High-intensity dynamic human muscle performance enhanced by a metabolic intervention

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ABSTRACT

STEVENS, B. R., M. D. GODFREY, T. W. KAMINSKI, and R. W. BRAITH. High-intensity dynamic human muscle performance enhanced by a metabolic intervention. *Med. Sci. Sports Exerc.*, Vol. 32, No. 12, 2000, pp. 2102–2108. **Purpose**: The purpose of this study was to quantify the effects of a metabolic treatment on human muscle dynamic performance (strength, work, and fatigue) measured under conditions of acute, exhaustive high-intensity anaerobic isokinetic exercise. **Methods**: Unilateral prefatigue and postfatigue peak torque and work values were measured in the quadriceps femoris of 13 subjects using a computer-controlled isokinetic dynamometer, over a 23-d interval. The two experimental treatments were: 1) a glycine and L-arginine salt of α-ketoisocaproic acid calcium ("GAKIC"); and 2) isocaloric sucrose (control). Based on a randomized double-blind cross-over repeated measures design, measurements were made before and during an exhaustive anaerobic fatigue protocol to calculate a *F*atigue *R*esistance *I*ndex (FRI = [peri-exhaustion torque] ÷ [baseline peak torque]), as well as total work. **Results**: The FRI and total work for each of the exhaustion sets measured at 0, 5, and 15 min after oral GAKIC treatment were greater than values obtained for isocaloric control treatment ($P < 0.02$). GAKIC treatment increased the mean resistance to fatigue (FRI) up to 28% over isocaloric control. Overall gain in total muscle work attributable to GAKIC was 10.5 ± 0.8% greater than control, sustained for at least 15 min. After 24 h, both GAKIC and control concentric forces returned to the same absolute values ($P > 0.05$): mean FRI = 0.42 ± 0.05 and mean total work = 4600 ± 280 J. There were no significant differences attributable to random order of testing. **Conclusions**: Compared with isocaloric carbohydrate, oral GAKIC treatment increased muscle torque and work sustained during intense acute anaerobic dynamic exercise; additionally, it increased overall muscle performance by delaying muscle fatigue during the early phases of anaerobic dynamic exercise. **Key Words:** MUSCLE, ACUTE, FATIGUE, EXERCISE PERFORMANCE

Muscle performance can be impaired during both acute and chronic phases of intense exercise. Dynamic high-intensity anaerobic use of skeletal muscle rapidly leads to fatigue and reduction in muscle force and work (11) and thus diminished athletic performance. Deleterious effects occurring during this phase propagate to long-term training results.

Various ergogenic supplements have been tried with limited success in attempts to enhance muscle performance during acute and long-term exercise (2,8,12,33–36). Advances in quantifying the effects of training or metabolic treatments on acute anaerobic dynamic skeletal muscle performance have been hindered by the difficulty of reproducibly and objectively measuring performance parameters during this phase of exercise (1,2,26,26).

Recently, our lab (17) and others (reviewed in (19,21,37)) have approached this problem in athletes by employing objective techniques in isolated muscle groups undergoing concentric and/or eccentric contractions. Isolated muscle studies are useful in systematically assessing the modular components of the whole body physiological response to exercise. The present study employs an isokinetic dynamometer in conjunction with a testing protocol that permits meaningful, reproducible performance assessments of the isolated quadriceps muscle group.

In vitro experiments and hospital studies have demonstrated (6,14,16,25) that metabolic manipulation of nitrogen metabolism using selected ketoacids alone, or in conjunction with certain amino acids, can reduce various acute pathophysiological effects of trauma, including clinically dysfunctional skeletal muscle. In the light of these findings, we hypothesized that an orally administered ketoacid/amino acid combination metabolic treatment would improve the

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dynamic performance of skeletal muscle during acute, anaerobic, exhaustive dynamic exercise in healthy adult males, compared with control isocaloric sucrose treatment. To test this hypothesis, the treatment effects were assessed using our Fatigue Resistance Index (FRI) (17), in conjunction with concentric force and total work obtained for dynamically fatigued isolated human quadriceps femoris.

