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Pharmacokinetics, safety, and effects on exercise performance of L-arginine α-ketoglutarate in trained adult men

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Abstract

Objective: We evaluated the pharmacokinetics, safety, and efficacy of L-arginine α-ketoglutarate (AAKG) in trained adult men.

Methods: Subjects participated in two studies that employed a randomized, double-blind, controlled design. In study 1, 10 healthy men (30–50 y old) fasted for 8 h and then ingested 4 g of time-released or non–timed-released AAKG. Blood samples were taken for 8 h after AAKG ingestion to assess the pharmacokinetic profile of L-arginine. After 1 wk the alternative supplement was ingested. In study 2, which was placebo controlled, 35 resistance-trained adult men (30–50 y old) were randomly assigned to ingest 4 g of AAKG (three times a day, i.e., 12 g daily, $n = 20$) or placebo ($n = 15$). Participants performed 4 d of periodized resistance training per week for 8 wk. At 0, 4, and 8 wk of supplementation the following tests were performed: clinical blood markers, one repetition maximum bench press, isokinetic quadriceps muscle endurance, anaerobic power, aerobic capacity, total body water, body composition, and psychometric parameters tests. Data were analyzed by repeated measures analysis of variance.

Results: In study 1, significant differences were observed in plasma arginine levels in subjects taking non–timed-release and timed-release AAKG. In study 2, significant differences were observed in the AAKG group ($P < 0.05$) for 1RM bench press, Wingate peak power, blood glucose, and plasma arginine. No significant differences were observed between groups in body composition, total body water, isokinetic quadriceps muscle endurance, or aerobic capacity.

Conclusion: AAKG supplementation appeared to be safe and well tolerated, and positively influenced 1RM bench press and Wingate peak power performance. AAKG did not influence body composition or aerobic capacity.

Keywords: Sports nutrition; Ergogenic aid; Resistance training; Supplementation; Exercise

Introduction

L-arginine is a "semi-essential" amino acid used by all cells [1]. It plays a critical role in cytoplasmic and nuclear protein syntheses, the biosynthesis of other amino acids, creatine synthesis, and the urea cycle. In this essential biochemical pathway, urea is synthesized from arginine to enable the body to remove excess ammonia, which is toxic to cells. L-arginine is classified as a glucogenic amino acid

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because it can be metabolized into α-ketoglutarate (AKG) and enter the citric acid cycle. In addition, L-arginine has been reported to improve the immune response and increase the release of growth hormone and insulin [2].

In one of its most important roles, L-arginine serves as a precursor for the biosynthesis of nitric oxide (NO), an endogenously produced, cellular signaling molecule that is involved in a variety of endothelium-mediated effects in the vasculature [3,4]. NO serves as a second messenger to trigger blood vessel dilation and increase blood flow. Nisoli et al. [5] recently reported that NO stimulates the synthesis of mitochondria. Although NO was first identified in endothelial cells [6], the generation of NO from L-arginine occurs in a variety of other cell types including skeletal muscle [3,7,8].

Nitric oxide is produced endogenously from L-arginine in a complex reaction that is catalyzed by the enzyme NO synthase [9]. The other product that is formed in this reaction is citrulline. L-arginine is the only endogenous nitrogen-containing substrate of NO synthase and thus governs the production of NO. In consequence, under certain conditions, the plasma concentration of L-arginine may be rate limiting for NO production. Research has indicated that exogenously administered L-arginine promotes NO-mediated biological effects [10]. Studies in animals have reported that acute and long-term administration of L-arginine improves blood flow and vascular health [11]. In humans, studies have reported beneficial effects after oral L-arginine supplementation including improved blood flow, reductions in blood pressure, and improved immune function [11,12]. Theoretically, L-arginine supplementation may support general health, enhance blood flow to tissues, and/or enhance protein synthesis [13,14]. These outcomes would potentially be beneficial for athletes undergoing heavy resistance training. However, the available literature on the effects of L-arginine on exercise and/or training adaptations is limited and conflicting [15].

α-Ketoglutarate is a five-carbon dicarboxylic acid produced in the citric acid cycle from the oxidative decarboxylation of isocitrate. In a subsequent decarboxylation reaction, AKG is converted to succinyl coenzyme A, a reaction catalyzed by the AKG dehydrogenase complex. Hammarqvist et al. [16] reported that AKG supplementation after surgery limited postoperative decreases in the concentration of glutamine and other amino acids. Ornithine AKG has been reported to modulate protein tissue metabolism in rats with burn injury and muscle-wasting conditions [17,18]. In addition, Czernichow et al. [19] reported that intergastrically administered ornithine AKG improved the early adaptive hypertrophic response to resection in rats. Theoretically, if oral AKG or ornithine AKG supplementation influences protein metabolism or catabolism, then it may help athletes undergoing intense training increase muscle mass and/or promote positive training adaptations. However, it should be noted that some studies have involved enteral administration of AKG or ornithine AKG and less is known regarding the effects of oral arginine, AKG, or ornithine AKG supplementation on protein metabolism. Wiren et al. [20] reported that oral supplementation of AKG after surgery had no significant effects on protein metabolism or catabolism in patients undergoing elective abdominal surgery. In consequence, some have doubted the potential value of orally supplemented AKG as a means of modulating protein synthesis [21].

