Failure of Glycine-Arginine-α-Ketoisocaproic Acid to Improve High-Intensity Exercise Performance in Trained Cyclists

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Oral supplementation with glycine-arginine-α-ketoisocaproic acid (GAKIC) has previously been shown to improve exhaustive high-intensity exercise performance. There are no controlled studies involving GAKIC supplementation in well-trained subjects. The aim of the current study was to examine the effects of GAKIC supplementation on fatigue during high-intensity, repeated cycle sprints in trained cyclists. After at least 2 familiarization trials, 10 well-trained male cyclists completed 2 supramaximal sprint tests each involving 10 sprints of 10 s separated by 50-s rest intervals on an electrically braked cycle ergometer. Subjects ingested 11.2 g of GAKIC or placebo (Pl) during a period of 45 min before the 2 experimental trials, administered in a randomized and double-blind fashion. Peak power declined from the 1st sprint ($M \pm SD$; Pl 1,332 ± 307 W, GAKIC 1,367 ± 342 W) to the 10th sprint (Pl 1,091 ± 229 W, GAKIC 1,061 ± 272 W) and did not differ between conditions ($p = .88$). Mean power declined from the 1st sprint (Pl 892 ± 151 W, GAKIC 892 ± 153 W) to the 10th sprint (Pl 766 ± 120 W, GAKIC 752 ± 138 W) and did not differ between conditions ($p = .96$). The fatigue index remained at ~38% throughout the series of sprints and did not differ between conditions ($p = .99$). Heart rate and ratings of perceived exertion increased from the 1st sprint to the 10th sprint and did not differ between conditions ($p = .11$ and $p = .83$, respectively). In contrast to previous studies in untrained individuals, these results suggest that GAKIC has no ergogenic effect on repeated bouts of high-intensity exercise in trained individuals.

Keywords: ergogenic, fatigue, power, cycling sprints, trained subjects

The effects of amino acid supplementation on physical performance have been widely investigated, with some studies showing positive effects on strength (Crowe, Weatherson, & Bowden, 2006; Schena, Guerrini, Tregnaghi, & Kayser, 1992) and endurance performance (Blomstrand, Hassmen, Ekblom, & Newsholme, 1991), whereas other studies reported no ergogenic effect (Madsen, MacLean, Kiens, & Christensen, 1996; Pitkanen et al., 2003; van Hall, Raaymakers, Saris, & Wagenmakers, 1995). Despite conflicting results, amino acid supplementation among athletes is widespread (Ahrendt, 2001; Maughan, King, & Lea, 2004) in their belief that exercise performance will be enhanced. Two studies have examined the effects of a specific combination of amino acids, namely glycine, arginine, and α-ketoisocaproic acid (a breakdown product of leucine; GAKIC), on human performance during high-intensity short-duration exercise (Buford & Koch, 2004; Stevens,

Godfrey, Kaminski, & Braith, 2000). In the first study, Stevens et al. found that GAKIC supplementation increased muscle torque and work sustained during intense acute anaerobic dynamic exercise and increased overall muscle performance by delaying muscle fatigue during the early phases of anaerobic dynamic exercise. In a subsequent study, Buford and Koch appeared to support the previous findings by reporting that GAKIC supplementation attenuated the decline in mean power output during repeated cycling sprints. Both studies employed untrained individuals as subjects. Although these are the only two studies to have examined the effects of GAKIC on exercise performance, both reported ergogenic effects yet neither seems to provide a clear mechanism by which the components of GAKIC act (individually or synergistically). The amino acids making up GAKIC appear to be active in the metabolic pathways associated with the biosynthesis of creatine, protein, and nitric oxide (Campbell, La Bounty, & Roberts, 2004; Minuskin, Lavine, Ulman, & Fisher, 1981; Nair, Schwartz, & Welle, 1992). The effects of GAKIC may be to stabilize muscle pH or attenuate the rise in ammonia concentration (Greenstein, Birnbaum, Gullino, Otey, & Winitz, 1956) released from the purine nucleotide cycle (Meyer & Terjung, 1979; Terjung, Dudley, & Meyer,

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1985), possibly through alterations in the enzymatic pathways of nitrogen metabolism (Jeevanandam, Ali, Holaday, Weis, & Petersen, 1993; Sitren & Fisher, 1977). As such, GAKIC could enhance high-intensity exercise performance, because ammonia accumulation has been implicated in fatigue (Wilkinson, Smeeton, & Watt, 2010). GAKIC is marketed as a muscle-fatigue-toxin neutralizer (http://www.muscletech.com).