METHODS

Subjects. The study was approved by the university Institutional Review Board. All subjects completed a health inquiry questionnaire and signed a written informed consent agreement before participation. The male volunteers (N = 13; age 20.9 ± 1.9 yr; height 71.6 ± 2.1 cm; mean ± SEM) indicated that they were free from the following metabolic conditions: diabetes mellitus, aminoacidurias, including maple syrup urine disease, renal failure, muscle wasting, hypertension, abdominal radiotherapy or intestinal resection, fever, steroid or immunosuppressant use. Blood pressure, leg weight, body weight, and dietary recalls were monitored during the testing period. Consumed L-leucine, L-arginine, glycine, total protein, total calories, and total carbohydrate were calculated using the Minnesota Nutrition Data software (ver. 2.91) developed by the Nutrition Coordination Center at the University of Minnesota.

Treatments. Subjects were orally administered 355-mL low calorie cranberry juice containing either 11.20-g freshly dissolved powdered GAKIC, or 9.46-g sucrose isocaloric control. This was consumed in 3 equal aliquots over 45 min. The 11.20 g of GAKIC was comprised 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 dicalcium). The reagents were chemically pure U.S.P.-grade components packaged and labeled coded for double-blind testing.

Protocol. The overall design was a repeated measures, randomized, cross-over scheme conducted in a double-blind fashion, with fasted human subjects orally receiving both of two treatments. Force (torque) and total work measurements were obtained unilaterally in the quadriceps femoris muscle group in real-time using a computerized isokinetic dynamometer with appropriate software (Kin-Com 125AP; Chattanooga Group, Inc., Chattanooga, TN). This permitted continual assessment of each subject's ability to produce muscular force and work over time (17). Pilot studies were initially conducted to determine eccentric and concentric isokinetic fatigue conditions (i.e., reps; rate, etc.) and muscle performance values. The present study focuses on the concentric phase.

An overall concept diagram of the protocol is shown in Figure 1, with details given below. Each subject was required to make five visits to the testing facility, with three visits in the fasted state. The entire testing procedure spanned 23 d. Peak torque, average torque, and work values were analyzed from a full set of repetitions throughout every degree in the range of motion. The technician gave verbal encouragement throughout each repetition sequence. Twenty-four–hour dietary recalls were obtained preceding each visit. Subjects fasted during 12 h preceding visits 2, 3, 4, and 5.

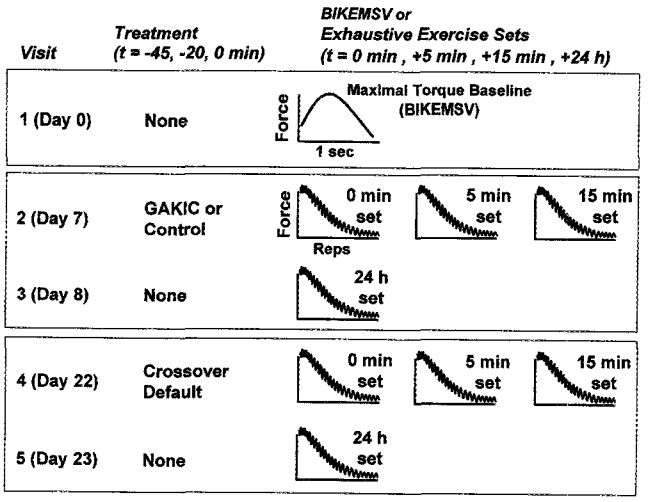

FIGURE 1—Testing protocol overview. This design was the basis for the double-blind cross-over repeated measures assessment of metabolic treatments on exhaustive anaerobic muscle torque, work, and fatigue resistance index. Specific details are given in the text.

FRI was assessed for each individual subject and for the group. One week preceding initial fatigue testing (day 0, Fig. 1), a Baseline Isokinetic Knee Extension Maximal Strength Value ("BIKEMSV"; peak torque, N·m) was obtained for each subject at a rate of $90°·s^{-1}$. During subsequent fatigue test bouts associated with the metabolic treatments (Fig. 1), each subject then performed another sequence of 35 continuous isokinetic concentric/eccentric knee extension repetitions at $90°·s^{-1}$. Peak forces for both the concentric and eccentric phases were analyzed during the peri-exhaustion phase—i.e., the last 5 repetitions of each 35-rep set. An average of the concentric five peak values was obtained, and the FRI was calculated for each bout by dividing this average peri-exhaustion torque value by the maximal force generated during the BIKEMSV assessment. FRI was obtained for each set in the protocol (0, 5, and 15 min; as well as 24 h). Total work (TW) values were also calculated for each 35 rep set.

