Nutritional and Metabolic Effects and Significance of Mild Orotic Aciduria During Dietary Supplementation With Arginine or Its Organic Salts After Trauma Injury in Rats

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The effects of acute food deprivation and subsequent refeeding with isonitrogenous oral liquid diets supplemented with arginine (ARG), ARG α-ketoglutarate (AKG), or ARG α-ketoisocaproate (AKIC) were examined in a Sprague-Dawley rat trauma model (bilateral femur fracture). Both control and trauma rats were starved for 2 days and then pair-fed for 4 days with one of four liquid isonitrogenous diets: diet 1 was a basal casein-based diet, and diets 2, 3, and 4 were the basal diet in which 10% of the nitrogen was replaced by ARG, AKG, or AKIC nitrogen. Two days of starvation resulted in a 13% loss of body weight and also a 27% decrease in the excretion of orotic acid (OA) in control and trauma rats. Although the ARG content of diets 2, 3, and 4 was the same, ARG- and AKIC-supplemented rats excreted significantly ($P < .05$) more OA than AKG-fed rats. The low level of OA excretion in AKG-fed rats indicates greater use of ARG for metabolic purposes, including efficient urea cycle operation. The metabolic adaptation and nutritional efficacy, ie, increased nitrogen retention, larger weight gain, and altered amino acid (AA) metabolism, of AKIC rats seem to be better than in ARG- or AKG-fed rats.

ARGININE (ARG), a nonessential amino acid (AA), plays a key role in the urea cycle, protein synthesis, biosynthesis of AAs and their derivatives, reparative collagen synthesis, and enhancement of immune function and promotion of nitrogen (N) retention in injury.[1] It is also the physiological precursor of nitric oxide, which induces vasodilation and inhibits platelet aggregation. ARG supplementation in amounts exceeding normal requirements were documented to benefit many pathological conditions by decreasing N losses after trauma,[1,2] improving the rate of wound healing,[3-5] stimulating growth hormone secretion,[6] reducing stress-induced thymic involution,[7] improving immune function after burn injury,[8] or surgery,[9] and suppressing tumor growth.[10] Traditional nutritional support alone in the early catabolic phase of severe injury did not reverse muscle protein catabolism even when high amounts of protein were given.[11,12] Selection of specific substrates is needed for their key role in the control of intermediary metabolism, since the efficacy of nutrition cannot be easily improved by quantitative modifications alone.

The 1:1 molar combination of basic L-ARG and organic α-ketoisocaproic acid (KIC), as in ARG α-ketoisocaproate (AKIC), used as a dietary supplement was found to improve N balance, particularly in traumatized rats.[13] Moreover, plasma AA patterns also demonstrated stimulation of net protein synthesis with AKIC,[13] and the mechanism seems to operate through the synergistic actions of the N-dense ARG and the N-free KIC. The 2:1 molar combination of L-ornithine and α-ketoglutaric acid as in ornithine α-ketoglutarate modifies AA metabolism and hormonal patterns in a way that was not seen when these were administered separately.[14,15] It appears that the anabolic actions of the basic amino acids ARG and ornithine could be further enhanced by their organic salts with KIC or α-ketoglutaric acid.

ARG deficiency impairs the capacity of the urea cycle to detoxify ammonia, allowing greater shunting of nitrogen into the pyrimidine pathway[16] and resulting in increased orotic acid (OA) excretion. Hence, ARG deficiency increases and ARG supplementation reduces OA excretion.[17,18] Bioavailability of the nitrogen-rich ARG in the diet could then be assessed by measuring OA excretion. ARG seems to segregate into two significant metabolic pools, one highly sequestered, related specifically to urea synthesis, and the other a systemic pool in equilibrium with plasma.[19] When intracellular ARG levels are low, intramitochondrial carbamylphosphate accumulates, diffuses into the cytosol, and increases pyrimidine biosynthesis, resulting in increased OA excretion. Mild oroticaciduria can thus be used as a method of determining in vivo amino acid imbalance. Excretion of OA is decreased in fasting normal subjects and increased during refeeding.[20] On the contrary, increased oroticaciduria is seen in fasting, hypermetabolic, severely injured trauma patients, and their response to feeding is a decrease in OA excretion[21] for injury normalization.

The objectives of the present study were to evaluate the significance of altered nutritional and metabolic responses in traumatized rats fed a liquid oral diet supplemented with ARG or its organic salts. We were able to examine the effect of ARG, ARG α-ketoglutarate (AKG), or AKIC on nitrogen metabolism, OA excretion, and muscle and plasma free-AA levels in starved-refed uninjured rats compared with similarly injured rats.

MATERIALS AND METHODS

Young male Sprague-Dawley rats (ACE Animals, Boyertown, PA) weighing 250 to 270 g were housed in individual metabolic cages (Plas-Labs, Lansing, MI) and kept in our well-ventilated, temperature- and humidity-regulated vivarium with a controlled 12-hour light-dark cycle. They were adapted to freely available liquid diet feeding (#F1259; Bioserv, Frenchtown, NJ) and water for 3 days. The animal

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research facility at St. Joseph's Hospital and Medical Center (Phoenix, AZ) is accredited by the American Association of Accreditation of Laboratory Animal Care. This research protocol was reviewed and approved by the Institutional Animal Care and Use Committee, and we adhered to the National Research Council's *Guide for the Care and Use of Laboratory Animals.* Forty-eight animals were divided into two groups: control (n = 24) and trauma (n = 24). Animals in each group were weight-matched and then assigned to one of four diets: basal, ARG, AKG, or AKIC. Food intake was determined daily by weighing the liquid-diet feeding tubes at the beginning and end of each 24-hour period, and urine output was collected from 8:00 AM to 8:00 AM for determination of 24-hour excretion of nitrogen, creatinine, and urea. Each day between 8:30 and 9:30 AM, the animals were weighed, urine output was measured and removed, and feeding tubes were changed.