Although previous research indicates that GAKIC supplementation improves high-intensity exercise performance (Buford & Koch, 2004; Stevens et al., 2000), the paucity of data warrants further investigation. Furthermore, both previous studies (Buford & Koch, 2004; Stevens et al., 2000) used untrained subjects and failed to incorporate more than one baseline trial, leaving the reliability of the data open to question. The subjects in Buford and Koch's study completed a modified Wingate test consisting of five sprints of 10 s. Although there were no statistical differences in peak power output after GAKIC supplementation, an increased power output was reported in the last of the five sprints. Conceivably, had the experiment been continued to include further sprints, an attenuation in the decline in peak power output may have been observed after GAKIC supplementation. Therefore, the aim of this study was to investigate the effects of GAKIC on fatigue during a series of 10 sprints in trained cyclists using an experimental design nearly identical to that in Buford and Koch's study. We hypothesized that GAKIC supplementation would enhance peak power output and attenuate the decline in power output during repeated sprints in trained cyclists, concurrent with previous research.

Methods

Subjects

Ten trained male cyclists (age 33 ± 6 years, weight 72.4 ± 8.9 kg, height 176 ± 1 cm) gave their written informed consent to take part in the current study, which was approved by the local ethics committee and performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). The sample size of the study was in accordance with the subject numbers used in the two previous studies involving GAKIC (Buford & Koch 2004; Stevens et al., 2000), as well as most previous relevant studies (see Hopkins, Schabort, & Hawley, 2001, for review). Subjects were in good health at the time of testing, trained almost daily, and participated regularly in local and national races.

All subjects completed two performance trials consisting of 10 repeated supramaximal sprints. All tests were conducted on an electronically braked cycle ergometer (Lode Excalibur, Lode BV, Groningen, The Netherlands) at standard room temperature (19–21 °C) with 1 week separating the trials. Before these performance trials, familiarization trials were completed until the variability of peak power output was within a 5% difference between the two consecutive trials. No subject had to complete a third familiarization trial to achieve less than 5% variability, in line with our previous experience with trained cyclists. Subsequently, all subjects completed two performance trials before which they ingested either GAKIC or placebo in a double-blinded crossover fashion and in accordance with previous studies (Buford & Koch, 2004; Stevens et al., 2000).

Experimental Procedures

Subjects reported to the laboratory on the day of testing after a 3-hr fast and having refrained from alcohol and strenuous exercise the day before. On arrival at the laboratory, body mass (Avery Weight-Tronix 3302, ABN, Birmingham, UK) and height (Invicta Plastics Ltd., Leicester, UK) were measured. In addition, a heart-rate monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was attached before each test. Subjects were also required to complete a 24-hr dietary record and reproduce this diet the day before each trial in an attempt to standardize the diet in terms of total energy intake, macronutrient intake, and quantity of specific amino acids (glycine, arginine, and leucine).

Experimental Design

Each subject ingested either 11.2 g of commercially available GAKIC (2.0 g glycine, 6.0 g L-arginine monohydrochloride, 3.2 g α-ketoisocaproic acid calcium salt; Iovate Health Sciences Research Inc., Mississauga, ON, Canada) or placebo (9.46 g sucrose, 3.2 g calcium carbonate) before the experimental performance trials. No attempt was made to verify the presence of the active ingredients. Supplements were dissolved in 450 ml of sugar-free fruit juice, and subjects were required to wear a nose clip to prevent differentiating between beverages, because GAKIC has a distinguishable and fairly unpleasant odor. It was confirmed via a simple discussion at the end of each experiment that subjects were not able to identify any differences between the treatments. The supplement was divided into three equal aliquots of 150 ml and ingested 45, 30, and 10 min before exercise. Timing of ingestion and supplementation dose were in line with those used in the two previous studies (Buford & Koch, 2004; Stevens et al., 2000). After ingestion of the third and final beverage, subjects commenced a warm-up consisting of 5 min of cycling at 100–150 W, followed by a familiarization sprint of 5 s. The warm-up also consisted of stretching exercises depending on each individual. Subsequently, subjects performed a series of 10 maximal-effort 10-s sprints, separated by 50-s rest intervals. Each sprint was a modified 10-s Wingate test performed using a resistance of 0.8 N/kg. Power output was analyzed using standard computer software (Version 1, Lode BV, Groningen, The Netherlands). This analysis enabled calculation of both 10-s mean power output (the average power output during each sprint) and 10-s peak power output (the highest power during each sprint). Furthermore, the fatigue index was determined as follows: Fatigue index % = [(peak power – minimum power)/peak

