Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise

D. A. MacLEAN, T. E. GRAHAM, AND B. SALTIN
School of Human Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada; and Department of Physiology III, Karolinska Institute, S-11486 Stockholm, Sweden

MacLean, D. A., T. E. Graham, and B. Saltin. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am. J. Physiol.* 267 (*Endocrinol. Metab.* 30): E1010–E1022, 1994.—In this study, five men exercised the knee extensor muscles of one leg for 60 min (71 ± 2% maximal work capacity) with and without (control) an oral supplement (77 mg/kg) of branched-chain amino acids (BCAA). BCAA supplementation resulted in a doubling ($P < 0.05$) of the arterial BCAA levels before exercise (339 ± 15 vs. 822 ± 86 μM). During the 60 min of exercise, the total release of BCAA was 68 ± 93 vs. 816 ± 198 μmol/kg ($P < 0.05$) for the BCAA and control trials, respectively. The intramuscular BCAA concentrations were higher ($P < 0.05$) for the BCAA trial and remained higher ($P < 0.05$) throughout exercise. In both trials, substantial quantities of NH_3 were released, and when NH_3 production equivalent to IMP accumulation was subtracted the net NH_3 production was 1,112 ± 279 and 1,670 ± 245 μmol/kg ($P < 0.05$) for the control and BCAA trials, respectively. In contrast, the release of the essential amino acids (EAA) was much lower for the BCAA than the control trial ($P < 0.05$). When the BCAA were subtracted from the EAA (EAA − BCAA), the total release of EAA minus BCAA was lower ($P < 0.05$) for the BCAA (531 ± 70 μmol/kg) than the control (924 ± 148 μmol/kg) trial. These data suggest that BCAA supplementation results in significantly greater muscle NH_3 production during exercise. Furthermore, the increased intramuscular and arterial BCAA levels before and during exercise result in a suppression of endogenous muscle protein breakdown during exercise.

essential amino acids; purine nucleotide cycle; total adenine nucleotides; inosine monophosphate

SKELETAL MUSCLE produces a large quantity of ammonia (NH_3)[1] during prolonged submaximal exercise (5, 10, 21, 22). However, the source of this NH_3 is the subject of some debate. Several researchers have attributed the NH_3 produced during a submaximal exercise bout to the same mechanism responsible for NH_3 production during intense exercise (5). This mechanism involves the deamination of AMP to IMP and NH_3 as one of the steps in the purine nucleotide cycle (PNC). The primary function of the PNC is to help maintain the energy state of the cell by removing AMP and allowing the adenylate kinase reaction ($2ADP \leftrightarrow ATP + AMP$) to move in the direction of ATP production. It is therefore probable that this mechanism for NH_3 production may be limited during a prolonged submaximal exercise bout.

On the other hand, it has been suggested that the degradation of amino acids, specifically the branched-chain amino acids (BCAA; i.e., isoleucine, leucine, and valine), may be a potential source of NH_3 during prolonged submaximal exercise (21, 22). Previous studies have demonstrated that BCAA are removed by active skeletal muscle during exercise (2) and that their oxidation increases as exercise progresses (6, 11, 32). However, until recently, they have never been considered a significant source of NH_3 production. Wagenmakers et al. (29), in a case study, administered BCAA to a McArdle's patient (myophosphorylase deficiency) before exercise and reported significantly higher venous plasma NH_3 concentrations during exercise. MacLean and Graham (21) administered BCAA to normal subjects and also reported significantly higher venous plasma NH_3 and glutamine levels. However, the authors were unable to precisely determine the mechanism responsible for the increased NH_3 production during exercise.

The purpose of the present study was to examine the effects of BCAA supplementation on amino acid and NH_3 metabolism in exercising humans. The one-legged extensor model was employed to obtain measurements of amino acid and NH_3 flux as well as intramuscular concentrations. These determinations allowed a more accurate estimation of the contribution to NH_3 production from the two potential mechanisms.

MATERIALS AND METHODS

Subjects. The experimental protocol was approved by the Swedish Ethics Committee. Five healthy male subjects were informed of the purposes and risks of the study. The subjects were 18–30 yr of age (mean = 24.4 ± 1.9 yr), weighed 64–85 kg (mean = 72.6 ± 4.3 kg), and were 180–192 cm in height (mean = 185.2 ± 2.2 cm).