The reported physiological and biochemical effects of L-arginine and AKG have apparently served as the rationale behind the development and marketing of a number of NO potentiating dietary supplements to resistance-trained athletes. These supplements are purported to stimulate NO production, improve blood flow to muscle during resistance training, increase protein synthesis, and/or reduce catabolism, leading to greater training adaptations. Although there is some supportive theoretical rationale, it is unclear whether oral dietary supplementation of L-arginine and AKG influence training adaptations in resistance-trained athletes. Therefore, this study evaluated the pharmacokinetics, safety, tolerability, and effects on exercise performance of L-arginine/α-ketoglutarate (AAKG) in trained adult men.

Materials and methods

Two separate studies were conducted to assess the pharmacokinetic profile of ingesting two forms of AAKG in the blood (study 1) and the effects of dietary supplementation of AAKG on training adaptations in resistance-trained men (study 2). The following paragraphs describe the methods employed in each study.

Study 1: pharmacokinetic profile

Ten male subjects volunteered to participate in this phase of the study (37 ± 5 y old, 180 ± 5 cm, 85 ± 17 kg). The study was conducted using a double-blind, randomized, and crossover design separated by 1 wk. All testing sessions were started at the same time of day for each testing session. All subjects were asked to replicate their diets for the 24-h period before each testing session. Participants came after fasting for 8 h and ingested 12 oz of non–sugar-fortified orange juice 2 h before donating their baseline blood sample. In a randomized and double-blind manner, participants ingested four caplets of non–time-released (NTR) AAKG (i.e., form of AAKG that is released immediately into the gut) or time-released (TR) AAKG (i.e., form of AAKG that is released from the delivery system matrix in a planned, predictable, and slower-than-normal manner). The total weight of each caplet was 1500 mg and consisted of 1000 mg of L-arginine/AKG and 500 mg of excipients including the time-released matrix material. These excipients included dicalcium phosphate, cellulose ethers and composites, sea-

weed extract, corn extract, pectin, and magnesium stearate. The formulation did not contain yeast, wheat, soy, sugar or other sweeteners, artificial flavors, colors, or preservatives. Ingestion of four caplets of AAKG provided approximately 2 g of L-arginine and 2 g of AKG.

After ingestion of the supplements, subjects donated blood samples at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. Four hours after ingestion of the supplementation, each participant was provided with an additional 12 oz of orange juice. Because the fasting and data collection protocol lasted 18 h, orange juice was provided to subjects as described above to prevent hypoglycemia and/or undue hunger pangs. Because both groups followed the same fasting protocol and ingested identical amounts of orange juice at the same time, provision of orange juice to the subjects would not have been a limiting factor in the ability to determine whether the different forms of AAKG had different time courses in the appearance of arginine in the blood.

The AAKG used for the pharmacokinetic study (study 1) and for the safety and efficacy study (study 2) was purchased from Rexim (Degussa-SAS, Paris, France). This material is of the highest purity commercially available (>99.0%) and produced in accordance with current good manufacturing practices. AAKG caplets were formulated and manufactured for the Medical Research Institute (MRI; San Francisco, CA, USA) in the United States in accordance with current good manufacturing practices. AAKG is currently manufactured and distributed in the United States by MRI under the trade name of NO2. The proprietary nature of this product is protected under U.S. patent number 6,905,707 (B2), and additional patents are pending. The quality and integrity of the active ingredient and finished product were assessed and certified by an independent, third-party analytical laboratory (Eurofins Scientific Inc., Petaluma, CA, USA). In addition, every finished lot of AAKG (NO2) is tested for the presence of illegal or otherwise unwanted substances under the terms and conditions of an agreement with Banned Substances Control Group (Los Angeles, CA, USA), an independent analytical laboratory certified and accredited by the International Olympic Committee (IOC).

Study 2: training study

Subjects

Thirty-five male, experienced, resistance-trained males volunteered to participate in the study. Subjects were informed of the risks and benefits and signed informed consent statements in adherence with the institutional review board of Baylor University. To qualify for participation, subjects had to be 30 to 50 y of age, been involved in a systematic resistance-training program for ≥1 y before the start of the study, and not have used any ergogenic aids within the previous 6 mo. Subjects who volunteered to participate in this study underwent two familiarization/practice exercise sessions to become accustomed to the exercise testing to be employed in the study. Demographic information of the subjects were 38.9 ± 5.8 y of age, 86 ± 13.7 kg of body weight, 178 ± 8.4 cm in height, and 18.8 ± 5% body fat. Subjects reported resistance training 4 ± 1 d/wk for 6 ± 3 h/wk.

Experimental design

Subjects reported to the laboratory to sign informed consent statements, complete medical and training history questionnaires, and become familiarized with the testing protocol. The subjects were then scheduled to participate in two practice testing trials to establish reliability in performing the exercise protocol. The testing protocol had the subjects perform a bench press, isokinetic leg extension test, and a Wingate anaerobic test. The procedures for these tests are described in detail below. After the practice trials were completed, the subjects were scheduled for baseline testing.