power] × 100, where the minimum power is the lowest power output after the subject achieved peak power output. After the 10 sprints, subjects were instructed to continue pedaling for at least 6–7 min to allow recovery to occur. Heart rate (HR) was recorded (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) and ratings of perceived exertion (RPE) determined using the Borg category scale (Borg, 1982) at the end of each sprint.

Data Analysis

All data are expressed as $M \pm SD$. After a test for the normality of distribution, all repeated-measures data (i.e., peak power, mean power, minimum power, fatigue index, HR, and RPE) were compared between the two treatments using a two-way repeated-measures analysis of variance. Other comparisons (i.e., total energy and macronutrients) were made using Student's paired t test. Statistical significance was set at $p < .05$. All statistical analysis was completed using the software package SPSS, version 11.0 (SPSS, Inc., Chicago, IL).

Results

Generally, all subjects tolerated the supplementation protocol well, with no reports of gastrointestinal distress. There were no significant differences in either macronutrient or micronutrient intake before either GAKIC or placebo trials. Detailed diet analysis is presented in Table 1. No significant differences were observed between GAKIC and placebo treatments in any of the measured performance variables. Peak power (Figure 1) declined from the first sprint (placebo 1,332 ± 307 W, GAKIC 1,367 ± 342 W) to the 10th sprint (placebo 1,091 ± 229 W, GAKIC 1,061 ± 272 W) and did not differ significantly between conditions ($p = .88$). Mean power (Figure 2) declined from the first sprint (placebo 892 ± 151 W, GAKIC 892 ± 153 W) to the 10th sprint (placebo

766 ± 120 W, GAKIC 752 ± 138 W) and did not differ between conditions ($p = .96$); the average coefficients of variation for the 10 sprints taking into account both trials were 2.1% and 2.2% for peak and mean power output, respectively (calculated as indicated in Hopkins et al., 2001). In general, the fatigue index (Figure 3) remained at ~38% throughout the series of sprints and did not differ between conditions ($p = .99$).

RPE increased from the first sprint (placebo 13 ± 3, GAKIC 13 ± 3) to the 10th sprint (placebo 19 ± 1, GAKIC 20 ± 1) and did not differ between treatments ($p = .11$). In addition, HR increased from the first (placebo 151 ± 10, GAKIC 152 ± 12 beats/min) to the 10th sprint (placebo 166 ± 11, GAKIC 164 ± 12 beats/min) and did not differ between conditions ($p = .83$).

Discussion

The findings of the current study suggest that GAKIC supplementation does not have an ergogenic effect on muscle power output or attenuation of fatigue during

Table 1   Comparison of Total Energy Intake, Macronutrients, and Specific Amino Acids ($M \pm SD$)

Variable	GAKIC	Placebo	p
Energy (kJ)	11,246 ± 3,084	10,629 ± 3,808	.46
Carbohydrates (g)	392 ± 110	406 ± 143	.71
Protein (g)	98 ± 38	90 ± 39	.26
Fat (g)	86 ± 44	66 ± 44	.60
Arginine (mg)	1,727 ± 1,158	1,400 ± 585	.34
Leucine (mg)	2,358 ± 1,755	2,496 ± 1,602	.81
Glycine (mg)	1,393 ± 925	1,360 ± 1,023	.93

