Glycine-Arginine-α-Ketoisocaproic Acid Improves Performance of Repeated Cycling Sprints

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ABSTRACT

BUFORD, B. N., and A. J. KOCH. Glycine-Arginine-α-Ketoisocaproic Acid Improves Performance of Repeated Cycling Sprints. *Med. Sci. Sports Exerc.*, Vol. 36, No. 4, pp. 583–587, 2004. **Purpose:** The purpose of this study was to determine the effect of glycine-arginine-α-ketoisocaproic acid (GAKIC) supplementation on repeated bouts of anaerobic cycling performance. **Methods:** Ten men completed a randomized, double-blinded, placebo-controlled exercise protocol of two sessions separated by 7 d. Plasma lactate was analyzed in blood collected 45 min before exercise (REST) and 5 min postexercise (POST). Subjects consumed either 11.2-g GAKIC or placebo (PLC) during a 45-min period between the REST and exercise. Mean power, peak power, and fatigue values were assessed from five supramaximal, 10-s cycle ergometer sprints, separated by 1-min rest intervals. Data were analyzed using repeated measures ANCOVA. **Results:** A significant treatment × time interaction ($P = 0.039$) was observed for the change in mean power output over the five sprints between the GAKIC and PLC treatments. *Post hoc* analyses revealed a greater retention of mean power ($P = 0.038$) between sprints 1 and 2 after GAKIC (-1 ± 9 W) versus PLC treatment (-47 ± 18 W). No other performance variables differed between PLC and GAKIC. POST lactate was increased ($P < 0.001$) above REST, but there was no difference between treatments ($P = 0.936$). **Conclusion:** These data support an ergogenic effect of GAKIC for attenuating the decline in mean power during repeated bouts of supramaximal exercise. **Key Words:** EXERCISE PERFORMANCE, FATIGUE, LACTATE, SUPPLEMENTATION, AMINO ACIDS

Rapid loss of muscular power output is a hallmark of exhaustive dynamic exercise (13). Many dietary supplements have been used in an attempt to enhance the retention of muscle power during short-term and high-intensity exercise. Some of these supplements, including creatine monohydrate and β-hydroxy-β-methylbutyrate (HMB) (17), have been shown to provide an ergogenic effect during high-intensity exercise.

Manipulation of nitrogen metabolism through the administration of ketoacids has been demonstrated to reduce some of the acute effects of trauma, including clinically dysfunctional skeletal muscle (18). A recent study by Stevens et al. (19) tested the hypothesis that an amino acid/ketoacid mixture could improve the performance of skeletal muscle during exercise. The authors reported that a glycine-arginine-α-ketoisocaproic acid mixture (GAKIC) significantly increased work output and delayed muscle fatigue during exhaustive isokinetic knee extensions. The findings of their study indicated that GAKIC imparted significant increases in torque production and resistance to fatigue during exercise, suggesting that it may play a useful role as a dietary aid for strength/power athletes. The precise mechanisms through which GAKIC may have imparted these performance gains are unknown. However, the time frame during which the effects of GAKIC were observed suggests possible involvement of metabolic pathways associated with branched chain amino acid (leucine) metabolism, biosynthesis, and utilization of creatine and/or HMB (19,20), and stabilization of muscle pH and ammonia (the purine nucleotide cycle end-product (10)).

Several studies have indicated that conventional isokinetic exercise may not apply directly to other dynamic exercise modes (1,9,15). In attempts to overcome this, Lewis and Fulco (13), Stevens et al. (19), Kaminski (12), and others have utilized specialized approaches to quantifying exhaustive exercise by employing fatigue indexes. Such indexes account for dynamic changes in peak torque and power output compared with the unfatigued state. Nonetheless, isokinetic dynamometry using multiple knee extension sets of 70 s each (19,12) may have limited the practical applicability of previous findings using GAKIC. Therefore, to more closely approximate the effects of GAKIC ingestion on performing a task more closely related to many real world athletic endeavors, we used a series of 10-s Wingate cycle ergometer bouts to test progressive anaerobic fatigue (11,22). Many sporting events (football, basketball, volleyball, etc.) consist of repeated bouts of high-intensity fatiguing movements, each lasting in duration from seconds to less than a minute (3). Therefore, we tested the effects of

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GAKIC on the performance of anaerobic exercise in a more applicable athletic setting.