On day 1, all rats (in batches of six because of cage restrictions) were anesthetized with an intraperitoneal injection of ketamine hydrochloride 0.1 mg/g body weight (Ketalar 50 g/L; Parke-Davis, Morris Plains, NJ), and the animals were weight-matched. Half of the animals (n = 24) received closed bilateral femoral fractures involving standard-force fracture at the midshaft of the femur by twice releasing the arm of a common spring-loaded rat trap onto the femur. This ensured uniform closed fracture and soft-tissue damage. The other half (n = 24) acted as weight-matched, pair-fed controls for each traumatized animal. The animals were then returned to individual cages and had free access to water but were deprived of food for 2 days. This condition was imposed to simulate the conditions for human subjects treated in intensive care trauma units.

On the third day, feeding was started and continued for 4 days. One set of 12 rats (six control and six trauma rats) in two batches were fed a casein-based basic oral liquid diet (#F1259; BioServ) that provided 6.5 g N/L and 4,187 kJ (1,000 kcal)/L (diet 1). The amino acid composition of this diet is listed in Table 1. It contained 18% protein, 35% fat, and 47% carbohydrate. The remaining three sets of 12 animals were each fed different oral liquid test diets. These isonitrogenous test diets contained the basic diet, but with 650 mg N/L replaced by nitrogen from either ARG, AKG, or AKIC. The test diet therefore had 10.0% of the nitrogen content replaced by either ARG (diet 2), AKG-N (diet 3), or AKIC-N (diet 4). Diets 2, 3, and 4 had the same amounts of ARG. AKIC (≥97% pure) was kindly supplied by Ross Laboratories (Columbus, OH); 97% pure L-ARG and αKG were obtained from Sigma Chemicals (St Louis, MO), and we prepared AKG from these pure components.

Table 1. AA Composition of Isonitrogenous Casein-Based Diets (mmol/L)

AA	Diet			
	(1) Basal*	(2) ARG	(3) AKG	(4) AKIC
Alanine	10.7	9.7	9.7	9.7
Glycine	11.6	10.4	10.4	10.4
Serine	19.1	17.2	17.2	17.2
Proline	31.0	27.9	27.9	27.9
Arginine	7.5	15.0	15.0	15.0
Histidine	6.3	5.7	5.7	5.7
Glutamic acid	48.3	43.5	43.5	43.5
Tyrosine	11.1	10.0	10.0	10.0
Aspartic acid	17.0	15.3	15.3	15.3
Cysteine	0.5	0.5	0.5	0.5
Ornithine	0	0	0	0
Valine	19.7	17.7	17.7	17.7
Leucine	22.2	20.0	20.0	20.0
Isoleucine	14.7	13.2	13.2	13.2
Phenylalanine	9.7	8.7	8.7	8.7
Threonine	13.2	11.9	11.9	11.9
Tryptophan	2.7	2.4	2.4	2.4
Methionine	7.9	7.1	7.1	7.1
Lysine	17.6	15.8	15.8	15.8
αKG	0	0	8.2	0
KIC	0	0	0	8.2

NOTE. Each milliliter provided 1 kcal (4.2 kJ) and 6.5 mg N. Energy sources: 18% protein, 35% fat, and 47% carbohydrate.
*Bioserv #F1259.

In each weight-matched group, control rats were pair-fed with the trauma rats by using BioServ's ARF/Israel simultaneous pair-feeding system (BioServe Product No. F7378, US Patent No. 4628866). This eliminated the gorging-fasting syndrome commonly associated with liquid-diet experiments. All animals had free access to water. On day 6 at the end of 4 days of oral feeding, food was withdrawn for 2 hours and the rats were anesthetized. Blood was collected by cardiac puncture, and the separated plasma was stored at −80°C for amino acid analysis. Forearm muscle tissues were excised, and blood was blotted out immediately, frozen by liquid N, and then stored at −80°C until analysis.

Total daily urinary nitrogen was determined using a chemiluminescence digital analyzer (Antek Instruments, Houston, TX). Urine urea and creatinine were determined using standard procedures (urease method for urea and picric acid method for creatinine) with the MicroCentrifugal Analyzer (Multistat Plus; Instrumentations Laboratory, Lexington, MA).

Individual free amino acids in plasma and dry muscle tissues were determined by the automated ion-exchange method with an amino acid analyzer (model 7300; Beckman Instruments, Palo Alto, CA). Briefly, 500 μL 4% (40 g/L) sulfosalicylic acid containing the internal standard amino-ethyl-L-cysteine was added to 500 μL plasma or 20 to 30 mg dry powdered tissue and vortexed slowly. It was kept in ice for 1 hour with occasional shaking, and then centrifuged at 10,000 × *g* for 10 minutes at 4°C. The supernatant was filtered through a 0.22-μm filter, and a 50-μL aliquot was injected for analysis. A calibration standard was analyzed after every six samples. The coefficient of variation of multiple analyses was less than 3%.

The colorimetric method of Harris and Oberholzer[22] was modified and used[20,21] to measure OA concentrations in urine samples. In this procedure, acidified (pH 2 to 3) urine in duplicate was passed through a cation-exchange column (Amberlite IRP-69; Sigma) to remove most of the interfering substances. OA in the eluate was converted to barbituric acid by bromination and reduction by ascorbic acid in a fume hood. *P*-Dimethylamino benzaldehyde in *n*-propanol (3%) was then added to form an orange product, and its optical density was measured at 450 nm by the Beckman DU-50 spectrometer. This assay procedure for OA was previously validated using high-performance liquid chromatography.[20]

Apparent daily nitrogen balance was calculated by subtracting urinary nitrogen excretion from dietary nitrogen intake. Because fecal output was small and constant, nitrogen losses from this source were considered negligible and were not included in nitrogen-balance calculations.[23] The apparent daily nitrogen balance was normalized to nitrogen intake, since the intake was not similar in the different dietary groups, although control and trauma rats in each group were pair-fed. The effects of trauma in relation to dietary supplementation were analyzed between corresponding groups.