Subjects were asked to not change their dietary habits in any way throughout the 8-wk investigative period. This was monitored by having each subject document dietary intake for 4 d (3 weekdays and 1 weekend day) before each testing session. In addition, each subject was instructed to fast for 12 h and not to perform any physical activity for the 2 d preceding each testing session. Upon entering the laboratory for baseline testing, each subject completed the 36-item Short-Form Health Survey (SF-36) quality-of-life inventory [22], the Profile of Mood States (POMS) psychological inventory [23], the Beck Depression Inventory [24], the Occupational Strain Questionnaire [25], and a Likert scale libido/energy questionnaire. After this, the following tests were conducted in the order listed: (1) body mass; (2) the donation of approximately 30 mL of fasting venous blood from an antecubital vein; (3) total body water; (4) body composition; (5) resting heart rate; and (6) resting blood pressure.

After these assessments, the subjects then began the performance tests. These tests were conducted in the following order, with the rest periods between tests listed in parentheses: one repetition maximum (1RM) bench press (5 min), isokinetic right quadriceps muscle endurance test (10 min), Wingate anaerobic capacity test (20 min), and a maximal cardiopulmonary aerobic capacity test. Rest periods were based on the estimated time for the energy system utilized to recover adequately [26]. Follow-up testing was conducted at the midway point of the study (4 wk) and at the end of the investigative period (8 wk). The 4-wk and 8-wk testing sessions were identical and compared with baseline testing. On a weekly basis, each participant was asked to complete a weekly follow-up assessment chart that addressed possible symptoms or side effects related to the supplementation and training.

Supplementation protocol

Subjects were assigned in a double-blind and randomized manner to ingest a dextrose placebo (PLA, $n = 15$) or AAKG ($n = 20$). All supplements were supplied to the

investigators in a double-blinded fashion by MRI. Subjects were instructed to ingest four caplets of the supplements three times per day (12 caplets daily) for the duration of the 8-wk investigation. Each 1.5-g caplet provided 0.5 g of L-arginine and 0.5 g of AKG in a 1:1 ratio. The remaining 0.5 g in each caplet contained the TR and other excipients required to produce a caplet form of the supplement. Therefore, ingestion of 12 caplets/d provided approximately 6 g of L-arginine and 6 g of AKG. Supplements were tested for purity and the presence of unwanted and/or banned substances by an IOC-certified laboratory (Banned Substances Control Group). Subjects were instructed to ingest their supplements 30 min before breakfast, 1 h before lunch, and late afternoon (~1500 h). Subjects were also informed to take their supplements on an empty stomach and not to ingest them with food. This precaution was taken to eliminate the competitive inhibition of amino acids in their transport across the blood-brain barrier [27].

Training protocol

All subjects were required to follow the same workout routine. Participants were required to participate in a 4-d/wk resistance-training program split into two upper and two lower body workouts per week for a total of 8 wk. This 8-wk training protocol was periodized in 4-wk increments consisting of selected exercises for the following muscle groups: chest (two exercises for a total of six sets), back (two exercises for a total of six sets), shoulders (one exercise for a total of three sets), biceps (one exercise for a total of three sets), triceps (one exercise for a total of three sets), abdominals and quadriceps (two exercises for a total of six sets), hamstrings (two exercises for a total of six sets), calves (one exercise for a total of three sets), and lower back (one exercise for a total of three sets). Each exercise consisted of three sets of 10 repetitions (weeks 1–4) or 8 repetitions (weeks 4–8) performed with as much weight as the participant could perform per set (typically 70–85% 1RM). Rest periods between exercises lasted no longer than 3 min and the rest periods between sets lasted no longer than 2 min [26]. The resistance-training workout was approximately 1 h in duration. In addition to the resistance-training program, each participant was instructed to perform some type of aerobic activity three times per week for 30 min in duration. Subjects were informed that the intensity of effort must result in a heart rate of 70% of maximal heart rate for the 30-min duration.

Procedures

Subjects recorded the amount of weight lifted and the number of repetitions performed on training cards. Training sessions were monitored by a training partner or fitness instructor who signed off that the session was completed. Total training volume was calculated by multiplying the number of repetitions by the amount of weight lifted for each exercise. Total daily lifting volume was calculated for each subject per exercise session and for the entire training program. Subjects recorded all caloric intake from food and fluids for 4 d (including 1 weekend day) before each testing session. Dietary records were interpreted by a registered dietitian and analyzed with ESHA Food Processor 8.3 (ESHA Research Inc., Salem, OR, USA). Subjects were instructed to maintain their normal diet throughout the supplementation and training periods. Resting heart rate was determined by palpation of the radial artery using standard procedures and resting blood pressure was also assessed using standard procedures [28]. Total body mass was measured on a calibrated digital scale with a precision of ±0.02 kg (Health-O-Meter, Bridgeview, IL, USA). Total body water was estimated with a Xitron Multi-frequency analyzer (Xitron Technologies Inc., San Diego, CA, USA). Bioelectrical impedance analysis has been determined to be a valid measurement for total body water [29]. Whole-body composition measurements (excluding cranium) were determined with a Hologic 4500W Dual-Energy X-ray Absorptiometer (DEXA; Hologic, Bedford, MA, USA) by using procedures previously described [30,31]. This test evaluates body composition and body density by scanning the entire body with a low dose of radiation and takes approximately 6 min. The DEXA scans regions of the body (right arm, left arm, trunk, right leg, and left leg) to determine bone mass, fat mass, and lean mass within each region. The scanned bone, fat, and lean masses for each region are then subtotaled to determine whole-body values (excluding cranium). Percentage of body fat was determined by dividing the amount of fat mass by the total scanned mass (bone mass, fat mass, and lean mass). Test-retest reliability studies performed on male athletes with this DEXA machine yielded mean deviation for total bone mineral content and total fat free/soft tissue mass of 0.31%, with a mean intraclass correlation of 0.985.