To illustrate the use of the FRI in this study, a conceptual scheme comparing the FRI for two exhaustive exercise sets is shown in Figure 2. In this example, Set A and Set B could represent two treatment groups, or they could represent two different bouts associated with a given treatment. The example given here represents a single subject exerting a 250 N·m maximal representing BIKEMSV. In the example of Figure 2, the mean peak torque for the last 5 repetitions of the 35 rep set is given as 75 N·m for example treatment A, and 125 N·m for treatment B. This results in example FRI values of 0.30 and 0.50, respectively. The ensuing data are based on similar calculations (Fig. 2) obtained for each subject at each visit (Fig. 1).

Visit 1. The unilateral quadriceps femoris BIKEMSV was measured during the first visit. This was subsequently used to calculate FRI for both control and GAKIC treatment groups.

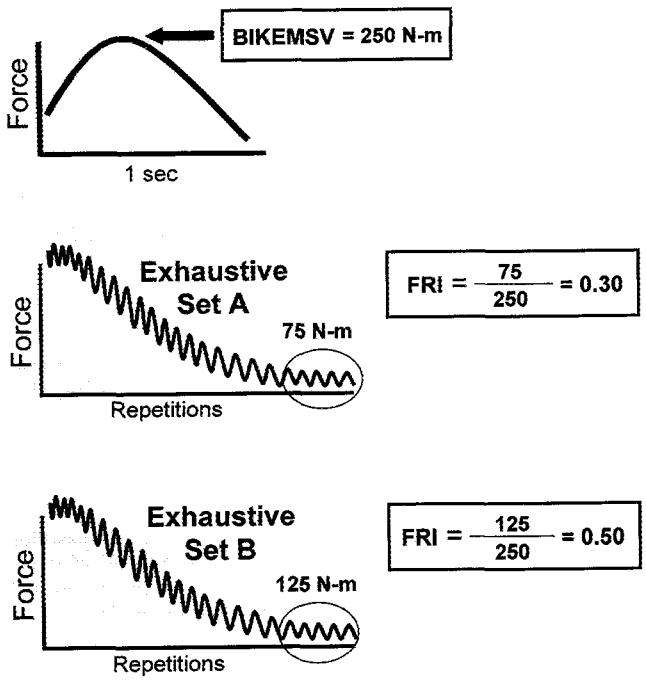

FIGURE 2—Concept of Fatigue Resistance Index assessment. The concept is shown comparing two exhaustive exercise sets to BIKEMSV for one subject, with calculations of FRI for each case. In this example, the 90°/s exhaustive exercise protocol described above is executed for treatment set A or treatment set B regimens. The Baseline Isokinetic Knee Extension Maximal Strength Value (BIKEMSV) for this hypothetical subject is 250 N·m. The mean peak torque for the last 5 repetitions of the 35 rep set is 75 N·m for set A, and 125 N·m for set B. This results in example FRI values of 0.30 and 0.50, respectively.

Visit 2. Resting blood pressure was measured, then subjects engaged in a practice/warm-up session comprised of 3 submaximal consecutive concentric and eccentric repetitions, followed by a second set of 3 maximal concentric and eccentric repetitions. This practice session preceded delivery of GAKIC or control. GAKIC or sucrose control was then administered in three equally aliquoted doses at t = −45 min, −20 min, and 0 min before dynamometer exercise. Blood pressure was measured again at −20 min. Once the final t = 0 dose of GAKIC or control was administered, subjects immediately performed 35 consecutive right knee concentric and eccentric contractions using maximal effort at a speed of 90°/s. The 35 rep exercise/fatigue set was then repeated at +5 and +15 min after the t = 0 final administration of GAKIC or control.

Visit 3. After a 24-h period without delivery of GAKIC or control, subjects returned for another testing bout. Subjects ate *ad libitum* and completed dietary recalls for this 24-h period. The subjects performed another set of 35 concentric and eccentric knee extension repetitions without GAKIC or control treatment.

Visit 4. After a 14 d rest, after another 24-h dietary recall and 12-h fast, the same subjects were assigned to crossover treatments and repeated the protocol in a manner identical to visit 2.

Visit 5. Visit 5 was identical to visit 3.

Statistics.