Unless otherwise noted, values listed are the mean ±SEM. Statistical analyses were made using two-way classification ANOVA with the two classifications defined as group (control and trauma) and feeding conditions.[24] A preselected contrast was performed: (1) control versus trauma for each of four diets; (2) control, basal diet versus ARG, AKG, or AKIC; and (3) trauma, basal diet versus ARG, AKG, or AKIC.

RESULTS

Metabolic and nutritional parameters and growth indices of the animals are summarized in Table 2. Control rats were pair-fed with respective weight-matched trauma animals. The starting weight of the rats was 265 ± 2 g. Deprivation of food for 2 days with free access to water resulted in a 13% loss of body weight in control and trauma rats. Trauma rats were not immobilized at any time and displayed little hindrance to motion from the bilateral femoral fractures except in the ability to exert force with the hindlimbs. Four days of refeeding after 2 days of fasting resulted in a greater gain of body weight in control rats than in trauma rats. Trauma rats gained significantly less body weight than the corresponding control rats, although the difference did not obtain statistical significance in rats fed the basal diet or AKG. The weight gain per day was higher in AKIC-fed control and trauma rats compared with respective ARG-fed rats. This was also reflected in the higher food intake, although control rats were pair-fed with the respective trauma rats. Relative daily food intake (2.3 ± 0.1 mg N/g body weight) was similar for all rats except those fed AKIC. On the first day of feeding, all control and trauma rats were able to ingest about 90% of their last 3 days' average consumption. The protein efficiency ratio (PER), defined as the gain in body weight per gram of nitrogen consumed, differed significantly ($P < .005$) only in the basal diet group between control and trauma rats. Diet had no effect on PER; however, PER was generally lower in trauma rats of each group.

Apparent nitrogen balance was relatively better in control rats compared with trauma rats when expressed as milligrams of N per day. Relative nitrogen retention (milligrams of N retained per gram of nitrogen intake) was significantly ($P < .05$) higher in control rats fed AKG and AKIC compared with the basal diet. However, in trauma groups, it was higher by 12% in ARG-fed rats, 14% in AKG-fed rats, and 33% in AKIC-fed rats compared with trauma rats fed the basal diet. Trauma rats, in general, excreted less creatinine (11%) and more urea (9%) than uninjured rats. The ratio of urinary urea nitrogen to total nitrogen excreted did not change with trauma. Control and trauma rats fed AKIC excreted significantly more urea than the corresponding ARG-fed rats. Since N excretion also increased, the ratio of urea N to total N did not change with trauma. In AKIC-fed rats, this ratio was significantly increased compared with rats fed basal diet in both trauma and control groups due to a decrease in total N excretion and hence a better N retention. There was no significant correlation between excretion of OA and urea in any of the groups.

Daily OA excretion by trauma and control rats is illustrated in Fig 1. A body weight loss of 13% due to 2 days of food deprivation was accompanied by a decreased (27%) excretion of OA in control and trauma rats. Altered OA excretion due to starvation and refeeding four isonitrogenous diets in injured and uninjured rats is shown in Fig 2. Compared with respective control and trauma rats fed basal diet, OA excretion was increased 30% and 10% in ARG-fed rats, significantly ($P < .025$) decreased (30% and 25%) in AKG-fed rats, and significantly ($P < .01$) increased (25% and 37%) in AKIC-fed rats. Supplementing 10% of nitrogen intake with ARG nitrogen in trauma rats had no significant effect on OA excretion. However, there were profound effects on OA excretion in both control and trauma rats when the same amount of ARG nitrogen was given as organic salts: OA excretion decreased 32% with AKG and increased 25% with AKIC. Both changes were statistically significant ($P < .05$).

Free AA levels in plasma and forearm muscle tissues are

Table 2. Nutritional and Growth Indices in Growing Traumatized Rats Starved and Refed Four Isonitrogenous Diets

	Basal Diet		ARG		AKG		AKIC	
Index	Control	Trauma	Control	Trauma	Control	Trauma	Control	Trauma
Body weight (g)								
Initial	261 ± 3	263 ± 4	265 ± 4	265 ± 4	266 ± 3	267 ± 3	274 ± 6	266 ± 3
2 d starved	224 ± 4	227 ± 4	236 ± 4	231 ± 3	232 ± 2	229 ± 3	234 ± 3	238 ± 3
4 d refed	282 ± 6	275 ± 4	275 ± 4‡	259 ± 4	280 ± 4	270 ± 5	302 ± 6†	288 ± 4†
Body weight gain								
g/d	14.6 ± 1.8	11.3 ± 1.7	10.7 ± 1.4	10.0 ± 2.0	11.9 ± 2.1	10.1 ± 2.0	17.1 ± 1.4†‡	12.6 ± 0.3
g/g N In	23.5 ± 0.9‡	18.0 ± 0.6	19.0 ± 2.3	17.2 ± 3.2	19.7 ± 3.5	18.8 ± 3.6	23.9 ± 2.2	18.6 ± 2.4
Food intake								
mg N/d	623 ± 14	608 ± 12	556 ± 18	587 ± 13	594 ± 18‡	538 ± 13†	728 ± 22*†	704 ± 14*†
mg N/g body weight	2.3 ± 0.05	2.4 ± 0.08	2.1 ± 0.07	2.3 ± 0.05	2.2 ± 0.07	2.1 ± 0.06	2.6 ± 0.09*†	2.6 ± 0.04*†
N balance								
mg N/d	289 ± 22	236 ± 16	288 ± 19	255 ± 21	314 ± 19‡	240 ± 19	410 ± 23*†	361 ± 15*†
mg N/g N In	466 ± 38	383 ± 25	507 ± 24	429 ± 30	522 ± 20‡	438 ± 28	557 ± 16*‡	509 ± 14
Excretion								
Creatinine (mg/d)	7.6 ± 0.1‡	6.9 ± 0.1	8.1 ± 0.8	6.6 ± 0.1	8.5 ± 0.1	8.5 ± 0.1†	8.2 ± 0.2	7.8 ± 0.1†
N (mg/d)	336 ± 15	363 ± 25	277 ± 10‡	323 ± 15	288 ± 10	318 ± 14	303 ± 12‡	353 ± 11
Urea (mg N/d)	256 ± 10	273 ± 10	220 ± 13	254 ± 12	249 ± 10	260 ± 16	273 ± 11†	300 ± 9†
Urea N/total N (%)	76.5 ± 2.7	76.2 ± 2.1	79.8 ± 3.6	80.2 ± 2.8	87.1 ± 2.6*	81.8 ± 3.1	90.2 ± 1.1†	85.4 ± 1.3*
OA (μg/d)	111 ± 10	116 ± 8	144 ± 9*	127 ± 11	79 ± 10*†	75 ± 6*†	145 ± 8*	162 ± 11*†