Preexperimental procedure. The subjects were familiarized with the Krogh ergometer modified for one-legged knee extension exercise, as previously described (3). With this exercise model, electromyographic activity is absent in the hamstrings and the glutei muscles (3). Thus the external work done for knee extension is exclusively performed by the quadriceps femoris muscle. The subjects performed an incremental maximal leg work capacity test with their dominant leg to determine the maximal work load of the knee extensors, and this ranged from 40 to 80 W (mean = 64 ± 6.8 W). The subjects were instructed to continue to ingest a normal mixed diet and to abstain from any strenuous physical activity for 2 days before the experiment.

Experimental protocol. The subjects reported to the laboratory after an overnight fast. Teflon catheters were inserted below the inguinal ligament in the femoral artery and vein of the leg to be exercised and advanced proximally so that the tips of the arterial and venous catheters were located ~2 cm proximal and 2 cm distal to the inguinal ligament, respectively. The subjects were moved to the exercise apparatus and rested while baseline (0-min) arterial and venous blood samples were drawn simultaneously and a muscle biopsy obtained from the

[1] In physiological conditions, both ammonia and the ion ammonium exist. In this paper, NH_3 represents the sum of both forms.

vastus lateralis with suction. Blood flow was determined by indicator-dilution technique (31) using indocyanine green dye infused into the femoral artery and measured in the femoral vein.

The subjects exercised by kicking at ~70–75% (mean = 70.5 ± 2.2%) of their one-legged maximal knee extension capacity for 1 h. Arterial and venous blood samples were taken at 5, 15, 30, 45, and 60 min of exercise, and blood flow determinations were made immediately after each blood sample. Muscle biopsies were obtained at 5 and 60 min of exercise, and expired air was collected at 15, 45, and 60 min of exercise. Heart rate was monitored throughout the experiment and recorded. The selection of the dominant or nondominant leg was randomized.

After the 60 min of exercise the subjects were moved to the preparation room where Teflon catheters were inserted as described above into the femoral artery and vein of the contralateral unexercised leg. The subjects rested supine for ~45 min, and simultaneous arterial and venous blood samples were then taken (−45 min, before BCAA supplementation). The subjects then consumed a 77 mg/kg supplement of BCAA administered in two equal doses of 38.5 mg/kg at 45 and 20 min (i.e., −45 and −20 min) before the onset of exercise. The 500-mg capsules of the commercially available (Quest) BCAA supplement were reported to contain only the three BCAA in the following proportions; 220, 150, and 130 mg L-leucine, L-valine, and L-isoleucine, respectively. This was confirmed by dilution of the capsules in water and with analysis by high-performance liquid chromatography (HPLC; as described later). After the 45-min supplementation period, the subjects exercised the second leg for 60 min at the same work intensity as the first leg. Blood, muscle, and cardiorespiratory samples were obtained at the same time points as in the first trial.

Analyses. The fractions of expired O_2 and CO_2 were determined with an Applied Electrochemical S-3A O_2 analyzer and infrared (Beckman LB-2) systems, respectively. Expired volumes were determined with a Parkinson-Cowan volumeter. The analyzers were calibrated with known gas concentrations, and the volumeter was calibrated with a Tissot spirometer.

Blood samples for plasma were drawn with syringes treated with heparin; blood samples for serum were drawn with untreated syringes. Blood (100 μl) was quickly added to 500 μl of 0.3 M $HClO_4$. These samples and the remaining arterial and venous blood samples were both immediately centrifuged, and the supernatant was collected and stored at −80°C. The whole blood extracts were analyzed enzymatically (4) in triplicate for glucose and lactate with a fluorometer. Plasma was analyzed enzymatically in triplicate (fluorometer) for NH_3 (18) and for hypoxanthine and urate by HPLC using a modified version (14) of the method described by Wung and Howell (33). Plasma amino acids were analyzed in duplicate by prior derivatization with phenyl isothiocyanate (13) and HPLC. Serum was analyzed enzymatically in duplicate for glycerol (4) with a fluorometer and in duplicate for free fatty acids (FFA; Wako free fatty acids kit 990–75401) and urea (Sigma urea nitrogen kit 640) with a Beckman Du-70 spectrophotometer. Arterial hematocrit was determined by high-speed centrifuge to document changes in plasma volume.