The purpose of the current study was to determine the effects of GAKIC on a series of five supramaximal sprints on a cycle ergometer. It was hypothesized that GAKIC supplementation would attenuate the loss of power output and reduce fatigue over the course of repeated exhaustive exercise bouts each lasting 10 s in duration.

METHODS

Subjects. Ten healthy college male subjects (age = 21 ± 1 yr, height = 179 ± 5 cm, 85.95 ± 16.77 kg) were recruited to participate in the study. All subjects reported engaging in planned high-intensity exercise (resistance exercise or sprints) at least 3 $d \cdot wk^{-1}$. Subjects provided written informed consent in accordance with Truman State University's policies for use of human subjects. Truman State University's Institutional Review Board approved all data collection and subject recruitment procedures.

Subjects visited the laboratory on three occasions. The first visit was used to explain the experimental procedures and collect anthropometric data. Two to four days after the first visit, subjects returned to the human performance laboratory for the second test session. The second and third sessions consisted of experimental treatments (GAKIC or placebo) and were randomly assigned in a double-blind, crossover fashion, separated by 3–14 d (mean = 7 d). Stevens et al. (19) previously demonstrated that residual effects of the GAKIC were not evident 24 h after ingestion; thus, we deemed the 72 h (minimum) window between treatments acceptable.

Initial testing. All subjects indicated that they were free of diabetes mellitus, phenylketonuria, or any other metabolic disorders. Anthropometric data (height and weight) were obtained using a wall-mounted stadiometer and a calibrated digital scale, respectively. The session was also used to familiarize the subjects with the testing equipment and provide instruction on how to complete the 24-h dietary records.

Pretesting dietary controls. Before each experimental treatment, subjects completed a 24-h dietary record. Dietary records were analyzed for total energy (kJ) content, protein (g), carbohydrates (g), fat (g), and for concentration (mg) of the specific amino acids L-leucine, glycine, and L-arginine. Dietary analyses were accomplished using a commercially available software package (Nutritionist IV, First DataBank, San Bruno, CA).

Experimental treatments. Upon arriving at the laboratory in a 12-h fasted state, subjects rested for 10 min in a supine position. A 4-mL preexercise blood sample (REST) was then collected from an antecubital vein. After collection of the blood sample, subjects consumed the treatment beverage (GAKIC or placebo) in three equal aliquots over a 45-min period. Treatments were administered in a randomized, repeated measures, double-blinded fashion. The GAKIC supplement consisted of glycine-L-arginine-α-ketoisocaproic acid (2.0-g glycine plus 6.0-g L-arginine monohydrochloride plus 3.2-g α-ketoisocaproic acid calcium salt). The isocaloric placebo (PLC) was composed of 9.46 g of sucrose. The dosing regimen employed was identical to the methodology reported by Stevens et al. (19). Briefly, 11.2 g of dry GAKIC powder or 9.46 g of sucrose placebo was blended into 355 mL of chilled cranberry juice. The mixture was then divided into three equal portions, which were then served at 45, 30, and 10 min before exercise. The beverages were served in opaque containers and subjects were instructed to pinch their noses as they drank in an effort to disguise differences between PLC and GAKIC and maintain the double blind.