Subjects abstained from exercise for 48 h before testing and provided fasting blood samples (8 h) via venipuncture from an antecubital vein in the forearm according to standard phlebotomy procedures. Serum and plasma blood samples were collected into two 10-mL serum separation tubes and two 5-mL anticoagulant tubes containing K_3 (ethylenediaminetetra-acetic acid). One serum separation tube was inverted several times and quickly centrifuged (1318*g*) for 20 min on a bench top centrifuge. The other serum separation tube was allowed to sit at room temperature until the sample clotted and was then centrifuged. Plasma and serum samples were transferred into microcentrifuge tubes and frozen at −80°C for subsequent analyses. A serum sample and whole blood from the tube containing ethylenediaminetetra-acetic acid were refrigerated and shipped in cold containers to Quest Diagnostics (Dallas, TX, USA) for clinical analysis. A complete clinical chemistry panel was run on serum samples using the Olympus AU5400 (Diamond Diagnostics, Holliston, MA, USA) according to standard clinical procedures. Cell blood counts with percent differentials were run on whole-blood samples using a Coulter GEN S (Diamond Diagnostics) analyzer according

to standard procedures. Test-test reliability (within and between) of performing these assays ranged from 2% to 6% for individual assays, with an average variation of ±3%. Samples were run in duplicate to verify results if the observed values were outside control values and/or clinical norms according to standard procedures.

Plasma samples were shipped frozen to the Nichols Institute (San Diego, CA, USA) for plasma L-arginine analysis according to blood collection and handling procedures outlined by the Nichols Institute. The specific methods for the assay of L-arginine are proprietary in nature but basically involve being analyzed by a Waters 2690 Alliance high performance liquid chromatographic (HPLC) system (Waters, Milford, MA, USA). When using the Waters Pico-Tag method, amino acids are precolumn derivatized with phenylisothiocyanate and separated by reversed-phase chromatography. The interassay precision (coefficient of variation) of L-arginine analysis by this method is typically 5–9%.

Plasma agmatine was analyzed by a high performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) method recently developed by Song et al. [32]. Pretreatment of plasma samples were as follows: 50 μL of trichloroacetic acid solution (30% w/v) was added to 150 μL of plasma sample and vortexed thoroughly. This mixture was placed on ice for approximately 30 min and centrifuged (20 937*g*) at 9000 rpm for 10 min. Twenty microliters of borate buffer (100 nM at pH 9.0) and 60 μL of 7-flouro-4-nitrobenzoxadiazole solution (10 mM in acetonitrile) were added to 10 μL of supernatant. This mixture was vortexed and heated at 65°C for 20 min in a dry heating block. After heating, the derivative solutions were cooled in running tap water and kept at 5°C until analysis. Portions (5 μL) of the solution were injected for HPLC-MS/MS analysis with an Autoinjector. This method has an intraday precision of 3.6% and interday precision of 5.7%.

Upper body strength tests were performed on the Nebula (Versailles, OH, USA) flat bench. Participants were instructed to conduct two warm-up sets with approximately 50% of their estimated 1RM. After the two warm-up sets, resistance was continually added until subjects obtained their 1RM. Three minutes of rest was allowed between each attempt. Subjects then performed successive 1RM lifts starting at about 70% of anticipated 1RM and increasing by 5–10 kg until participants reached their 1RM. Test-test reliability of performing these strength tests in our laboratory on resistance-trained subjects yielded low mean coefficients of variation and high reliability for the bench press (1.9%, intraclass $r = 0.94$).

Before the isokinetic leg extension was conducted, subjects warmed up on a stationary bicycle for 2–3 min at a self-selected pace. Subjects then performed five warm-up repetitions on the Biodex (Biodex Medical Systems, Shirley, NY, USA) isokinetic leg extension machine. After these warm-up repetitions, subjects performed unilateral leg extensions (right leg) for one set of 50 repetitions [33] at 180 degrees/s. Test-test reliability has been shown to be 0.78 to 0.82 [34]. Subjects then warmed up for 2 min at a self-selected pace on a stationary bicycle ergometer before performing the Wingate test. This warm-up was continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. The Wingate anaerobic capacity test was performed on the LODE (Amsterdam, Netherlands) with a resistance of 0.7 Nm/kg. Test-test variability in performing repeated Wingate tests in our laboratory yielded correlation coefficients of $r = 0.98 \pm 15\%$ for mean power. The maximal cardiopulmonary test used the Bruce protocol. Metabolic gases were obtained with the Parvo Medics 2400 TrueMax metabolic measurement system (Sandy, UT, USA) on a Trackmaster TMX425C treadmill (Newton, KS, USA). The mean coefficient of variation (assessing maximum oxygen consumption) for this protocol was 6.5% (range, 2–14%) [35].