The number of subjects recruited (N = 13) exceed the calculated sample size requirement from power analysis, using the following conditions. These parameters were derived from our preliminary experiments using our leg extension force apparatus and protocol: the detectable percent difference in means between two groups was better than a FRI = 5, with a population standard deviation of ± 5. With the power set at 0.80 and with α = 0.05, then the required N ≥ 10 for repeated measures paired t-tests.

Significant differences in the values of FRI and TW between the GAKIC trials and the isocaloric carbohydrate control trials were assessed using repeated measures ANOVA or paired t-tests, calculated with SigmaStat statistical software. Also assessed was the treatment order. An *a priori* α = 0.05 was used for all comparisons.

RESULTS

Subject parameters. There were no significant differences between the randomly assigned control versus GAKIC treatment periods, with respect to systolic blood pressure (117.2 ± 2.8 vs 118.8 ± 2.8 mm Hg), diastolic blood pressure (73.6 ± 2.3 vs 73.2 ± 2.5 mm Hg), body mass (82.93 ± 12.3 vs 82.74 ± 12.0 kg), and leg weight (84.8 ± 8.9 vs 86.2 ± 11.4 N). Furthermore, there were no significant differences between the GAKIC and control treatment test periods concerning dietary levels of L-leucine, L-arginine, glycine, total protein, total carbohydrate, and total kcal for the 24-h intervals preceding the testing days (Table 1).

FRI enhancement by GAKIC treatment. The concentric FRI, shown in Figure 3, represented peri-exhaustion force expressed as a fraction of BIKEMSV (17). The overall

TABLE 1. Subjects' dietary intake of selected nutrients during protocol.

Treatment, Visit No. (Day of Protocol)	L-Leucine (g)	L-Arginine (g)	Glycine (g)	Protein (g)	Total Carbohydrate (g)	Total kcal
CONTROL treatment, visits 2 or 4 (days 7 or 22)	8.1 ± 1.4	5.4 ± 1	4.3 ± 0.9	102 ± 18	331 ± 42	2531 ± 363
GAKIC treatment, visits 2 or 4 (days 7 or 22)	8.6 ± 1.6	6.0 ± 1.3	4.9 ± 1.3	109 ± 21	301 ± 33	2385 ± 293
CONTROL treatment, visits 3 or 5 (days 8 or 23)	8.8 ± 0.9	5.8 ± 0.6	4.5 ± 0.5	112 ± 10	387 ± 36	2690 ± 230
GAKIC treatment, visits 3 or 5 (days 8 or 23)	8.0 ± 2.0	5.3 ± 1.1	3.8 ± 0.8	101 ± 23	328 ± 62	2534 ± 439

For each nutrient, there were no significant differences between any treatment group means by visit; means ± SD.

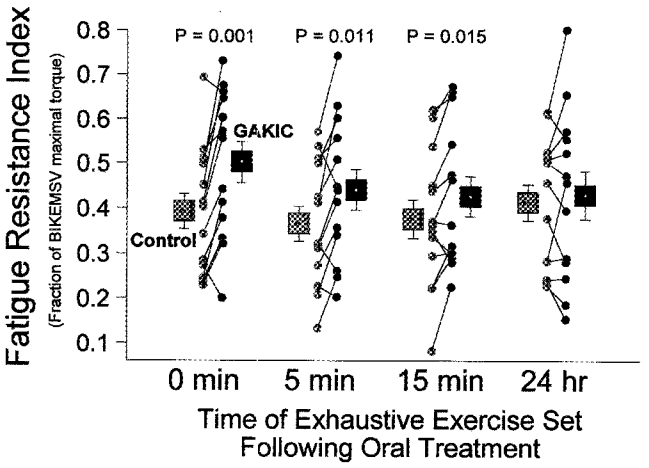

FIGURE 3—Concentric muscle fatigue resistance enhanced by GAKIC treatment. The Fatigue Resistance Index is shown for each subject (*gray and black filled circles*), as well as their collective mean ± SEM (*boxes*), at each time period. The FRI reflects torque of fatigued muscle concentric movement as a fraction of the maximal torque (BIKEMSV). Means were significantly different by repeated measures crossover ANOVA at the 0, 5 and 15 min periods, but not at the 24-h point.