NOTE. Results are the mean ± SEM (n = 6 per group).

*$P \leq .05$ *v* basal diet.

†$P \leq .05$ *v* ARG.

‡$P \leq .05$ *v* corresponding trauma.

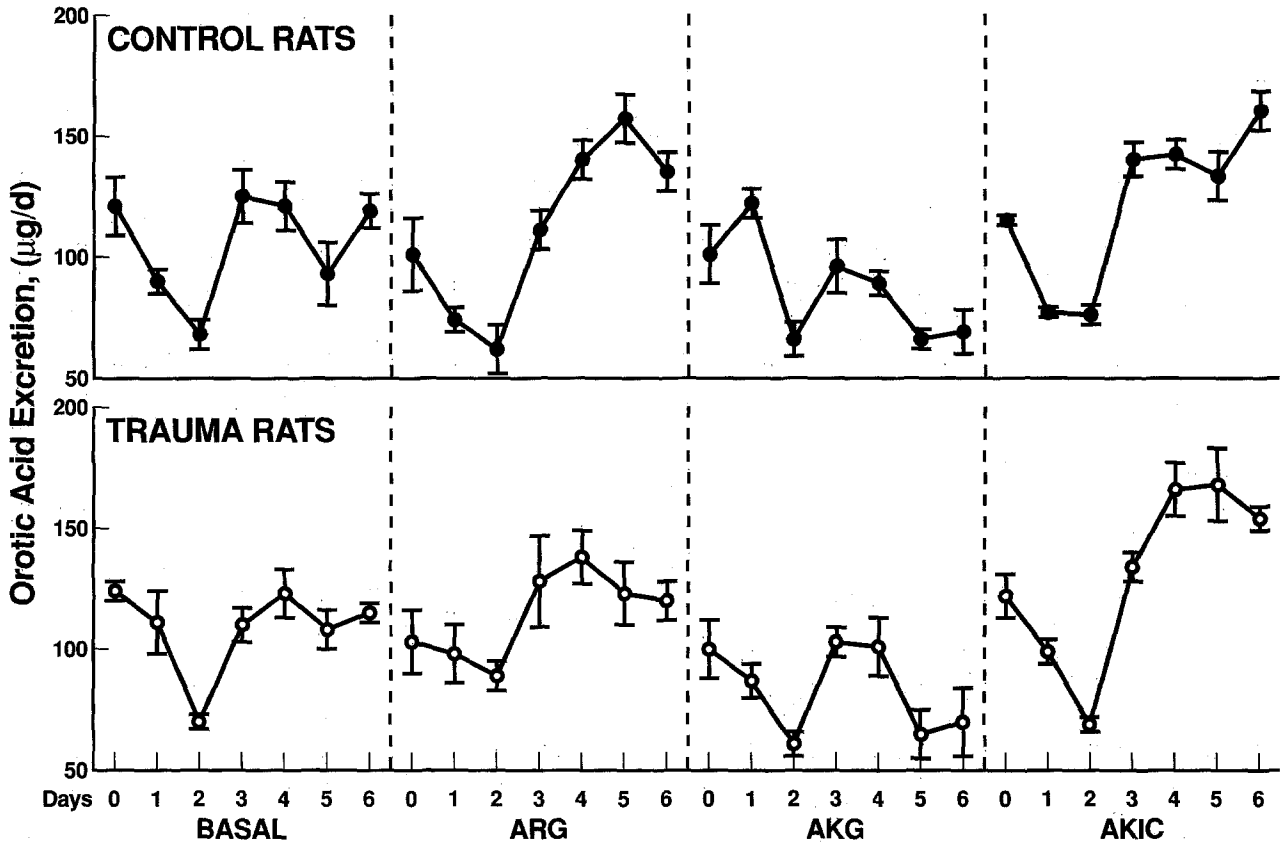

Fig 1. OA excretion (μg/d) in control and trauma rats starved for 2 days (days 0 to 2) and fed basal or test diets, ARG, AKG, or AKIC, for 4 days (days 3 to 6). Results are the mean ±SEM; n = 6 for each day and each diet.

listed in Tables 3 and 4, respectively. Contrary to what is seen in rats fed ARG and AKG, plasma and muscle free AA levels did not show statistically significant differences in rats fed AKIC versus basal diet. Muscle total AA (TAA) levels were decreased due to supplementation with ARG or its salts. Hypoglutaminemia due to trauma was shown in all groups, although statistically significant differences were not attained. Muscle glutamine levels were also decreased due to trauma except in AKIC-fed rats when glutamine levels were already significantly reduced. Plasma tryptophan levels were significantly increased due to ARG or its salts. On the other hand, muscle tryptophan levels were significantly increased only in AKG-fed rats. Plasma lysine, alanine, serine, and tyrosine levels were unchanged due to dietary supplementation. Taurine is the major AA (37% ± 2% of TAA) present in muscle, which was not affected by AKG feeding but was reduced by half in both ARG- and AKIC-fed rats. Plasma taurine levels, on the other hand, were significantly reduced (38% ± 2%) in AKG-fed control and trauma rats. Plasma levels of phenylalanine or tyrosine did not change due to trauma, increasing only in ARG-fed control rats.