The SF-36 was designed for use in clinical practice and research and includes one multi-item scale that assesses health-related quality of life via eight health concepts [22,36]. The POMS questionnaire is a standardized and validated scale that allows subjects to self-rate on six mood states [23]. The Occupational Roles Questionnaire measures stressful work roles [25]. The Beck Depression Inventory is a 21-item self-report rating inventory that measures characteristic attitudes and symptoms of depression [24,37]. The libido/activity questionnaire was composed of seven questions with Likert-scale rankings from 0 (very poor) to 10 (very good). The seven questions contained in this assessment included such items as positive attitude about training, ability to recover from training sessions, body satisfaction, sexual desire, erectile function, quality of sleep, and feeling of energy when waking up.

Statistical analysis

In study 1, data were analyzed by a 2 (groups) × 10 (samples) repeated measures analysis of variance (ANOVA) to determine whether there were any differences in appearance of arginine in the blood between the NTR and TR versions of AAKG. Area under the curve analyses from the pharmacokinetic study was determined with PK Solutions 2.0 (Summit Research Services, Montrose, CO, USA). In study 2, data were analyzed by a 2 × 3 repeated measures ANOVA. Delta scores (post minus pre values) were calculated on selected variables and analyzed using repeated measures ANOVA. SPSS 11.5 for Windows (SPSS, Inc., Chicago, IL, USA) was used to analyze data in both phases of the study. Post hoc procedures were conducted when necessary by using least-significant difference post hoc procedures. Statistical significance was accepted at $P < 0.05$. Data are presented as means ± standard deviations.

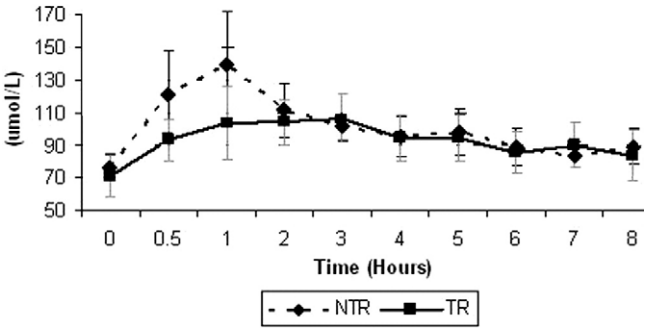

Fig. 1. Plasma arginine levels across all time points in subjects taking non–time-released (circles) and time-released (squares) forms of L-arginine α-ketoglutarate.

Results

Study 1: pharmokinetic profile

Figure 1 shows that a significant interaction was observed across groups in arginine levels over the 8-h observation. However, no significant differences were observed across groups in mean changes in arginine levels (NTR 26.6 ± 11 versus TR 23.9 ± 12 μmol/L, $P = 0.62$) or accumulated area under the curve (NTR 788 ± 63 versus TR 741 ± 79 $\mu mol \cdot h^{-1} \cdot L^{-1}$, $P = 0.17$) during the 8-h observation period. These findings indicate that the pattern of appearance of arginine differed in the blood between the TR and NTR forms of AAKG but that similar amounts of arginine appeared in the blood over time.

Study 2: training study

Medical monitoring

No significant clinical side effects, related or unrelated to the study, were reported to the research nurse by any subject throughout the entire course of the study. All subjects tolerated the training and supplementation protocols without any problems.

Training and diet

No statistically significant differences were observed across groups in total lifting volume during the training period (AAKG 350 336 ± 67 867 versus PLA 386 990 ± 83 087 kg, $P = 0.51$). There were also no statistically significant interactions observed across groups in absolute caloric intake ($P = 0.52$), relative caloric intake ($P = 0.90$), carbohydrate intake ($P = 0.26$), protein intake ($P = 0.71$), or fat intake ($P = 0.10$).

Blood analysis

No significant differences were observed between groups in lipid profiles, liver enzymes, renal function (creatinine, serum urea nitrogen), electrolytes, markers of cytolysis (creatine kinase, lactate dehydrogenase), calcium, total protein, albumin, globulin, total bilirubin, alkaline phosphatase, nitrate/nitrite, agmatine, red blood cells, hematocrit, or white blood cells. Table 1 presents data for glucose, hemoglobin, and plasma arginine. Fasting plasma arginine levels were significantly increased from baseline (108 ± 17 to 126 ± 21 μmol/L) and were significantly higher than PLA values (106 ± 14 μmol/L) after 8 wk of supplementation. Fasting glucose levels were significantly increased from 90 ± 10 to 97 ± 8 mg/dL during the course of the study. However, glucose values remained within normal ranges and were not significantly different from PLA values (96 ± 7 mg/dL) after 8 wk of supplementation. A significant interaction was observed among hemoglobin values as a result of values in the PLA group decreasing (14.9 ± 0.9 to 14.5 ± 0.9 g/dL), whereas values in the AAKG group were maintained (15.0 ± 0.7 to 15.2 ± 1.0 g/dL). All hematologic values remained within normal clinical ranges.