The muscle biopsies were immediately frozen in liquid N_2, removed from the needle, and stored at −80°C. However, the muscle biopsies at rest had a small piece immediately removed before freezing for fiber type determination (26). A 5- to 8-mg portion of the frozen biopsy was weighed at −20°C, extracted in 3 M $HClO_4$ for 20 min, and neutralized with 2 M $KHCO_3$. The neutralized extract was immediately assayed for NH_3 by the method of Kun and Kearney (18) with a fluorometer. All samples for the experiment were analyzed at the same time, and analysis was complete within 1 h of neutralization. The remaining extract was used for lactate determination (4).

The remainder of the frozen muscle sample was freeze-dried and then dissected free of visible blood, connective tissue, and other nonmuscle elements. A 2- to 3-mg portion of this freeze-dried sample was homogenized for 1 min in 100 μl deionized (Milli Q) H_2O and then centrifuged for 3 min. The supernatant was used for determination of free amino acids using the method of Heinrikson and Meredith (13) and HPLC. A 1.5- to 2-mg portion of freeze-dried muscle was extracted in 1 ml 2 M HCl and incubated for 2 h at 85–90°C. The muscle and acid were weighed before and after incubation to document any possible fluid loss due to evaporation. After incubation, the extract was neutralized with 1 ml 2 M NaOH and centrifuged for 15 min at 15,000 revolutions/min. The supernatant was used in duplicate for the fluorometric determination of glycogen using an enzymatic glucose assay (4). The remainder of the muscle was extracted with 0.5 M $HClO_4$ (1.0 mM EDTA), neutralized with 2.2 M $KHCO_3$, and analyzed for ATP, ADP, AMP, and IMP by HPLC (27). A portion of the extract was used for the enzymatic determination of phosphocreatine (PCr) and creatine (Cr), as described by Harris et al. (12). Muscle metabolite contents were corrected to total Cr and expressed per kilogram dry muscle. Both muscle NH_3 and lactate are expressed per kilogram wet muscle.

Calculations. Thigh volume was calculated by using the thigh length, three circumferences, and three skinfold measurements (15), and muscle mass was estimated from a regression equation (3). The total adenine nucleotide (TAN) pool was calculated by summing ATP, ADP, and AMP. The uptake and/or release of O_2, glucose, lactate, NH_3, FFA, hypoxanthine, urate, and amino acids was calculated by multiplying the blood or plasma flow by the arteriovenous difference in concentration and were expressed per kilogram muscle. The total exchange of lactate, NH_3, and amino acids was estimated for each subject by averaging the exchange between consecutive sample points and then multiplying this value by the duration between the two sample points (e.g., averaging flux between 5 and 15 min and then multiplying by 10). These values were summed to obtain an estimate of the total exchange (always termed total release or uptake). In some cases, it was necessary to calculate the net production of a measured parameter. To obtain this estimate, the intramuscular concentrations were converted to wet weight using the wet-to-dry weight ratios. The overall shift in the intramuscular concentration (muscle accumulation) was then calculated, and this was added to the total release/uptake to obtain the net production (always termed net production, release, or uptake). BCAA were calculated by summing isoleucine, leucine, and valine, essential amino acids (EAA) by summing the BCAA, threonine, methionine, phenylalanine, tryptophan, and lysine, and total amino acids (TAA) by summing all amino acids except hydroxyproline and 3-methylhistidine.

Statistics. Because the BCAA trial always followed the control trial, any potential effects of the previous exercise bout and the effects of BCAA supplementation were analyzed by a one-way repeated-measures analysis of variance (ANOVA) between time points −45 min (BCAA leg) and 0 min (control and BCAA legs). Each subject exercised at the same relative work load; treatment effects were therefore analyzed with a paired *t*-test at each time point during exercise. To assess the effects of exercise, the data from *time 0* to *60* were analyzed with an ANOVA approach. If significance was indicated, a Tukey's (honest significant difference) post hoc point-to-point comparison test was used to determine where the significance occurred. Significance was accepted at $P < 0.05$, and all values are means ± SE.