ARG or its salts generally increased (18% to 188%) plasma and muscle ARG and ornithine levels in both uninjured control and injured trauma rats. Increased levels of the three urea cycle AAs, ARG, ornithine, and citrulline, were consistently seen in AKG-supplemented rats. Interestingly, OA excretion in these AKG-fed rats was significantly less than in the other groups. Irrespective of diet, trauma results in increased levels of plasma and decreased levels of muscle ARG levels. This seems to indicate the compartmentalization of the ARG pool. However, plasma and muscle citrulline levels were decreased due to trauma in all dietary regimens studied. Ornithine levels were changed little due to trauma, except in muscle tissues of AKG- and AKIC-fed rats.

DISCUSSION

Elevated urinary OA has been proposed to result from decreased urea cycle capacity and impaired urea synthesis because of a deficiency of either ARG or other urea cycle substrates.[25] The decreased OA excretion seen in fasting may be due to many factors, including diminished clearance, reduced synthesis with unchanged or decreased metabolism, and maintained production with increased metabolic utilization.[20] Severe acute trauma in humans is accompanied by parallel increases in the excretion of OA and uric acid.[21] These enhanced pyrimidine and purine responses are in conjunction with the known trauma effects of increased protein mobilization, nitrogen excretion, and hyperaminoaciduria.[26] Dietary feeding in 7-day fasted normal subjects restores the decreased OA excretion on the first day of feeding, and then increases above the basal level on subsequent days.[20] Balanced nutrition given to severely injured trauma patients did not appreciably change the already elevated

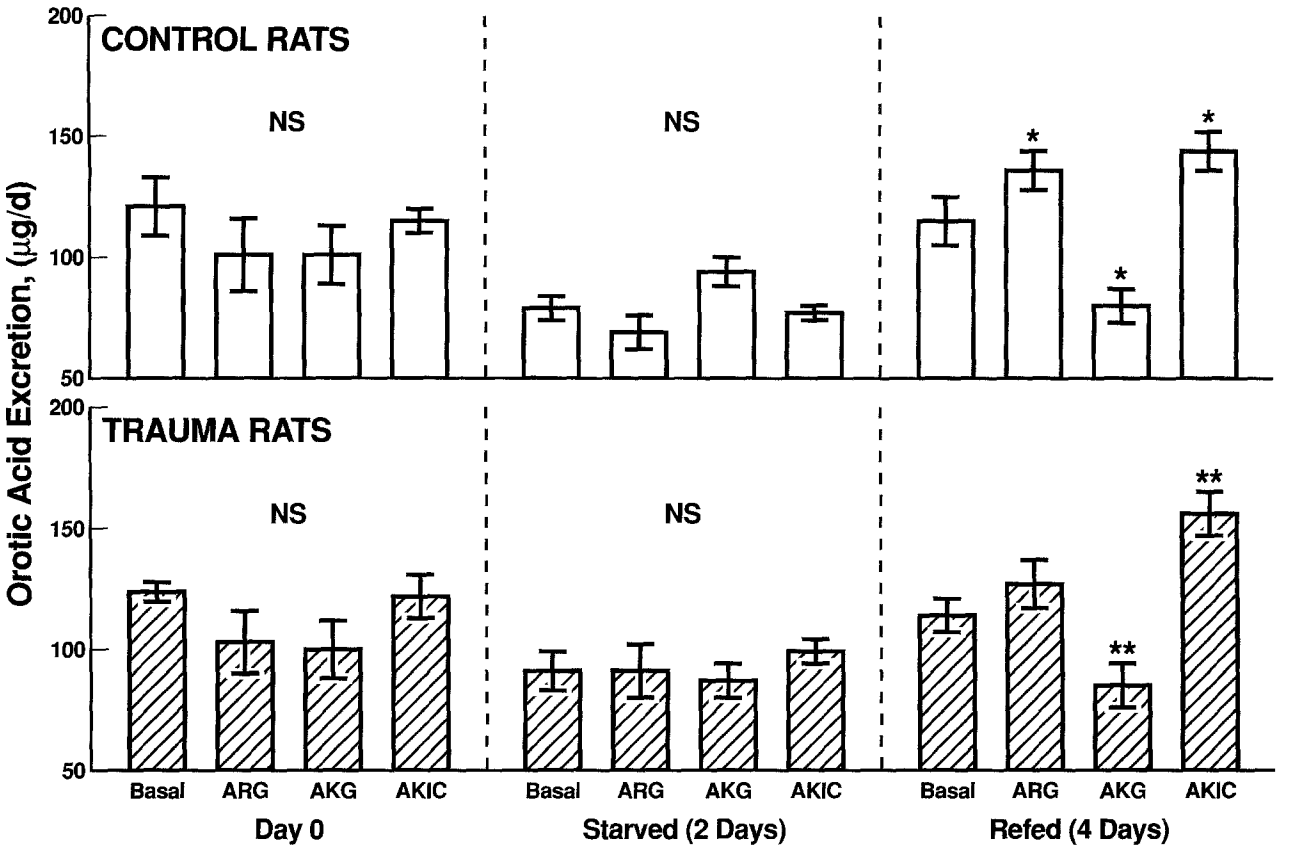

Fig 2. Altered daily OA excretion due to starvation (2 days) and refeeding (4 days) in control and trauma rats. Diet 1, basal diet (BioServe #F1259); diet 2, ARG; diet 3, AKG; diet 4, AKIC. Results are the mean ±SEM; n = 6. *$P \leq .05$ *v* basal diet 1 (refed), control rats; **$P \leq .05$ *v* basal diet 1 (refed), trauma rats; NS, not significantly different ($P > .05$, trauma *v* control).