Table 1
Selected blood markers

	AAKG	Placebo	P (group × time)
Glucose (mmol/L)			
T1	5.0 ± 0.56	5.2 ± 0.33	
T2	5.1 ± 0.44	5.4 ± 0.5	
T3	5.4 ± 0.44*	5.3 ± 0.39	0.03
Hemoglobin (g/dL)			
T1	15.0 ± 0.7	14.9 ± 0.9	
T2	15.2 ± 0.9	14.9 ± 1.0	
T3	15.2 ± 1.0*	14.5 ± 0.9	0.04
Plasma arginine (μmol/L)			
T1	108 ± 17	105 ± 18	
T2	122 ± 30	112 ± 22	
T3	128 ± 21*	106 ± 14	0.01

AAKG, L-arginine/α-ketoglutarate; T1 to T3, time 1 to time 3
* $P < 0.005$, different from baseline or from placebo.

Muscular strength and muscular endurance

The analysis of 1RM bench press (Fig. 2) over 8 wk showed a significant difference between groups (AAKG 8.82 ± 7.33 versus PLA 2.67 ± 9.11 kg, $P = 0.03$). No

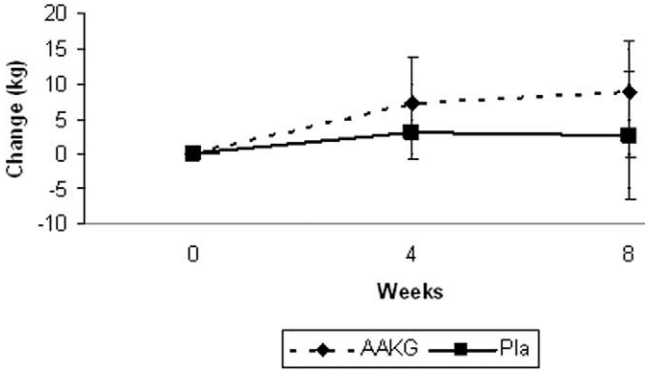

Fig. 2. Changes in 1RM strength for bench press in subjects taking L-arginine α-ketoglutarate (circles) and placebo (squares).

Table 2
Wingate anaerobic power indices.

	AAKG	Placebo	*P* (group × time)
Peak power (W)			
T1	1251 ± 236	1271 ± 257	
T2	1291 ± 254	1282 ± 219	
T3	1331 ± 242*	1202 ± 241	0.005
Time to peak power (s)			
T1	3.77 ± .55	3.83 ± 1.02	
T2	3.80 ± .80	4.12 ± 0.84	
T3	3.88 ± .48*	3.32 ± 1.25	0.050
Rate to fatigue (W/s)			
T1	34.9 ± 8.9	35.6 ± 8.6	
T2	36.4 ± 10.0	35.6 ± 9.1	
T3	37.6 ± 8.8*	31.9 ± 9.5	0.005

AAKG, L-arginine/α-ketoglutarate; T1 to T3, time 1 to time 3
* AAKG greater than placebo at T3 ($P < 0.05$).

significant difference was observed between groups in these isokinetic right quadriceps muscle endurance variables: peak torque, time to peak torque, total work, and work fatigue.

Anaerobic power and aerobic capacity

Table 2 presents anaerobic power data for the AAKG and PLA groups. Significant differences were observed between groups in peak power and rate to fatigue. A trend was observed between groups in time to peak power and fatigue index. No significant differences were observed between groups in mean power, minimum power, or total work.

Table 3 presents selected aerobic capacity variables. No significant differences were observed between groups in time to exhaustion, oxygen consumption (milliliters per kilogram per minute), minute ventilation, metabolic equivalents, respiratory exchange ratio, systolic or diastolic blood pressure, or maximal heart rate.

Body composition

Table 4 presents body composition results. No significant differences were observed between groups in changes in total body mass (AAKG 0.6 ± 0.3 versus PLA 0.2 ± 0.1 kg, $P = 0.53$), fat mass (AAKG 0.7 ± 0.4 versus PLA −0.6 ± 0.3 kg, $P = 0.25$), lean mass (AAKG 0.8 ± 0.1 versus PLA 0.9 ± 0.4 kg, $P = 0.90$), or percentage of body fat (AAKG −0.5 ± 0.2% versus PLA −1.0 ± 0.2%, $P = 0.31$).

Psychometric analysis

No significant differences were observed for the SF-36 health-related quality-of-life domains. Likewise, no significant differences were observed between groups for the six mood states of the POMS questionnaire. For the Occupational Roles Questionnaire, no significant differences were observed between groups for five of the scales, but there was a significant interaction between groups for the role overload scale (AAKG −1.5 ± 5 versus PLA 3.4 ± 8, $P = 0.04$). This shows an improvement by decreasing the feelings that workload is increasing, unreasonable, and unsupported by needed resources. In relation to the Beck Depression Inventory, no significant differences were observed between groups (AAKG −2.3 ± 5 versus PLA −0.2 ± 4, $P = 0.20$). No significant differences were observed between groups in the libido questionnaire.

Table 3
Aerobic capacity variables.