RESULTS

Morphology, O_2 consumption, and blood flow data. There were no significant differences in quadriceps muscle mass or fiber type distribution between the control and BCAA legs. The control leg quadriceps muscle mass was 3.56 ± 0.23 kg and consisted of 57 ± 6% type I, 30 ± 4% type IIa, and 13 ± 4% type IIb muscle fibers. Meanwhile, the BCAA leg quadriceps muscle was 3.58 ± 0.25 kg and consisted of 51 ± 6% type I, 37 ± 7 type IIa, and 12 ± 4% type IIb muscle fibers. Pulmonary and muscle O_2 consumption was not significantly different between trials and did not vary during the exercise period. The respective means for these parameters were 14.0 ± 1.6 and 167.0 ± 31.7 $ml \cdot min^{-1} \cdot kg^{-1}$ for the control trial and 14.1 ± 1.1 and 165.8 ± 24.6 $ml \cdot min^{-1} \cdot kg^{-1}$ for the BCAA trial. Similarly, there were no significant differences between trials in blood or plasma flow, nor did these parameters vary during exercise. The respective means for these variables were 1.34 ± 0.07 and 0.75 ± 0.03 $l \cdot min^{-1} \cdot kg^{-1}$ for the control trial and 1.36 ± 0.08 and 0.76 ± 0.03 $l \cdot min^{-1} \cdot kg^{-1}$ for the BCAA trial.

Blood, plasma, and serum metabolites. There were no significant differences in arterial plasma NH_3 concentrations (Fig. 1) from −45 to 0 min for the control trial. At the onset of exercise, both the arterial and NH_3 flux (Fig. 1) levels were significantly elevated and remained elevated for both trials. The arterial NH_3 concentrations were consistently higher for the BCAA trial throughout the experiment but were only significantly higher at 45 and 60 min. Similarly, NH_3 efflux was dramatically increased as early as 5 min, and there were no differences between trials until 60 min when the NH_3 efflux was significantly higher for the BCAA trial. The intramuscular NH_3 (Table 2) concentrations were not significantly different between trials, and despite the large degree of NH_3 efflux, the intramuscular concentrations were only doubled over 0 min by the end of 60 min of exercise.

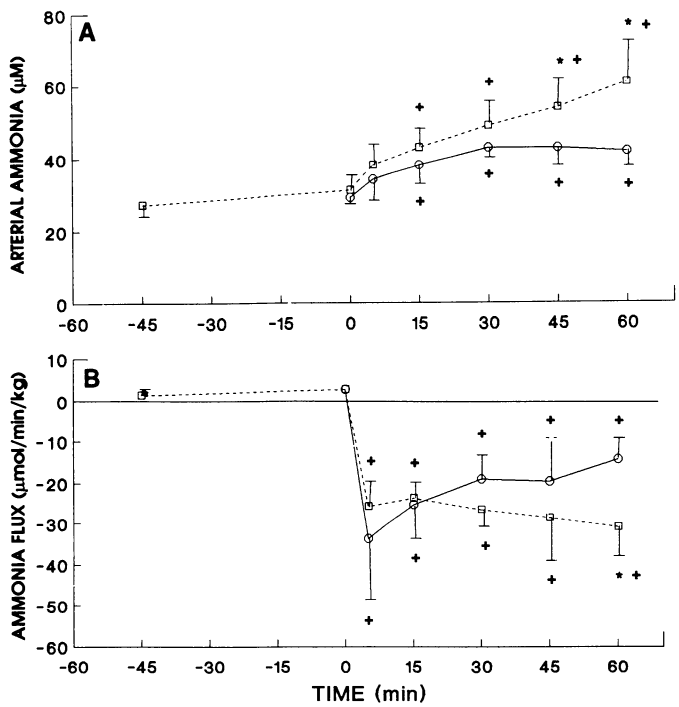

Fig. 1. Summary of plasma NH_3 responses for the experiment. *A*: arterial NH_3, which demonstrated increasing concentrations as exercise progressed. *B*: muscle NH_3 flux. Negative value indicates efflux from muscle. □, BCAA leg; ○, control leg. *Significantly different from control leg; + significantly different from 0 min, $P < 0.05$.