excretory rate of OA.[21] In the present study, we investigated the biological significance of OA excretion in starved and refed traumatized rats. Under these experimental conditions, in 6 days the fasting and trauma effects on OA excretion seemed to be ameliorated to normal conditions as observed in injured patients.[21]

Studies in mature rats show that the rates of repletion from severe undernutrition and recovery from trauma are significantly accelerated by supplemented dietary ARG.[27] We provided sufficient protein ad libitum; however, the PER (gain in body weight per gram of nitrogen consumed) was 15% lower in trauma rats compared with control rats in each dietary group. Relative nitrogen retention (milligrams of N retained per gram of N intake) was improved in both control and trauma rats during dietary supplementation, and became significantly greater in rats fed AKG and AKIC compared with basal diet. The decreased plasma and muscle glutamine levels due to trauma could not be normalized by ARG or its salts. However, in AKIC-fed rats, muscle glutamine levels in trauma rats show an increasing trend toward normalization. Plasma and muscle citrulline levels were decreased due to trauma in all dietary groups studied. Ornithine levels were changed little due to trauma, except in muscle tissues of AKG- and AKIC-fed rats.

Supplementation of isonitrogenous diets with ARG, AKG, or AKIC changes the urinary excretion of OA significantly in healthy, growing rats and in severely injured rats. Although the ARG-N content was the same in these three diets, ARG and AKIC rats excreted significantly ($P < .05$) more OA than AKG-fed rats. The low level of OA excretion in AKG-fed rats indicates greater availability of ARG for metabolic purposes and more efficient urea cycle operation. This happens concurrently with the increased plasma levels of the three urea cycle–generated AAs, ARG, ornithine, and citrulline, that were consistently seen in AKG-supplemented rats compared with basal diet–fed rats.

In the liver, ARG metabolism plays a pivotal role in the urea cycle, the rate of which is conditioned not only to metabolize extra nitrogen to ornithine and urea, but also to maintain acid-base homeostasis.[28] In the intestine, ARG is transformed into citrulline, which is then taken up mainly by the kidney to form ARG and is subsequently released to peripheral tissues. Supplementation with ARG or its salts generally increased plasma and muscle ARG and ornithine levels in both injured and uninjured rats. Plasma citrulline levels were increased due to dietary supplementation; however, muscle citrulline levels were decreased in ARG- and AKIC-fed rats and increased in AKG-fed rats. Increased levels of the three urea cycle AAs, ARG, ornithine, and citrulline, were consistently seen in AKG-supplemented rats. Interestingly, excretion of OA in these AKG-fed rats was significantly less than in the other groups.

Table 3. Plasma Free AA Levels (μmol/L) in Control and Trauma Rats on Four Isonitrogenous Diets

	Basal Diet		ARG		AKG		AKIC	
AA	Control	Trauma	Control	Trauma	Control	Trauma	Control	Trauma
EAA								
Val	200 ± 8	200 ± 11	187 ± 13	159 ± 15†	171 ± 5*	195 ± 22	169 ± 10*	153 ± 14†
Leu	154 ± 6	142 ± 9	159 ± 10	126 ± 8	122 ± 6*‡	119 ± 13	128 ± 7*	113 ± 10†
Ile	92 ± 3	87 ± 4	82 ± 5	69 ± 3†	69 ± 3*‡	70 ± 9	75 ± 4*	67 ± 6†
Phe	45 ± 3	44 ± 3	58 ± 4*	51 ± 3	48 ± 2‡	46 ± 4	42 ± 1‡	39 ± 2§
Thr	363 ± 27	347 ± 19	300 ± 12*	256 ± 15†	222 ± 10*‡	264 ± 26†	304 ± 12	274 ± 16†
Trp	83 ± 8	71 ± 9	221 ± 13*	202 ± 15†	97 ± 8‡	91 ± 7§	92 ± 6‡	77 ± 4§
Met	40 ± 1	40 ± 6	46 ± 2*	43 ± 2	49 ± 2*	59 ± 6†§	40 ± 3	41 ± 4
Lys	504 ± 22	610 ± 59	546 ± 27	574 ± 14	466 ± 24	631 ± 58	520 ± 21	555 ± 19
NEAA								
Ala	454 ± 24	526 ± 44	428 ± 28	428 ± 22	360 ± 71	447 ± 65	430 ± 26	469 ± 28
Gly	240 ± 14	219 ± 8	291 ± 20	257 ± 12†	187 ± 10*‡	245 ± 27	185 ± 15*‡	169 ± 8†§
Ser	219 ± 10	222 ± 6	277 ± 16	248 ± 9	216 ± 9	280 ± 34	212 ± 7‡	204 ± 9§
Gln	550 ± 25	496 ± 37	535 ± 30	405 ± 26	436 ± 73	526 ± 33	485 ± 11	429 ± 19
Pro	197 ± 16	258 ± 36	191 ± 14	173 ± 10†	138 ± 32	151 ± 57	232 ± 22	241 ± 22§
Arg	104 ± 9	136 ± 15	163 ± 19*	172 ± 14	139 ± 14	208 ± 29†	209 ± 10*‡	194 ± 13†
His	53 ± 1	61 ± 3*	77 ± 1*	71 ± 2†	66 ± 3*‡	75 ± 6†	53 ± 1‡	49 ± 3†§
Tau	228 ± 13	242 ± 21	242 ± 22	162 ± 14†	144 ± 5*‡	146 ± 5†	217 ± 31	173 ± 12†
Glu	156 ± 13	131 ± 15	191 ± 28*	140 ± 12	102 ± 27	91 ± 15§	88 ± 7*‡	68 ± 8†§
Tyr	61 ± 11	72 ± 10	68 ± 3	68 ± 4	60 ± 4	68 ± 9	49 ± 3‡	55 ± 5
Orn	34 ± 2	42 ± 5	69 ± 7*	56 ± 4†	64 ± 14*	74 ± 9†	51 ± 3*	55 ± 3†
Cit	75 ± 4	66 ± 4	97 ± 2*	84 ± 5†	76 ± 2‡	88 ± 10†	81 ± 3‡	66 ± 4§
Asn	46 ± 5	56 ± 5	41 ± 2	39 ± 1†	37 ± 1	52 ± 7	54 ± 4	60 ± 11
Cys	36 ± 2	36 ± 6	10 ± 5*	8 ± 6†	47 ± 2*‡	50 ± 8†	49 ± 7‡	58 ± 6†
Asp	14 ± 1	15 ± 2	19 ± 2*	15 ± 1	14 ± 1	6 ± 2†§	10 ± 1‡	11 ± 4
ΣBCAA	445 ± 15	429 ± 23	428 ± 26	354 ± 13†	362 ± 11*‡	384 ± 43	371 ± 14*‡	346 ± 21†
ΣEAA	1,480 ± 64	1,542 ± 94	1,600 ± 56	1,479 ± 37	1,243 ± 28*‡	1,475 ± 134	1,268 ± 43*‡	1,230 ± 53†§
ΣNEAA	2,467 ± 81	2,581 ± 74	2,679 ± 115	2,311 ± 73†	1,985 ± 189‡	2,509 ± 250	2,367 ± 100	2,299 ± 122†
ΣTAA	3,947 ± 144	4,123 ± 163	4,279 ± 163	3,789 ± 110	3,228 ± 180‡	3,983 ± 382	3,666 ± 141	3,529 ± 172†