After 45 min, subjects followed a 2-min warm-up protocol on a cycle ergometer. For the warm-up, the cycle ergometer was set at a fixed resistance of 2.0 kp. Subjects were instructed to alternate their pedal cadence between a self-selected, relaxed pace and all-out sprint in the following pattern: 30 s relaxed then 10 s sprint, 20 s relaxed then 10 s sprint, 20 s relaxed then 15 s sprint, and ending with 15 s relaxed. Three minutes after the warm-up, subjects performed a series of five, 10-s sprints on a Monark model 818E cycle ergometer. Each sprint was separated by a 50-s rest interval. During the rest intervals, subjects remained seated on the cycle ergometer and were permitted to backpedal (against zero resistance) to prevent cramping in the thighs. Immediately after the last sprint, a postexercise blood sample (POST) was collected. Subjects were free to leave the laboratory after collection of the postexercise blood sample.

Performance variables. Each sprint was a modified 10-s Wingate test, performed using a resistance of 0.8 $N \cdot kg^{-1}$ body mass. Although higher resistance levels have been shown to produce maximum power outputs (8), the Monark model 818E ergometer is limited to a maximum resistance setting of 7 KP, which was the impetus for our choice. Subjects initiated the test from a dead stop, the resistance on the ergometer's friction belt having been preset by the testers immediately before the test. The Monark ergometer was equipped with a computer-interfaced optical sensor to allow the measurement of pedal revolutions (SMI Optosensor 2000, SMI, St. Cloud, MN). This sensor fed data into an IBM-compatible microcomputer allowing the analysis of power output data using the SMI Power software program. This software calculates power outputs for each second of work. Mean power was defined as the average power output (mean of 10 values) over each 10-s Wingate test. The single highest second of power output per second of each 10-s test was termed peak power. Fatigue index was the ratio between peak power value and the minimum value and was determined from the following equation: fatigue index % = [(peak power − minimum power)/peak power] × 100. The minimum power value used in the equation was the lowest power output after the subject achieved peak was used rather than an overall minimum, because the when sprints are initiated from a dead stop, the power output during the first second is often the lowest recorded. Using the minimum power recorded after the attainment of peak power provided an indication of the decrease in power output due to fatigue during the 10-s sprint. Figure 1 pro-

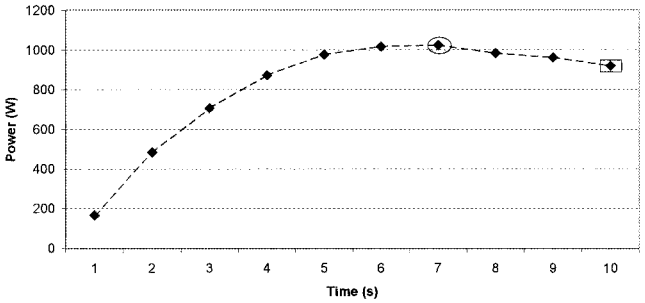

FIGURE 1—Sample output from a single 10-s sprint. The *circled point* indicates peak power. The *point enclosed in a box* indicates the minimum power output after the peak.

vides a sample graph of the output from a 10-s sprint. Intraclass correlations among the five sprint trials revealed a high level of reliability for all the performance variables measured (r = 0.987 for peak power, r = 0.976 for mean power, and r = 0.8522 for fatigue index,).

Blood collection and analysis. All blood samples were collected from an antecubital vein by a trained phlebotomist using a sterile needle with a Vacutainer. Samples were collected into 4-mL heparinized Vacutainers; 750 μL of whole blood was transferred to microcentrifuge tubes containing 750-μL chilled 1 M perchloric acid and mixed using an air-displacement pipet. The microcentrifuge tube was then centrifuged at room temperature at 1000 $\times$ *g*. After centrifuging for 10 min, the deproteinized plasma was extracted and frozen at −100°C for later analysis. Samples were analyzed for lactate using standard spectrophotometry techniques (Sigma kit no. 735-10, St. Louis, MO).