Table 1. *Arterial concentrations and leg exchange of metabolites*

Time, min	Trial	Arterial, mM			Leg Exchange, $mmol \cdot min^{-1} \cdot kg^{-1}$	
		Lactate	FFA	Glycerol	Lactate	FFA
−45	B	0.8 ± 0.1	0.62 ± 0.11	0.19 ± 0.02	−0.05 ± 0.02	0.01 ± 0.01
0	C	0.7 ± 0.1	0.41* ± 0.05	0.10* ± 0.01	−0.03 ± 0.01	0.01 ± 0.01
	B	0.7 ± 0.1	0.70† ± 0.08	0.16† ± 0.01	−0.04 ± 0.02	0.01 ± 0.01
5	C	2.9‡ ± 0.4	0.41 ± 0.06	0.12 ± 0.01	−2.16‡ ± 0.38	0.07‡ ± 0.02
	B	2.6‡ ± 0.3	0.66† ± 0.13	0.20† ± 0.03	−1.14† ± 0.19	0.05‡ ± 0.02
15	C	3.8‡ ± 0.6	0.44 ± 0.08	0.16‡ ± 0.03	−1.20‡ ± 0.49	0.06‡ ± 0.02
	B	3.0‡ ± 0.4	0.87† ± 0.19	0.26†‡ ± 0.04	−0.63‡ ± 0.11	0.10‡ ± 0.02
30	C	3.8‡ ± 0.6	0.78‡ ± 0.13	0.27‡ ± 0.01	−0.98‡ ± 0.37	0.12‡ ± 0.04
	B	3.1‡ ± 0.5	0.87‡ ± 0.16	0.28‡ ± 0.02	−0.44‡ ± 0.21	0.11‡ ± 0.02
45	C	2.6‡ ± 0.4	0.69‡ ± 0.08	0.27‡ ± 0.02	−0.87‡ ± 0.40	0.13‡ ± 0.03
	B	2.4‡ ± 0.5	0.96‡ ± 0.12	0.34‡ ± 0.03	−0.47‡ ± 0.15	0.16‡ ± 0.03
60	C	2.1‡ ± 0.3	0.88‡ ± 0.17	0.36‡ ± 0.06	0.15 ± 0.16	0.12‡ ± 0.02
	B	2.1‡ ± 0.4	1.15‡ ± 0.17	0.41‡ ± 0.04	−0.31 ± 0.23	0.21‡ ± 0.05

Values are means ± SE; $n = 5$ subjects. Whole blood lactate and serum free fatty acid (FFA) and glycerol. C, control leg; B, branched-chain amino acid (BCAA) leg. Positive value indicates uptake; negative value indicates release. *Significant difference between −45 and 0 min; †significant difference from control leg; and ‡significant difference from 0 min, $P < 0.05$.

The arterial lactate concentrations (Table 1) were consistently higher for the control trial than the BCAA trial, but this was not found to be significant. In contrast, the lactate release (Table 1) for the control trial was also consistently higher than the BCAA trial, and this was found to be significant at 5 min. Despite these differences, the intramuscular lactate (Table 2) levels were not significantly different between trials. When the intramuscular shifts in lactate were taken into consideration, the net lactate production for 60 min was 58.0 ± 19.4 and 34.1 ± 1.8 mmol/kg for the control and BCAA legs, respectively. Despite this large difference, this was not significant.

There were no significant differences between trials in glucose metabolism. The arterial glucose concentrations did not vary in either trial, whereas leg uptake was significantly increased by 5 min and remained elevated