group BIKEMSV maximal torque was 240.7 ± 9.4 N·m. Comparing treatment groups, the greater FRI values with GAKIC indicate that this treatment was associated with the ability to sustain significantly greater muscle force (torque), as well as the ability to significantly reduce acute muscle fatigue ($P < 0.02$). Mean significant differences were sustained at 0 min (control FRI = 0.392 ± 0.039 vs GAKIC FRI = 0.502 ± 0.046), 5 min (control FRI = 0.364 ± 0.039 vs GAKIC FRI = 0.440 ± 0.045), and 15 min (control FRI = 0.375 ± 0.043 vs GAKIC FRI = 0.426 ± 0.045) for these same-subject repeated measures cross-over paired comparisons, as shown in Figure 3. After 24 h, the treatment groups were not significantly different, with overall mean FRI = 0.421 ± 0.050.

Gain in fatigue resistance directly attributable to GAKIC treatment. Values were obtained as the percent increase in concentric mean FRI for GAKIC treatment compared with isocaloric carbohydrate treatment, as shown in Figure 4. The significant ($P \leq 0.05$) percent gains were 28 ± 7% at 0 min, 21 ± 7% at 5 min, and 14 ± 5% at 15 min. After 24-h recovery, values were not significantly different from zero.

Total work performed during each fatigue set for the concentric phase. The data of Figure 5 indicated that GAKIC treatment means were significantly greater than control means ($P < 0.05$) by repeated measures comparisons of the 35-repetition fatigue sets at 0, 5, and 15 min. After 24 h, the effect of GAKIC treatment was restored to the control mean value of TW = 4600 ± 280 J, with no significant difference between treatment groups.

The GAKIC effect: muscle work gain directly attributable to GAKIC treatment. The difference in total concentric muscle work output was obtained for carbohydrate compared with GAKIC treatments, as shown in Figure 6. The GAKIC treatment values were significantly greater than carbohydrate treatment group values ($P \leq 0.05$) at 0, 5, and 15 min. GAKIC treatment increased the mean TW per set by 12%, 9%, and 11% above isocaloric carbohydrate values ($P \leq 0.05$) at these respective time points (Fig. 6). The overall mean percent gain in muscle total work attributable to GAKIC treatment was 10.5 ± 0.8% retained for at least 15 min. After 24 h, work gain values were not significantly different.

No treatment order effects. Analysis of values by order of treatment in the repeated measures cross-over design (i.e., GAKIC in either first or second set of trials) indicated no significant differences at any test time point for either FRI values or total work. This validated the protocol's randomly assigned treatment order.

DISCUSSION

A single bout of exhaustive exercise—indeed any acute muscle trauma—perturbs inter- and intra-organ metabolic and energetic events, resulting in attenuated performance and recovery (4,30). The present study was designed to test our hypothesis that metabolic intervention with oral delivery of GAKIC can improve isolated quadriceps concentric muscle performance during the acute anaerobic phase of exhaustive isokinetic exercise.

Overall, the results demonstrated that GAKIC significantly ($P \leq 0.05$) improved performance compared to control isocaloric carbohydrate treatment. The salient findings can be summarized as follows: 1) GAKIC treatment increased the ability to sustain muscle force (concentric torque) up to at least 28% during intense acute anaerobic muscle exercise; 2) GAKIC treatment increased the ability to sustain muscle total work by up to at least 12% during intense anaerobic muscle exercise; and 3) GAKIC increased the overall muscle performance by delaying muscle fatigue during the early phases of anaerobic exercise through at least 15 min.

FRI. The FRI test instrument (17) used in this study quantified the maximal exerted force sustained under fatiguing conditions as a percent or fraction of maximal force

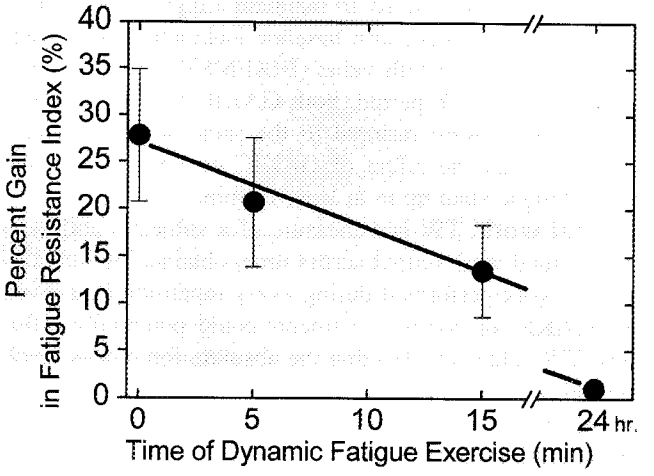

FIGURE 4—Gain in concentric fatigue resistance directly attributable to GAKIC treatment compared to isocaloric control. The group maximal torque value was 241 ± 9 N·m.