Data analysis. All data are presented as means ± standard deviation. Dietary variables (total energy, macronutrient, and selected amino acid concentrations) were compared between the 24 h before each of the two treatments using paired samples *t*-tests. Performance variables (peak power, mean power, and fatigue index) were compared between GAKIC and PLC using a 5 $\times$ 2 (5 sprints $\times$ 2 treatments) repeated measures ANCOVA. Treatment order was the covariate in the analysis. Posthoc analyses were accomplished using paired contrasts to compare changes in a given performance variable from one sprint to the next between GAKIC and PLC treatments (i.e., comparing the change in mean power output from sprint 1 to sprint 2 between GAKIC and PLC). Plasma lactate concentrations were compared using a 2 $\times$ 2 (treatments $\times$ time) repeated measures analysis of variance (ANOVA). Statistical significance was set at the 0.05 level of confidence.

TABLE 1. Comparison of total energy, macronutrient and selected amino acid content of pretest diets 24-h prior to GAKIC or placebo treatments (N = 10); values are mean ± SD.

Variable	GAKIC	Placebo	*P*
Energy (kJ)	9557 ± 3386	11,639 ± 3,022	0.126
Carbohydrates (g)	305 ± 133	200 ± 126	0.189
Protein (g)	99 ± 40	131 ± 60	0.120
Fat (g)	76 ± 28	87 ± 48	0.508
Arginine (mg)	1277 ± 1253	1070 ± 1117	0.556
Leucine (mg)	5526 ± 3438	7193 ± 2999	0.162
Glycine (mg)	1014 ± 933	836 ± 852	0.555

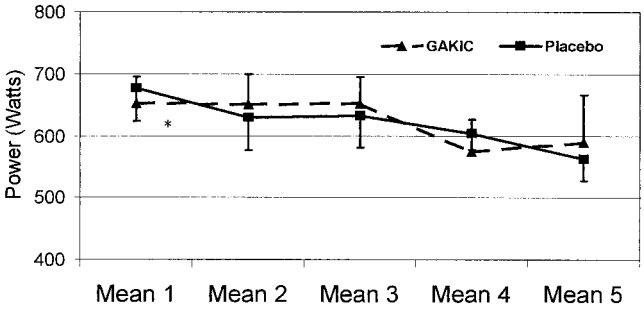

FIGURE 2—Mean power outputs over five repeated cycling sprints with GAKIC and placebo treatments (N = 10, mean ± SD). *Repeated measures ANCOVA indicated an overall significant difference (P = 0.039) between GAKIC and PLC over the course of five sprints, with a significantly (P = 0.038) smaller decline in power output for GAKIC compared with PLC between sprints 1 and 2.

RESULTS

Dietary analyses. Paired samples *t*-tests revealed no significant differences in total energy, macronutrient, or amino acid intake between the diet records 24 h before GAKIC or PLC treatment. Table 1 provides a summary of the dietary analysis before each test condition.

Performance variables. Two $\times$ 5 repeated measures ANCOVA indicated a significant difference between PLC versus GAKIC in the pattern of change in mean power output over the five sprints (P = 0.039, Fig. 2). *Post hoc* analyses revealed that the decrease in mean power output between sprints 1 and 2 was significantly (P = 0.038) less with GAKIC than with PLC (−1 ± 9 W vs. −47 ± 18 W respectively). There were no treatment $\times$ time effects observed for the variables of peak (P = 0.487) power output (Fig. 3) or fatigue index (P = 0.537, Fig. 4).

Plasma lactate. Repeated measures ANOVA revealed that POST lactate concentration were significantly (P < 0.001) increased above REST lactate concentration, with no difference between treatments (P = 0.936, Fig. 5).

DISCUSSION

The results of the present study indicate that GAKIC ingestion significantly attenuates the drop in mean power output associated with repeated sprints of anaerobic cycling.