	AAKG	Placebo	*P* (group × time)
Time to exhaustion (min)			
T1	11.0 ± 1.2	10.5 ± 1.1	
T2	11.3 ± 1.1	11.0 ± 1.3	
T3	11.6 ± 1.4	11.0 ± 1.5	0.76
Oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$)			
T1	41.0 ± 6.1	40.4 ± 5.1	
T2	42.4 ± 7.0	41.8 ± 5.9	
T3	43.8 ± 6.1	42.2 ± 6.0	0.39
Minute ventilation			
T1	94.7 ± 14.8	93.4 ± 13.9	
T2	98.1 ± 14.8	96.0 ± 12.4	
T3	95.1 ± 24.4	97.3 ± 15.3	0.62
METS			
T1	11.7 ± 1.7	11.5 ± 1.5	
T2	12.1 ± 2.0	11.9 ± 1.7	
T3	12.5 ± 1.8	12.0 ± 1.7	0.41
Respiratory exchange ratio			
T1	1.19 ± 0.07	1.14 ± 0.07	
T2	1.20 ± 0.06	1.17 ± 0.05	
T3	1.20 ± 0.07	1.15 ± 0.07	0.86

AAKG, L-arginine/α-ketoglutarate; METS, metabolic equivalents; T1 to T3, time 1 to time 3

Table 4
Body composition variables

	AAKG	Placebo	*P* (group × time)
Lean mass (kg)			
T1	59.8 ± 8.0	63.6 ± 9.4	
T2	60.0 ± 8.0	64.3 ± 9.3	
T3	60.6 ± 7.9	64.5 ± 8.9	0.90
Fat mass (kg)			
T1	13.9 ± 6.6	15.7 ± 4.7	
T2	14.8 ± 6.2	15.4 ± 4.9	
T3	14.6 ± 6.3	15.1 ± 4.9	0.25
Total body mass (kg)			
T1	77.0 ± 12.7	81.9 ± 12.7	
T2	77.1 ± 12.6	82.2 ± 13.0	
T3	77.6 ± 13.0	82.1 ± 12.5	0.53
Body fat (%)			
T1	18.7 ± 5.8	19.1 ± 3.8	
T2	18.7 ± 5.6	18.6 ± 3.8	
T3	18.2 ± 5.5	18.1 ± 3.9	0.31

AAKG, L-arginine/α-ketoglutarate; T1 to T3, time 1 to time 3

Discussion

This study examined the hypothesis that L-arginine in combination with AKG might serve as an effective ergogenic aid for resistance-trained athletes. The theoretical rationale was based on research indicating that L-arginine may increase NO production and/or protein synthesis and that AKG may increase protein synthesis and/or reduce catabolism during training [14,15]. Although numerous NO-potentiating supplements containing L-arginine and/or AKG are marketed to resistance-trained athletes, no study has evaluated the potential ergogenic value of AAKG supplementation. In consequence, this is the first report of the development, use, and clinical assessment of a TR formulation of AAKG as a potential ergogenic aid in this population.

To examine the potential ergogenic value, we conducted two studies to examine the pharmacokinetics, safety, tolerability, and effects of AAKG on training adaptations in trained adult men. The first study was conducted to examine the pharmacokinetic profiles of ingesting NTR and TR forms of AAKG. Results showed that plasma arginine levels peaked to a greater degree after ingesting the NTR version of AAKG but that the total area under the curve for plasma arginine was similar between groups. These findings indicated that that NTR and TR forms of AAKG have different pharmacokinetic patterns that may affect arginine release, uptake, and/or physiologic effect over time. However, it should be noted that orange juice, which was provided to the subjects during the course of the observation period in an effort to prevent hypoglycemia during the 18-h fasting period, contained macro- and/or micronutrients that may have influenced arginine levels to some degree because observed peak values were not increased in an overly impressive manner. Although this does not detract from the finding that plasma arginine profiles differed between the TR and NTR forms of arginine supplements, the absolute values observed may have been influenced to some degree. Additional research should examine the physiologic effect of more rapid or delayed appearance of arginine in the plasma in response to NTR and TR forms of AAKG.

The present study is the first to examine the safety and efficacy of AAKG supplementation during resistance training in well-trained men. Results indicated that AAKG supplementation (12 g/d for 8 wk) was well tolerated and produced no significant changes in liver enzymes, liver or kidney function, or hematologic profiles. Moreover, no serious side effects were observed. These results are in good agreement with the history of safety and tolerability of L-arginine, whether administered by intravenous infusion or orally [4].

In terms of potential ergogenic value during training, the major finding of this study was that AAKG treatment resulted in significantly greater gains in 1 RM bench press and anaerobic power in resistance-trained men. These findings may be of interest to athletes interested in gaining strength and/or anaerobic power during training and warrant additional research to examine the possible mechanisms of action and potential ergogenic value of AAKG supplementation on additional anaerobic strength and power indices. However, AAKG supplementation did not promote statistically significant changes in fat-free mass, muscular endurance, or aerobic capacity. These findings indicate that, although there was evidence of greater gains in strength and anaerobic power, the improved performance capacity did not promote muscle hypertrophy or improve body composition results during training. These findings do not support contentions that AAKG promotes lean tissue accretion during resistance training. However, it is possible that the sample and/or use of well-trained resistance-trained subjects who are more resistant to gains in muscle mass during training as opposed to untrained subjects may have influenced the results.