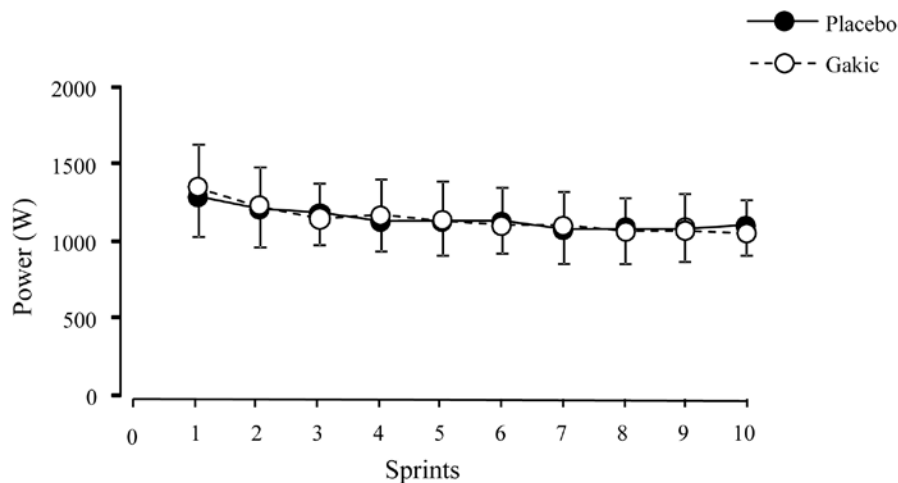*Note*. GAKIC = glycine-arginine-α-ketoisocaproic acid.

Figure 1 — Peak power outputs over 10 repeated cycling sprints with glycine-arginine-α-ketoisocaproic acid (Gakic) and placebo treatments ($N = 10$, $M \pm SD$).
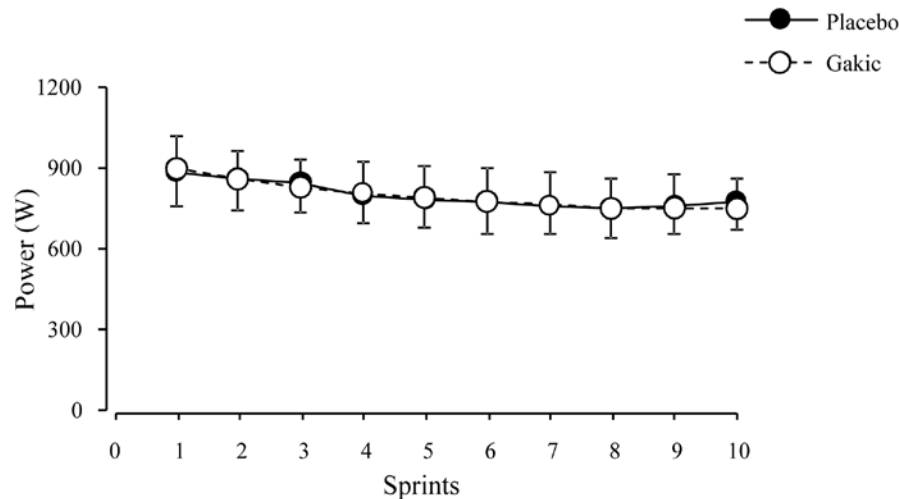
Figure 2 — Mean power outputs over 10 repeated cycling sprints with glycine-arginine-α-ketoisocaproic acid (Gakic) and placebo treatments ($N = 10$, $M \pm SD$).
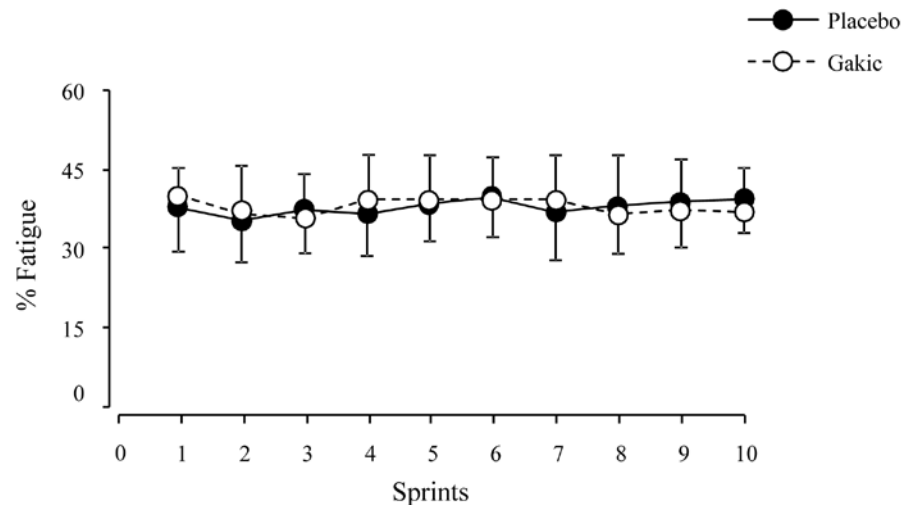