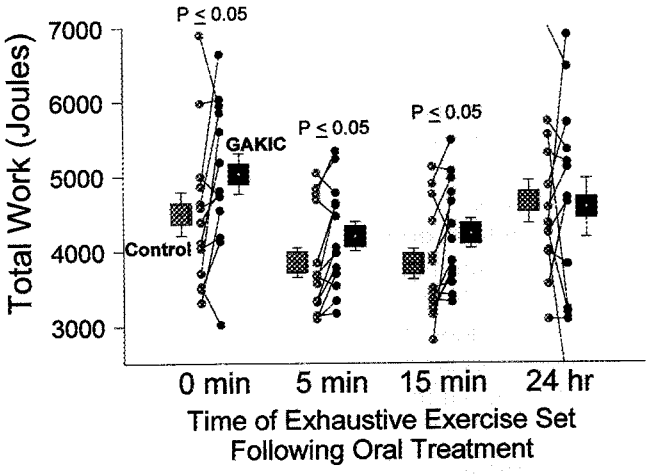

FIGURE 5—Concentric phase total work. Quadriceps femoris total work performed during each fatigue set for the concentric phase was obtained with GAKIC or control treatment. Total work is shown for each subject (*gray and black filled circles*), as well as their collective mean ± SEM (*boxes*), at each time period. Means were significantly different by repeated measures crossover ANOVA at the 0, 5 and 15 min periods, but not at the 24-h point.

exerted under fresh conditions for each individual over a period of time. The FRI provided a meaningful measure of resistance to fatigue by allowing each subjects to perform the same fatigue protocol of four 35-repetition high-intensity sets of 90°/s knee extensions, regardless of fatigue rate (Fig. 2). This is in contrast to other methods which have attempted to quantify exercise fatigue (e.g., bicycle ergometers, free weight trials, vertical leap tests, and treadmill running) that are not as sensitive to acute changes in muscle force. Inasmuch as the data were not influenced by the random order of treatment (GAKIC or control given first; $P > 0.05$), the absence of potential crossover "learning effect" artifact biases in this study further validated the results.

It is notable that GAKIC treatment significantly ($P < 0.02$) increased the mean FRI above control FRI for each of the 35-repetition sets obtained at 0, 5, and 15 min after oral treatment, compared with isocaloric carbohydrate control treatment (Fig. 3). These data indicated that GAKIC treatment increased the ability to maintain a higher percentages of the subjects' concentric baseline isokinetic knee extension maximum strength value (BIKEMSV) over these periods. After a 24-h period, both GAKIC and control concentric forces were restored to the same absolute values, indicating that the effect of GAKIC occurred during the initial fatigue state up to at least 15 min.

Total work. TW is a measure of a subject's ability to sustain total work output across time, obtained by integrating the work performed during every repetition in a given set. GAKIC or control treatments could potentially influence TW values by affecting the absolute force peak level, the ability to sustain a given force peak level, or by changing the magnitude of sustained force output during each repetition. During the 0, 5, and 15 min sets, GAKIC treatment resulted in greater concentric TW values compared with isocaloric carbohydrate treatment. The treatment effects were not significantly different after 24 h, reinforcing the notion that GAKIC influences the early phases of acute anaerobic fatigue.

Role of GAKIC. GAKIC is a glycine and L-arginine salt of α-ketoisocaproic acid. The physiological effects of GAKIC treatment likely affected metabolic pathways individually or synergistically associated with L-arginine, glycine, and α-ketoisocaproate (KIC) as the ketoacid parent of the branched chain amino acid L-leucine (10,20,28,30–34). Therefore, it was important to maintain equal intake of these dietary sources during both treatment testing periods, as was demonstrated in Table 1. In addition to maintaining equal dietary intake of L-leucine, L-arginine, glycine, total protein, total calories, and total carbohydrate (Table 1), the additional physical parameters of systolic and diastolic blood pressure, body mass, and leg weight were also maintained throughout the protocol (see Results).