Table 2. *Muscle metabolites*

Metabolite	Trial	Time, min 0	5	60
Glycogen, mmol/kg	C	374 ± 23	293 ± 29‡	91 ± 29‡
	B	330 ± 26	277 ± 22‡	82 ± 37‡
Lactate, mmol/kg	C	2.7 ± 0.2	12.0 ± 3.9‡	5.6 ± 1.2‡
	B	2.5 ± 0.6	9.8 ± 2.0‡	5.3 ± 1.2‡
NH_3, μmol/kg	C	256 ± 52	385 ± 58‡	492 ± 130‡
	B	250 ± 20	324 ± 37‡	520 ± 99‡
PCr, mmol/kg	C	86.0 ± 3.7	41.5 ± 9.3‡	52.2 ± 9.1‡
	B	87.7 ± 3.9	44.5 ± 5.9‡	56.9 ± 12.0‡
Cr, mmol/kg	C	47.7 ± 5.9	92.2 ± 10.1‡	81.5 ± 9.2‡
	B	45.9 ± 2.5	89.2 ± 6.2‡	76.8 ± 11.5‡
ATP, mmol/kg	C	23.2 ± 0.8	21.9 ± 1.1	21.7 ± 1.2
	B	22.1 ± 0.8	23.1 ± 0.8	22.2 ± 1.3
ADP, mmol/kg	C	4.3 ± 0.5	4.2 ± 0.3	4.7 ± 0.4
	B	4.1 ± 0.2	4.3 ± 0.2	4.1 ± 0.2
AMP, mmol/kg	C	0.16 ± 0.03	0.13 ± 0.02	0.15 ± 0.01
	B	0.15 ± 0.02	0.15 ± 0.01	0.18 ± 0.02
IMP, mmol/kg	C	0.35 ± 0.08	1.29 ± 0.79	1.12 ± 0.18‡
	B	0.50 ± 0.07	0.66 ± 0.13	0.94 ± 0.30‡
TAN, mmol/kg	C	27.7 ± 1.1	26.2 ± 1.3	26.5 ± 1.6
	B	26.3 ± 1.0	27.5 ± 0.9	26.5 ± 1.4

Values are means ± SE; n = 5 dry muscles except for lactate and NH_3, which are wet muscle. PCr, phosphocreatine; Cr, creatine; TAN, total adenine nucleotide pool (ATP + ADP + AMP). ‡Significant difference from 0 min, $P < 0.05$.

throughout the experiment for both trials (data not shown). The arterial FFA and glycerol concentrations were significantly elevated above 0 min during exercise for the control trial (Table 1). However, these parameters had not completely returned to resting values before the adminstration of the BCAA supplement or the onset of the second exercise bout. Thus the BCAA trial began with higher basal levels of these metabolites, and as a result the arterial FFA and glycerol concentrations were significantly higher for the BCAA trial at −45, 0, and 15 min, but there were no further significant differences (Table 1). Despite the higher arterial FFA concentrations, there were no significant differences in FFA uptake between trials (Table 1). FFA uptake was significantly increased after 15 min of exercise and remained significantly elevated throughout the remainder of the experiment for both trials.

There were no significant differences between groups in the arterial plasma hypoxanthine, urate, or serum urea concentrations (data not shown). Furthermore, these variables did not demonstrate any significant shifts during exercise. Similarly, there were no significant differences between trials in hypoxanthine or urate flux (data not shown). These variables did not show any consistent uptake or release and were highly variable between subjects.

Muscle metabolites. There were no significant differences between trials in any of the measured muscle metabolites (Table 2). Muscle glycogen was significantly decreased after 5 and 60 min of exercise for both trials. Exercise resulted in a significant decrease in PCr and a reciprocal increase in Cr by 5 min for both trials, and these metabolites remained significantly depressed throughout the experiment. There were no measurable shifts in ATP, ADP, AMP, or TAN during exercise for both trials. However, there was a significant increase in IMP after 60 min of exercise for both trials. The increase in IMP was very modest and corresponded to a 0.4–0.8 mmol/kg increase at 60 min.

Arterial amino acids. BCAA supplementation resulted in a significant increase in only the arterial plasma BCAA concentrations (Fig. 2). At the onset of exercise the arterial BCAA levels had more than doubled and remained significantly higher than control throughout the experiment. In contrast, the arterial BCAA concentrations for the control trial remained constant during the exercise bout. The arterial EAA and TAA levels were significantly higher for the BCAA trial compared with the control trial. When the BCAA concentrations were subtracted from the EAA concentrations (EAA − BCAA), significantly lower EAA minus BCAA levels were observed at 30, 45, and 60 min for the BCAA trial compared with the control trial (Fig. 2). There are five remaining EAA after the BCAA (threonine, methionine, phenylalanine, tryptophan, and lysine), and significantly lower arterial concentrations were observed for threonine, methionine, phenylalanine, and lysine in the later stages of exercise for the BCAA trial (Table 3). On the other hand, when the BCAA concentrations were subtracted from the TAA concentrations (TAA − BCAA), there were no significant differences between trials in these concentrations (Table 3).

