for 2 h followed by a new maximum incremental test (new MIT) to drive the athletes to exhaustion (MAX) after KAAA supplementation (experimental group - KEx, ●) or control supplementation (CEx, ○). The values are the means and the standard errors (SEM). (A) Glucose: resting values were KEx 4.62 ± 0.11 mmol/L and CEx 5.01 ± 0.18 mmol/L; (B) Lactate: resting values were KEx 1.60 ± 0.24 mmol/L and CEx 1.64 ± 0.26 mmol/L. The MAX moment varied for each athlete in a range from 124.1-128.4 min (125.6 ± 0.5). The error bars are not visible because they are inside the symbols. * The mean values were

significantly different from 0 min within the group; † The mean values were significantly different from 30 min within the group; ‡ The mean values were significantly different from 60 min within the group; $ The mean values were significantly different from 90 min within the group; # The mean values were significantly different from 120 min within the group.



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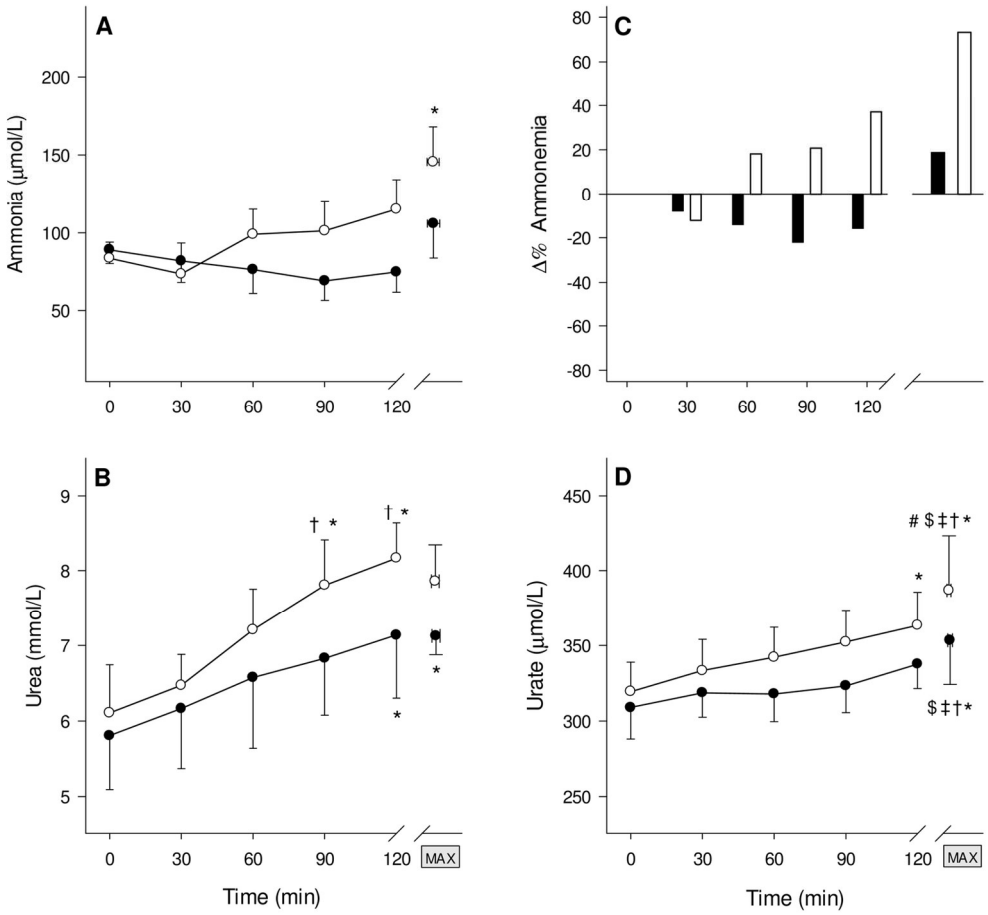

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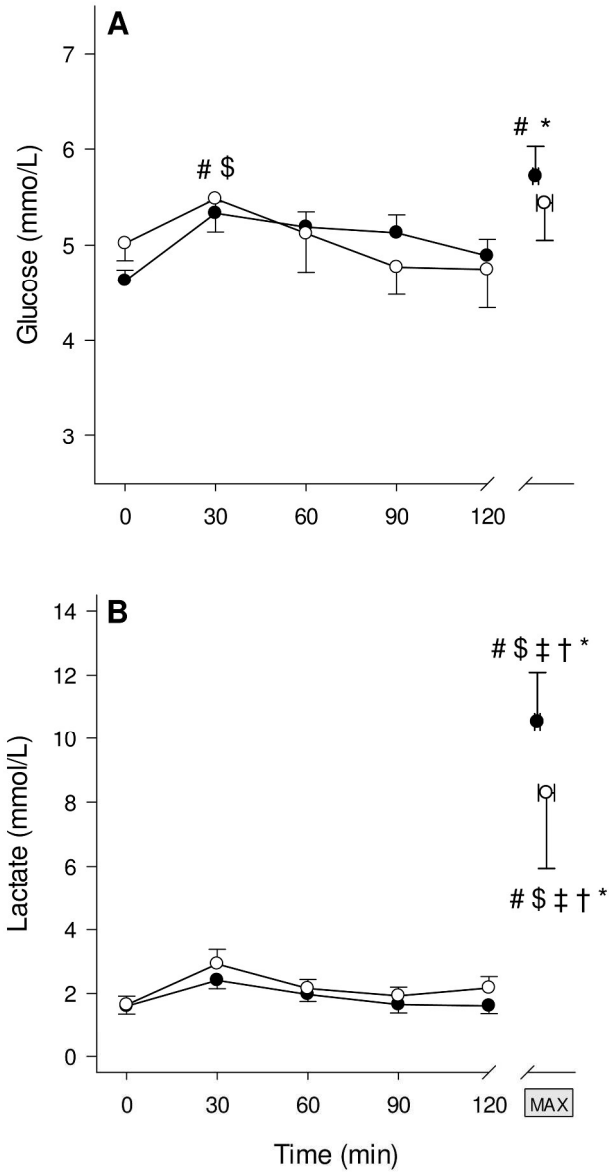

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Keto analogues and amino acids supplementation decreases ammonemia at high-intensity exercise without affecting performance under thermoneutral conditions.