The exact mechanism by which the components of GAKIC enhanced acute exhaustive anaerobic muscle

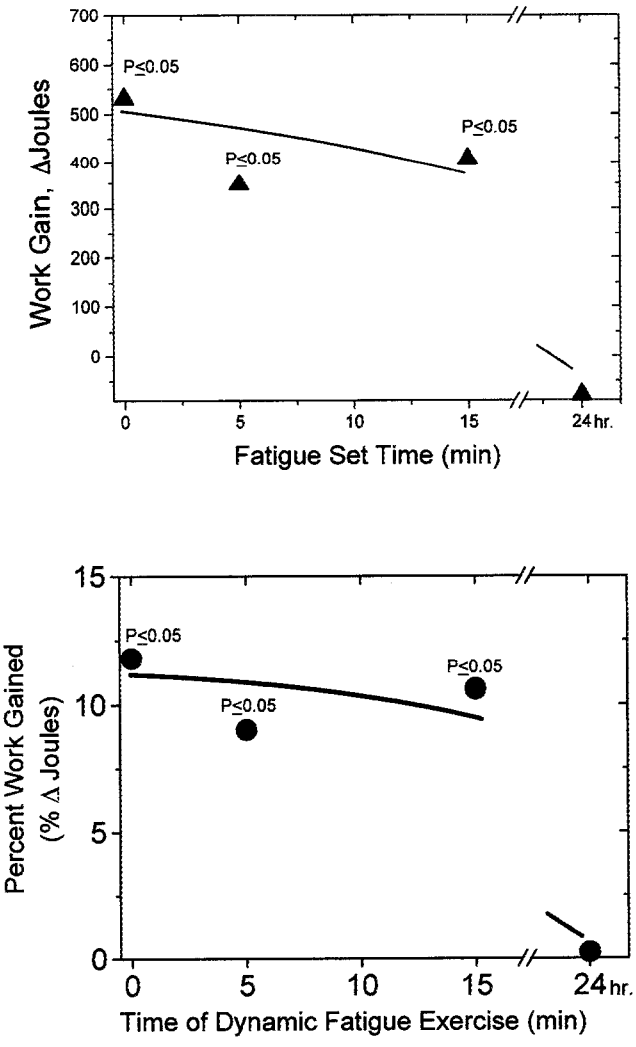

FIGURE 6—Concentric muscle work gain directly attributable to GAKIC treatment. A, The ΔJ value represents the gain in concentric work attributable to GAKIC, reported as the difference between GAKIC and control values. B, Percent increase in total work gained attributable to GAKIC. The overall mean percent gain in total muscle work attributable to GAKIC (over control values) was 10.5 ± 0.8% preceding the complete return to baseline work at 24 h.

performance is currently not known. However, the mechanism likely included effects on anaerobic energetics, modifications of muscle bed hemodynamics, metabolic acidosis, or ammonia levels, and/or enzymatic pathways concerning branched chain ketoacid and nitrogen metabolism (4,6,10,24,28,29,31–34,36).

The time frame of onset and duration of the effects of GAKIC (Figs. 3–6) are consistent with previously reported natural kinetics of endogenous KIC in humans. Fielding and coworkers (10) demonstrated in humans that endogenous levels of muscle KIC rose by about 50% during acute high-intensity cycle exercise, whereas natural levels of plasma KIC peaked at 15 min after the exercise period and returned to preexercise levels by 60 min. These data suggested that KIC diffused into the circulating blood from exercising muscle after a lag period (10). Hospital and *in vitro* studies of sepsis, muscle trauma, liver disease, and attendant central portal encephalopathy, or renal disease, have shown that administered combinations of ketoacids and amino acids improve muscle trauma recovery time, reduce serum ammonia, enhance acute and long-term injury repair (16), and stabilize excitatory central nervous system physiology (38).