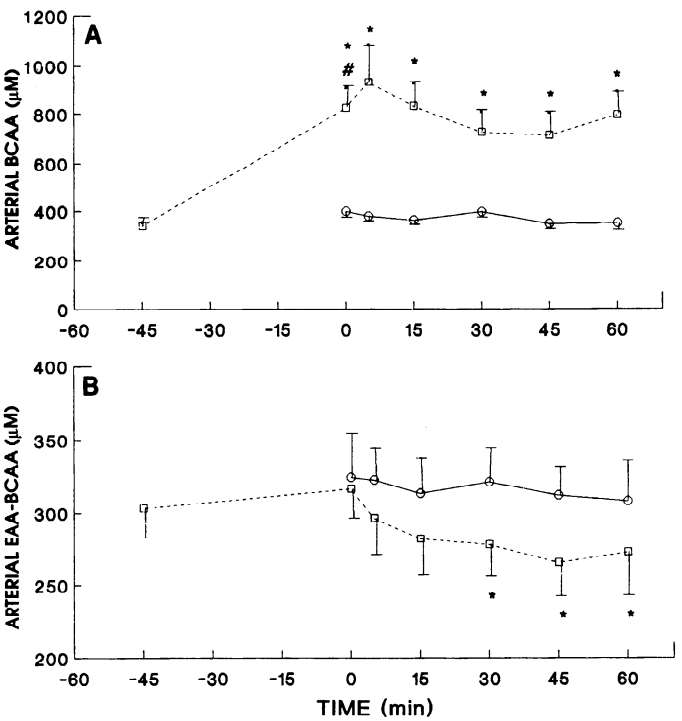

Fig. 2. Arterial BCAA (*A*) and EAA − BCAA concentrations (*B*) for the experiment during exercise. BCAA supplementation results in dramatically elevated arterial BCAA levels. □, BCAA leg; ○, control leg. *Significant difference from control leg; #significant difference between −45 and 0 min.