Figure 3 — Fatigue index over 10 repeated cycling sprints with glycine-arginine-α-ketoisocaproic acid (Gakic) and placebo treatments ($N = 10$, $M \pm SD$).

repeated sprints in trained cyclists. These results are in contrast to our initial hypothesis developed from previous literature that suggested that supplementation with GAKIC would enhance peak power output and attenuate the decline in power output during repeated high-intensity sprints. In the current study, GAKIC had no effect on peak power, mean power, minimum power, or fatigue index. In addition, no differences were found in HR or RPE between the two experimental conditions.

The data reported in the current study contradict both previous performance studies in which GAKIC was found to attenuate the decline in power output, improve muscle performance (Buford & Koch, 2004), and delay muscle fatigue, resulting in improved total work (Stevens et al., 2000) during high-intensity exer-

cise. In the initial study, Stevens et al. demonstrated that GAKIC supplementation increased muscle force production by up to 28%, increased total muscle work by at least 12%, and improved overall performance compared with placebo. Stevens et al.'s high-intensity experimental protocol consisted of 35 maximal, isokinetic repetitions of knee extensions at a rate of 90°/s. In the later study, Buford and Koch reported a significant difference in mean power output during high-intensity exercise consisting of five repeated sprints of 10 s on a cycle ergometer. On the other hand, a more recent study (Yarrow, Parr, White, Borsa, & Stevens, 2007) failed to find any ergogenic effect during moderate- or high-intensity exercise when the KIC component of GAKIC was examined in isolation.

In both previous studies (Buford & Koch, 2004; Stevens et al., 2000), it was hypothesized that the specific combination of amino and keto acids contained in GAKIC could work synergistically to improve high-intensity exercise performance through their relevant metabolic pathways. In theory this hypothesis could be proposed because the individual components of GAKIC have been shown to contribute to the detoxification of ammonia (Greenstein et al., 1956; Meneguello, Mendonca, Lancha, & Costa Rosa, 2003), which is released from the purine nucleotide cycle (Meyer & Terjung, 1979; Terjung et al., 1985) during high-intensity exercise or from the catabolism of protein and deamination of amino acids (Sitren & Fisher, 1977; van Hall, van der Vusse, Soderlund, & Wagenmakers, 1995). The detoxification of ammonia could theoretically enhance high-intensity exercise performance because ammonia accumulation has been shown to be linked to muscle fatigue (Wilkinson et al., 2010), although the precise mechanisms remain to be determined. However, when the effect of each GAKIC component on exercise performance was examined (Bower et al., 1995; Daly et al., 1988; Jeevanandam et al., 1993; Nair et al., 1992; Weimann et al., 1998), there was little evidence to support an ergogenic effect.