The observed biochemical and physiological effects of oral KIC on muscle recovery likely involve several control points (28,30), including key enzymes. The most critical enzymes are BCAA-aminotransferase (EC 2.6.1.42), the BCKA dehydrogenases (EC 1.2.4.4) family, L-leucine dehydrogenase (EC 1.3.1.9), and 3-methyl-2-oxobutanoate dehydrogenase (EC 1.2.4.2). Enzyme concentrations of BCAA-aminotransferase are relatively unregulated at fairly steady-state levels in muscle (13). Therefore, transamination is reversibly catalyzed by BCAA-aminotransferase activity through mass action of available concentrations of KIC, L-leucine, L-glutamate, α-ketoglutarate, and their ancillary metabolites (30). In contrast to unregulated BCAA-aminotransferase, the activity of BCKA-dehydrogenase (EC 1.2.4.4) is highly regulated during exercise (13) in muscle and liver, BCKA-dehydrogenase is a multienzyme complex that catalyzes the irreversible oxidative decarboxylation of BCKA, as it reduces NAD to NADH. The activity of BCKA-dehydrogenase greatly increases immediately after strenuous exercise, with subsequent return to resting baseline levels by 10 min postexercise (18). BCKA-dehydrogenase enzyme activity is regulated by an ATP phosphorylation (inactivation)-dephosphorylation (activation) mechanism. It is notable that KIC is a key stimulator of this enzyme complex, whereby it inhibits the ATP-mediated kinase allosteric inactivation of BCKA-dehydrogenase (3,18); the potency of KIC is several orders of magnitude greater than other BCKAs. BCKA-dehydrogenase activity is therefore mediated by exercise and nutritional factors at the levels of allosteric and substrate mass action.

KIC helps to reduce the endogenous ammonia that hinders muscle function. In the presence of the excessive free NH_3 liberated from the purine nucleotide cycle activated during exercise or from glutamate, L-leucine dehydrogenase (EC 1.4.1.9) provides a beneficial pathway that catalyzes the NH_3 amination of KIC to yield L-leucine. Matthews and coworkers (22) demonstrated that enterally administered KIC in humans is aminated to leucine in the splanchnic bed.

Recent whole animal evidence (15) suggests that enterally administered KIC may contribute to survival of septic rats by conversion to various keto energy alternatives; the survival benefits were specific to KIC rather than ketoacids in general. The enzyme 3-methyl-2-oxobutanoate dehydrogenase can catalyze the decarboxylation of α-ketoisocaproate, leading to pathways eventually creating acetoacetate. KIC can be hydrolyzed to β-hydroxy-β-methylvalerate by cytosolic dioxygenase (24).

L-arginine and glycine are the other components of GAKIC. The dibasic amino acid L-arginine is a "conditionally essential" amino acid that becomes essential under certain metabolic conditions including muscle trauma and injury (9,27). L-arginine can rapidly induce vasodilation in skeletal muscle via vascular smooth muscle nitric oxide biosynthesis (15). The blood flow/perfusion effects of administered L-arginine can be localized in muscle beds (23), as supported by data in the present study (Results text) showing the absence of systemic hypotension with GAKIC compared with control. L-arginine aides in conversion of ammonia to urea (15) and increases levels of pituitary hormones in human athletes, with the metabolic effects of arginine augmented by simultaneously administrating glycine (the third component of GAKIC) or branched chain amino acids (5,7). The present study with GAKIC employed the parent ketoacid (KIC) of one such branched chain amino acid, leucine, in conjunction with L-arginine and glycine.

Interestingly, the time frame during which GAKIC affects acute high-intensity exercise overlaps with that of creatine (12,34). In the light of known metabolic pathways converting arginine and/or glycine to creatine (28), and given the supporting role of KIC in stabilizing purine nucleotide cycle waste, it is tempting to suggest that components of GAKIC may work through muscle energetic pathways in common with creatine. However, additional *in vitro* and *in vivo* research is required to pursue this.

Conclusions. In conclusion, we have employed a specific testing protocol to demonstrate that GAKIC treatment, compared with isocaloric control, improves dynamic muscle function under intense exhaustive anaerobic conditions. In this exercise state, GAKIC treatment increases the ability to sustain muscle force, sustain total muscle work, and delay muscle fatigue. Gains in skeletal muscle strength and performance can be achieved over time by exploiting the fundamental "overload principle" of exercise physiology training, whereby muscle fibers are overloaded and forced to contract at maximal or near maximal tension for a given period of time. Therefore, in addition to its beneficial use in enhancing muscle performance during acute exhaustive activity, the results of this study suggest that GAKIC is useful in enhancing the long-term effects of high volume training and performance that are physiologically linked to the acute phase.

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