NOTE. Results are the mean ± SEM; n = 6 per group.

Abbreviations: EAA, essential AA; NEAA, nonessential AA; BCAA, branched-chain AA.

*Significantly different from control basal diet, $P \leq .05$.

†Significantly different from trauma basal diet, $P \leq .05$.

‡Significantly different from control ARG, $P \leq .05$.

§Significantly different from trauma ARG, $P \leq .05$.

In fasted trauma patients, we previously reported a 50% decrease in plasma glycine levels and a 200% increase in daily urinary glycine excretion rates.[26] In the present study of an ARG-fed rat trauma model, injury-induced significant depletion of glycine levels was notably absent in both plasma and muscle. Simultaneous addition of ARG and glycine to a relatively high-protein diet was needed to improve nitrogen balance in trauma rats and to promote optimum growth in healthy rats.[23] Supplementation with ARG alone did not promote better nitrogen retention but did improve growth posttrauma.[23] Addition of glycine along with ARG may improve nitrogen retention and promote growth. Less OA was excreted in rats fed casein supplemented with ARG and glycine than in those fed casein only.[29]

Comparison of AKG- and AKIC-supplemented rats indicates that AKIC rats (1) ate more food, retained more nitrogen, and gained more weight; (2) excreted more OA, urea, and total nitrogen; (3) had increased plasma levels of proline, threonine, and taurine; and (4) had decreased plasma levels of histidine, glutamic acid, tyrosine, and ornithine and muscle levels of TAA. Plasma essential AA, serine, and glycine levels in AKIC-fed trauma rats were the lowest observed among trauma rats, presumably due to increased use in protein synthesis. We have previously shown that AKIC is superior to OKIC (ornithine α-ketoisocaproate) in promoting protein metabolism, particularly in injured rats.[13] The mechanism seems to be an increased protein synthesis via the synergistic actions of ARG and KIC.

ARG deficiency leads to mild hyperammonemia.[18] Enhanced OA synthesis due to shunting of this excess nitrogen into pyrimidine biosynthesis is a well-established consequence of ARG deficiency,[30] which may also lead to mild insulin refractiveness.[31] The absence of dietary ARG during repletion significantly reduced the weight gain and efficiency of feed utilization with no apparent changes in feed intake.[31] L-ARG–derived nitrogen oxides mediate insulin release from pancreatic β cells.[32] ARG action on nitrogen metabolism could also be linked to its ability to stimulate growth hormone secretion[1,15] and polyamine synthesis.[15] Changes in plasma L-ARG due to diet or other factors would be expected to be a regulator of the oxidative L-ARG pathway in pancreatic β cells.[32] The availability of the ingested ARG may thus direct the metabolic pathway

Table 4. Front Leg Muscle AA Levels (μmol/g dry tissue) in Control and Trauma Rats on Four Isonitrogenous Diets