Arginine appears to be essential for human metabolism only under specific circumstances such us burns, trauma, cancer (Bower et al., 1995; Daly et al., 1988; Weimann et al., 1998), and other conditions of rapid growth, apparently via the production of a number of hormones such as growth hormone (Besset, Bonardet, Rondouin, Descomps, & Passouant, 1982). However, most studies using a variety of doses of L-arginine have failed to show any effect on altering nitric oxide production (Liu et al., 2009), influencing hormone levels (e.g., growth hormone), or indeed exercise performance (Liu et al., 2009; Marcell et al., 1999; McConell, Huynh, Lee-Young, Canny, & Wadley, 2006). In addition, large doses of arginine have been shown to cause gastrointestinal discomfort (unpublished data cited in Wagenmakers, 1999). On the other hand, glycine has an active role in the formation of creatine phosphate (as one of the three amino acids involved in synthesis of creatine), which is the main source of energy during intense exercise such as employed in the current study. The synthesis of creatine from arginine and glycine depends on the formation of guanidinoacetate, which is formed by the enzyme arginine:glycine amino-transferase (AGAT; Walker, 1979). Excess creatine leads to down-regulation of AGAT and in doing so inhibits the formation of guanidinoacetate (Stead, Au, Jacobs, Brosnan, & Brosnan, 2001). Therefore, the rate-limiting step in the formation of creatine is not the concentration of arginine but the formation of guanidinoacetate from AGAT (Campbell et al., 2004). Consequently, supplementation with arginine would not necessarily lead to a higher intramuscular creatine concentration. Previous studies involving KIC or leucine supplementation (individually or in conjunction with other amino acids such as the branched-chain amino

acids) have also failed to find any effect on aerobic or anaerobic performance (Madsen et al., 1996; Pitkanen et al., 2003; van Hall, Raaymakers, et al., 1995; Yarrow et al., 2007). Leucine supplementation seems ineffective in altering nitrogen balance (Sandstedt, Jorfeldt, & Larsson, 1992) or protein degradation (Sandstedt, Jorfeldt, & Larsson, 1992) or reducing the concentration of ammonia (MacLean, Graham, & Saltin, 1996; Madsen et al., 1996). Therefore, the underlying mechanism behind the performance-enhancing effects of GAKIC supplementation reported in the two aforementioned studies (Buford & Koch, 2004; Stevens et al., 2000) remains elusive.

Alternatively, and possibly most likely, the findings in the previous studies (Buford & Koch, 2004; Stevens et al., 2000) could be a result of the experimental design and methodology employed. For example, Stevens et al., using untrained subjects, failed to include baseline trials to establish the repeatability of the performance trials. It remains a distinct possibility that the differences in power output observed in the previous studies (Buford & Koch, 2004; Stevens et al., 2000) were caused by a lack of reliability between performance trials, especially since untrained subjects were used (Hopkins et al., 2001; Coleman, Wiles, Nunn, & Smith, 2005; Hebestreit, Dunstheimer, Staschen, & Strassburg, 1999). Although subjects' maximal oxygen uptake was not measured in the current study, peak power output is an established good predictor of maximal oxygen uptake (Hawley & Noakes, 1992). Peak power output of the first sprint in the current study was $1,297 \pm 263$ W for the placebo trial and $1,353 \pm 283$ W for the GAKIC trial, compared with approximately 750 W in the study by Buford and Koch, reflecting the clear difference in training status of the subjects between studies. Therefore, there are several possible explanations for the difference in reliability between athletes (used in the current study) and nonathletes (used in the previous studies by Stevens et al. [2000] and Buford & Koch [2004]). First, the frequent exposure to high-intensity exercise on an almost daily basis may eliminate performance variability (Hopkins et al., 2001). Second, physical conditioning during the course of a study should not change to the same extent in trained and nontrained individuals. Third, and possibly the most likely explanation, is the custom-to-detail approach typically associated with trained cyclists (e.g., using customized cycling shoes and pedals, fixed cycling position). Finally, assuming a constant random error contributed by equipment or examiners, this error will have less impact on overall outcome when expressed as a percentage of the higher power output of trained individuals (Hopkins et al., 2001). In the current study, variability in performance trials between familiarization trials was small (i.e., <5%), as would be expected from subjects who are well accustomed to the exercise protocols employed (Coleman et al., 2005; Hebestreit et al., 1999; Hopkins et al., 2001). The more stringent methodology used in the current study ensured that all possible confounding factors were minimized.

Conclusion

The data from the current study do not support the findings of previous studies (Buford & Koch, 2004; Stevens et al., 2000) that GAKIC supplementation will enhance high-intensity exercise performance. After we controlled for all possible confounding factors that could have adversely affected the results, GAKIC supplementation had no effect on peak power, mean power, or fatigue index. On the basis of this finding and those of previous studies involving the individual components of GAKIC, there appears to be little evidence to support the ergogenic effects of GAKIC during high-intensity exercise performance in well-trained subjects.

Acknowledgments

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