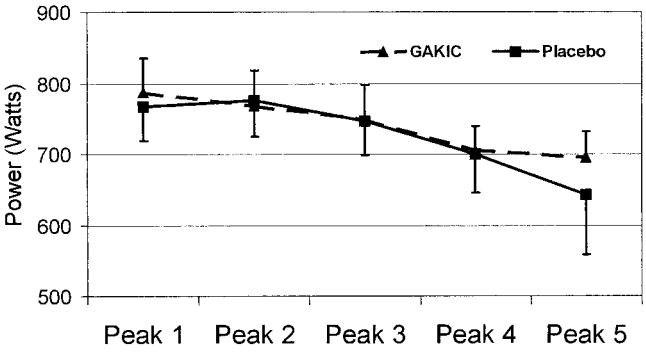

FIGURE 3—Peak power outputs over five repeated cycling sprints with GAKIC and placebo treatments (N = 10, mean ± SD). No between treatment differences were observed.

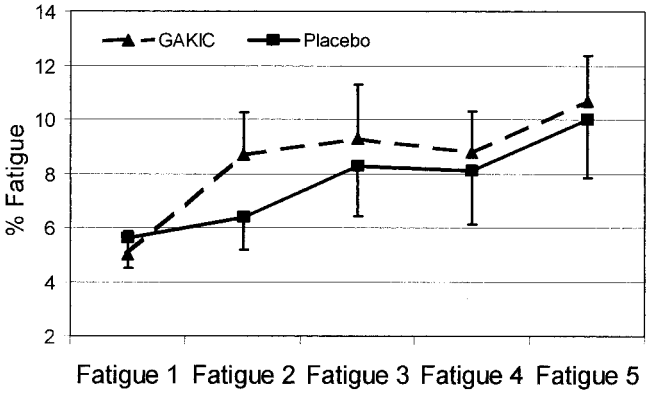

FIGURE 4—Fatigue index over five repeated cycling sprints with GAKIC and placebo treatments (N = 10, mean ± SD). No between treatment differences were observed.

These findings support the work of Stevens et al. (19), who found that GAKIC significantly improved total work performed on three sets of isokinetic knee extensions by an average value of 10.5% and improved resistance to fatigue by an average value of 21%.

Previous GAKIC results (19) were based on multiple sets of isokinetic knee extensions for durations of approximately 70 s each. In a preliminary experiment (Buford and Koch, unpublished data), we found that GAKIC ingestion did not affect performance variables (peak or mean power output, nor fatigue index) after a single 30-s Wingate test, suggesting that the duration and intensity of the exercise stimuli employed may be important. Therefore, for the present study we hypothesized that a GAKIC-induced ergogenic effect would be evident during the course of a repeated series of anaerobic cycle exercise bouts totaling 50 s in duration. The results of the present study support our hypothesis, in that GAKIC ingestion resulted in a significantly improved retention of mean power output between the first and second sprint of a series of five cycle bouts comprised of 10-s work and 50-s rest.

GAKIC is composed of glycine, L-arginine, and α-ketoisocaproic acid. Ingestion of GAKIC may affect metabolic pathways associated with L-arginine, glycine, and α-ketoisocaproate (KIC), the ketoacid parent of the amino acid L-leucine. Given this, it is possible that consumption of these three amino acids in the daily diet could have confounded the results of the present experiment. Dietary analysis indicated that during the experimental treatment, subjects maintained a constant intake of glycine, L-arginine, L-leucine, total protein, total calories, and carbohydrates. This similarity in pretest energy and nutrient content between treatments is paramount in ruling out any interfering effects of daily dietary intakes on the present results. From the dietary data, it is clear that the main difference between conditions is the KIC ingested during the GAKIC treatment.

The precise mechanism by which GAKIC improves exercise performance is currently unknown. GAKIC treatment is theorized to enhance anaerobic exercise performance through any one of several mechanisms including alterations in acidosis, ammonia and transamination waste products, or via alterations in the enzymatic pathways concerning nitrogen and branched chain ketoacid metabolism (2,4,6,16,21,23–26).