	Basal Diet		ARG		AKG		AKIC	
AA	Control	Trauma	Control	Trauma	Control	Trauma	Control	Trauma
EAA								
Val	1.39 ± 0.14	1.10 ± 0.16	0.66 ± 0.05*	0.57 ± 0.03†	0.10 ± 0.05*‡	1.10 ± 0.12§	0.71 ± 0.05*	0.70 ± 0.05†
Leu	0.19 ± 0.10	0.57 ± 0.08*	0.42 ± 0.04*	0.34 ± 0.39†	0.59 ± 0.08*	0.56 ± 0.07§	0.46 ± 0.05*	0.44 ± 0.06
Ile	0.49 ± 0.10	0.37 ± 0.05	0.22 ± 0.03*	0.19 ± 0.02†	0.36 ± 0.05	0.35 ± 0.07	0.34 ± 0.04	0.36 ± 0.06
Phe	0.35 ± 0.09	0.27 ± 0.03	0.20 ± 0.02	0.18 ± 0.02	0.29 ± 0.03	0.30 ± 0.05	0.18 ± 0.02*	0.17 ± 0.02
Thr	6.60 ± 0.57	4.78 ± 0.51*	3.34 ± 0.16*	3.16 ± 0.17†	4.47 ± 0.18*‡	4.03 ± 0.29	2.75 ± 0.14*‡	2.49 ± 0.12†§
Trp	0.18 ± 0.06	0.17 ± 0.04	0.11 ± 0.01	0.08 ± 0.01†	0.38 ± 0.01*‡	0.24 ± 0.07§	0.24 ± 0.09	0.16 ± 0.05
Met	0.28 ± 0.05	0.24 ± 0.03	0.13 ± 0.02*	0.13 ± 0.02	0.36 ± 0.07	0.40 ± 0.07	0.08 ± 0.01*	0.08 ± 0.00
Lys	5.90 ± 0.92	4.88 ± 0.52	4.14 ± 0.46	4.04 ± 0.47	6.24 ± 0.58‡	5.11 ± 0.52	4.04 ± 0.29	4.44 ± 0.52
NEAA								
Ala	14.51 ± 1.21	11.91 ± 1.05	9.72 ± 1.02*	8.41 ± 0.38†	11.31 ± 0.73*	12.82 ± 1.20§	8.83 ± 0.78*	9.02 ± 0.82
Gly	20.90 ± 2.44	17.33 ± 1.58	12.30 ± 0.66*	12.36 ± 0.66†	15.90 ± 0.91‡	16.70 ± 0.80§	5.54 ± 0.33*‡	6.14 ± 0.60†§
Ser	8.14 ± 0.61	5.36 ± 0.56*	4.32 ± 0.20*	3.75 ± 0.21†	5.87 ± 0.09‡	5.10 ± 0.26§	2.65 ± 0.17*‡	2.46 ± 0.14†§
Gln	24.96 ± 2.54	17.27 ± 1.59*	17.58 ± 1.12*	14.92 ± 1.01	19.71 ± 0.69	17.03 ± 1.26	8.52 ± 0.86*‡	9.80 ± 0.79†§
Pro	4.44 ± 0.39	3.60 ± 0.54	2.02 ± 0.27*	1.90 ± 0.14	2.65 ± 0.37*	2.95 ± 0.45	1.74 ± 0.19*	1.97 ± 0.12†
Arg	1.27 ± 0.15	1.05 ± 0.06	1.50 ± 0.22	1.25 ± 0.25	2.12 ± 0.17*‡	1.70 ± 0.13†	1.61 ± 0.18*	1.50 ± 0.20†
His	1.19 ± 0.10	0.97 ± 0.10	0.70 ± 0.04*	0.66 ± 0.04†	1.30 ± 0.15‡	1.19 ± 0.27	0.60 ± 0.03*	0.55 ± 0.04†
Tau	56.96 ± 5.19	54.15 ± 3.97	29.35 ± 0.58*	28.60 ± 1.03†	54.69 ± 4.95‡	60.96 ± 4.77§	26.32 ± 0.90*	26.43 ± 0.65†
Glu	9.53 ± 0.88	8.20 ± 0.88	3.59 ± 0.40*	4.33 ± 0.24†	5.11 ± 0.35‡	6.49 ± 0.54§	6.66 ± 0.80‡	6.22 ± 0.82§
Tyr	0.81 ± 0.09	0.64 ± 0.05	0.40 ± 0.05*	0.44 ± 0.02†	0.58 ± 0.02*‡	0.64 ± 0.03§	0.24 ± 0.01*‡	0.27 ± 0.02†§
Orn	0.29 ± 0.03	0.26 ± 0.02	0.20 ± 0.03*	0.20 ± 0.02	0.38 ± 0.07*‡	0.75 ± 0.04†§	0.28 ± 0.02	0.34 ± 0.03†§
Cit	1.76 ± 0.20	1.25 ± 0.13*	1.13 ± 0.07*	0.99 ± 0.05	1.92 ± 0.22*	1.64 ± 0.26§	0.79 ± 0.07*‡	0.66 ± 0.05†§
Asn	1.21 ± 0.09	—	0.97 ± 0.05*	0.76 ± 0.06	0.79 ± 0.06*	0.68 ± 0.07	0.28 ± 0.10*‡	0.28 ± 0.08§
Cys	—	—	0.02 ± 0.00	0.02 ± 0.00	0.46 ± 0.03‡	0.51 ± 0.06§	0.06 ± 0.01	0.07 ± 0.01
Asp	3.02 ± 0.49	2.38 ± 0.17	2.29 ± 0.07	2.19 ± 0.11	2.07 ± 0.18	2.64 ± 0.17	2.65 ± 0.17	2.46 ± 0.14
ΣBCAA	2.69 ± 0.39	2.04 ± 0.29	1.31 ± 0.12*	1.09 ± 0.07†	1.94 ± 0.17‡	2.02 ± 0.22§	1.51 ± 0.13*	1.50 ± 0.17†§
ΣEAA	12.05 ± 1.24	10.68 ± 1.07	9.50 ± 0.59	8.67 ± 0.62	13.43 ± 0.72‡	11.93 ± 0.74§	8.52 ± 0.42*	8.67 ± 0.65
ΣNEAA	147.0 ± 10.8	123.4 ± 7.6	85.6 ± 1.7*	80.8 ± 1.9†	124.2 ± 6.01‡	130.6 ± 8.09§	63.8 ± 2.0*‡	63.3 ± 1.96†§
ΣTAA	159.1 ± 10.2	134.1 ± 7.8	95.1 ± 2.1*	89.5 ± 2.37†	137.7 ± 2.41‡	142.5 ± 8.5	72.3 ± 2.3*‡	71.9 ± 2.5†§

NOTE. Results are the mean ± SEM (n = 6 per group).
*Significantly different from control basic diet, $P \leq .05$.
†Significantly different from trauma basic diet, $P \leq .05$.
‡Significantly different from control ARG, $P \leq .05$.
§Significantly different from trauma ARG, $P \leq .05$.

through anabolic hormones like insulin or growth hormone and hence enhance protein synthesis.

This study shows that the same amount of ARG given as organic salts of KG or KIC was better utilized with profound metabolic changes. More studies are needed to examine the optimal levels of supplementation for healthy and stressed conditions. OA excretion should be evaluated as a measure to identify when enhancement of ARG intake may prove beneficial.

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