We are aware of one other study that used resistance-trained men as subjects that investigated the effects of arginine on body composition and muscle function. Walberg-Rankin et al. [38] gave male weight trainers who were consuming a hypocaloric diet approximately 8 g of arginine daily for 10 d. The investigators concluded that arginine supplementation had no influence on fat or lean tissue loss, muscle function (as determined by biceps and quadriceps isokinetic testing), or overall growth hormone status. Results of the present study support these findings because 8 wk of AAKG supplementation did not significantly affect body composition, lower body muscle function as measured by quadriceps isokinetic testing, or anabolic hormonal profiles. Conversely, Santos et al. [39] examined the effects of short-term arginine supplementation (3 g/d for 15 d) on muscle function in healthy but untrained men. The researchers reported that arginine supplementation promoted an 8.5% improvement in isokinetic knee extension fatigue index. They concluded that arginine supplementation improved muscular resistance capacity to fatigue.

The significant effects observed from AAKG supplementation on anaerobic sprint capacity in the present study are interesting. To date, there has only been one study [40] that has evaluated the effects of an arginine-containing compound on anaerobic sprint performance. Buford et al. [40] examined the effects of ingesting 11.2 g of glycine-arginine-[α]-ketoisocaproic acid (GAKIC) 45 min before exercise on repetitive cycling sprint performance (5 $\times$ 10-s sprints with 1-min rest recovery between sprints). The researchers reported that GAKIC supplementation before exercise significantly improved retention of mean power during the first two 10-s sprints. They concluded that GAKIC appears to attenuate the decline in mean power during repeated bouts of supramaximal exercise. In the present study, subjects performed a 30-s Wingate anaerobic capacity test. This test assesses peak power and the ability to maintain power output during a prolonged sprint and thereby assesses phosphagen and glycolytic capacity. Re-

sults showed that AAKG supplementation increased peak power and thereby influenced rate of fatigue. The reason that AAKG increased peak power is unclear. Because arginine is one of three amino acids used in the synthesis of creatine [41] and low-dose creatine supplementation (e.g., 3 g/d for 4 wk) has been shown to increase muscle phosphocreatine concentrations [42], 6 g/d of L-arginine supplementation may have influenced phosphocreatine availability and thus anaerobic power indices [43,44]. Additional research should be conducted to examine the effects of AAKG supplementation on muscle phosphagen stores and anaerobic capacity.

Arginine supplementation has also been reported to increase aerobic capacity in patients with congestive heart failure, stable angina, and pulmonary hypertension. For example, Bronislaw et al. [45] studied the effects of arginine supplementation on exercise duration in patients who had congestive heart failure and concluded that arginine supplementation prolonged exercise duration. In another study, Ceremuzynski et al. [46] investigated the effects of oral supplementation with arginine on exercise capacity in patients with stable angina after myocardial infarction. Arginine supplementation resulted in an increase in mean exercise time to maximal ST-segment depression and an increase in the maximum workload (metabolic equivalents). Mizuno et al. [47] reported that infusion of arginine counteracted myocardial ischemia. Nagaya et al. [48] studied the effects of oral supplementation of arginine on exercise capacity in patients with pulmonary hypertension. A significant increase in peak oxygen consumption was observed, leading the investigators to conclude that arginine improves exercise capacity in patients with pulmonary hypertension. Results of the present study in healthy resistance-trained men do not support these findings.

Analysis of blood markers showed that AAKG supplementation had no influence on nearly all parameters studied and that all values remained within normal clinical ranges. These findings indicate that AAKG supplementation is relatively benign in terms of markers of clinical safety. A statistically significant increase was observed in plasma arginine and fasting glucose in the AAKG group. Increases in plasma arginine would be expected from the ingestion of 12 g of AAKG daily for 8 wk. The observed values of plasma arginine for the AAKG-supplemented group were in the upper end of the normal reference range for plasma arginine. Although the AAKG-supplemented group showed a significant increase in blood glucose as compared with the PLA group (7.7% versus 2.1%), the blood glucose values were within normal blood glucose reference ranges, within the observed standard deviation range of the baseline assays (8–10%), and not significantly different from PLA values after 8 wk of supplementation (i.e., AAKG 97 ± 10 versus PLA 96 ± 7 mg/dL). Hence, it is unclear whether the differences observed were directly related to AAKG supplementation, a result of natural changes in blood glucose levels, assay variability, and/or of any clinical significance. Interestingly, Siani et al. [49] reported that fasting glucose levels decreased by 4% ($P = 0.10$) in subjects consuming an arginine-rich diet (10 g/d) and by 9% ($P = 0.008$) in subjects supplementing their diet with 10 g/d of L-arginine for 1 wk. This reported reduction in fasting glucose in response to L-arginine supplementation was a primary reason that we provided orange juice to our subjects during the 18-h fasting and experimental pharmacokinetic trial. Obviously, further research is needed to examine the effects of AAKG supplementation on fasting glucose levels before any conclusions can be drawn.

In conclusion, AAKG supplementation appeared to be safe and well tolerated and positively influenced 1RM bench press and Wingate peak power performance. AAKG did not influence body composition or aerobic capacity. Further research is needed to examine the role of AAKG supplementation during training in untrained and trained populations before definitive conclusions can be drawn regarding its potential ergogenic value.

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