Buckspan et al. (4) have reported that KIC infusion decreased glucose uptake and increased lactate release in fasting subjects. One potential explanation of their findings is that KIC may enhance glycogen breakdown in skeletal muscle (4). Additionally, KIC infusion in isolated muscle preparations has been shown to inhibit the enzyme pyruvate dehydrogenase (5), which would shift the fate of pyruvate towards lactate dehydrogenase and lactate production. Given this, we hypothesized that GAKIC ingestion would result in a greater release of lactate. Plasma lactate concentrations were increased significantly above rest at postexercise, with no difference between GAKIC and PLC treatments. The lack of a treatment difference in lactate concentration suggests that GAKIC did not alter the rate of anaerobic glycolysis during the Wingate test. Thus, the present data provide no indication that orally ingested KIC altered glucose kinetics during repeated bouts of high-intensity exercise on a cycle ergometer, and the precise mechanism behind GAKIC's effectiveness remain unclear. Although the ingestion of carbohydrate, such as the sucrose

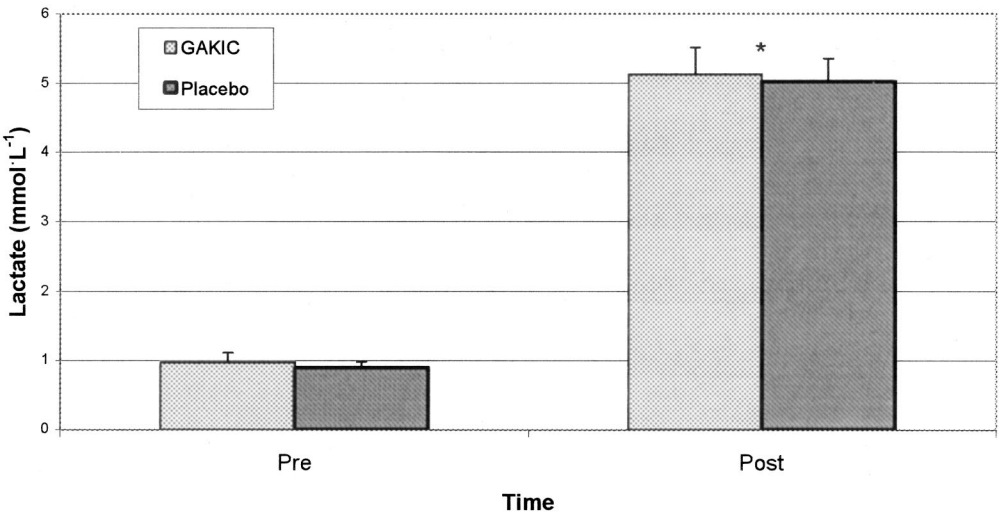

FIGURE 5—Plasma lactate values at rest and after five 10-s Wingate tests with GAKIC and placebo treatments (*N* = 10, mean ± SD). *Significant within-treatments difference from rest (*P* < 0.001).

placebo, could potentially lead to an increase in plasma lactate and confound the current findings, the small dosage (9.46 g sucrose) ingested in the present study makes this event unlikely. Previous research (7) has demonstrated no difference in lactate levels after vigorous anaerobic exercise between carbohydrate and placebo ingestion, after a much higher (1.0 g CHO·kg^{-1} body mass) carbohydrate dosage.

In conclusion, the present data obtained using repeated sets of Wingate cycle ergometry support and extend the previous isokinetic dynamometer findings of Stevens et al. (19), that GAKIC can serve as ergogenic aid to improve muscle performance in repeated, high-intensity, exercise. These findings reinforce the notion that GAKIC supplementation may be a useful aid for strength-power athletes, similar to creatine supplementation. Unlike creatine, which requires approximately 5 d of loading to produce improvements in high-intensity work (14), GAKIC appears to impart an ergogenic effect within minutes of consumption. This presents an obvious advantage for GAKIC supplementation. Future studies are needed to corroborate the effectiveness of GAKIC. Additionally, it would be of interest to determine if GAKIC supplementation in combination with creatine or HMB supplementation would yield an additive effect.

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