Table 3. *Some arterial plasma amino acids*

AA	Trial	Time, min						
		−45	0	5	15	30	45	60
Asp	C		4 ± 1	3 ± 1	5 ± 2	5 ± 1	6 ± 1	6 ± 1
	B	5 ± 1	7 ± 3	4 ± 1	4 ± 1	5 ± 1	5 ± 1	5 ± 1
Glu	C		57 ± 6	45 ± 5	43 ± 5	52 ± 6	49 ± 6	49 ± 8
	B	59 ± 8	56 ± 8	44 ± 6	45 ± 7	51 ± 6	48 ± 5	47 ± 11
Gln	C		548 ± 20	558 ± 23	565 ± 22	564 ± 24	568 ± 22	577 ± 37
	B	559 ± 23	581 ± 29	586 ± 24	588 ± 31	613 ± 34†	586 ± 43	643 ± 65
Ala	C		278 ± 25	329 ± 30‡	371 ± 37‡	424 ± 37‡	398 ± 31‡	358 ± 31‡
	B	281 ± 26	252 ± 26	304 ± 34‡	342 ± 45‡	373 ± 44†‡	341 ± 44†‡	336 ± 48‡
Thr	C		100 ± 15	97 ± 8	92 ± 10	92 ± 9	90 ± 7	87 ± 7
	B	94 ± 10	98 ± 11	83 ± 11†	79 ± 11	79 ± 9†	76 ± 10†	69 ± 8†
Met	C		27 ± 3	26 ± 3	25 ± 3	26 ± 2	27 ± 2	27 ± 3
	B	24 ± 2	24 ± 2	24 ± 3	23 ± 3	22 ± 3	20 ± 2†	23 ± 3†
Phe	C		43 ± 3	43 ± 2	44 ± 3	46 ± 2	44 ± 3	45 ± 3
	B	40 ± 2	43 ± 2	44 ± 3	39 ± 2	38 ± 3†	37 ± 3†	40 ± 5
Trp	C		3 ± 1	3 ± 1	4 ± 2	4 ± 1	5 ± 1	7 ± 3‡
	B	4 ± 1	4 ± 1	4 ± 2	4 ± 1	4 ± 1	5 ± 1	7 ± 1‡
Lys	C		151 ± 13	153 ± 12	147 ± 10	153 ± 12	147 ± 9	113 ± 30
	B	142 ± 9	147 ± 10	146 ± 12	136 ± 13	133 ± 11†	128 ± 13†	133 ± 20
Hyp	C		13 ± 3	13 ± 3	12 ± 2	13 ± 3	13 ± 3	14 ± 5
	B	11 ± 2	18 ± 3†	17 ± 3†	17 ± 3†	18 ± 2†	16 ± 3†	17 ± 14
3-MH	C		4 ± 1	5 ± 2	5 ± 1	6 ± 1	5 ± 1	4 ± 1
	B	5 ± 1	5 ± 1	5 ± 1	5 ± 1	6 ± 1	5 ± 1	6 ± 1
TAA	C		2,490 ± 141	2,509 ± 112	2,524 ± 125	2,638 ± 126	2,577 ± 129	2,562 ± 172
	B	2,380 ± 75	2,913 ± 146*†	2,998 ± 220†	2,885 ± 157†	2,876 ± 95†	2,748 ± 88	2,917 ± 137†
TAA-BCAA	C		2,090 ± 116	2,130 ± 92	2,162 ± 107	2,269 ± 105	2,229 ± 109	2,211 ± 147
	B	2,040 ± 67	2,092 ± 94	2,065 ± 112	2,056 ± 147	2,150 ± 124	2,036 ± 152	2,124 ± 220

Values are means ± SE; $n = 5$ subjects in μM. AA, amino acid; Asp, aspartate; Glu, glutamate; Gln, glutamine; Ala, alanine; Thr, threonine; Met, methionine; Phe, phenylalanine; Trp, tryptophan; Lys, lysine; Hyp, hydroxyproline; 3-MH, 3-methylhistidine; TAA, total amino acid; TAA-BCAA, total AA minus BCAA. *Significant difference between −45 and 0 min; †significant difference from control leg; and ‡significant difference from 0 min, $P < 0.05$.

Exercise after BCAA supplementation resulted in significantly lower arterial asparagine levels at 5, 30, and 45 min, arterial proline levels at 5, 30, and 60 min, and arterial tyrosine levels at 5, 30, 45, and 60 min compared with control (data not shown). In contrast the BCAA trial demonstrated significantly higher arterial hydroxyproline concentrations at 0, 5, 15, 30, and 45 min compared with control (Table 3). The BCAA trial was also characterized by consistently higher arterial glutamine concentrations and consistently lower arterial alanine levels compared with control (Table 3). However, glutamine and alanine were only found to be significantly different from control at 30 min and 30 and 45 min, respectively. There were no significant differences between trials in arterial aspartate (Table 3), glutamate (Table 3), 3-methylhistidine (Table 3), serine, glycine, taurine, histidine, arginine, tryptophan, and ornithine (data not shown).

Intramuscular amino acids. Most of the intramuscular amino acids are presented in Table 4. BCAA supplementation resulted in significantly higher intramuscular BCAA levels by the onset of exercise, and these levels remained significantly higher throughout exercise compared with control (Fig. 3). After the onset of exercise the BCAA concentrations for both trials were significantly elevated at both 5 and 60 min. Despite the increase in BCAA levels, there were no significant differences between trials in the EAA levels. However, both trials demonstrated a significant increase in EAA concentrations during exercise. When the BCAA were subtracted from the EAA, significantly lower intramuscular EAA minus BCAA concentrations were observed for the BCAA trial at 60 min (Fig. 3). This was further demonstrated by significantly lower intramuscular threonine, phenylalanine, and lysine concentrations at 60 min for the BCAA trial.

The BCAA trial was further characterized by significantly lower intramuscular glycine, histidine, and proline concentrations at 60 min compared with control. Furthermore, BCAA supplementation resulted in significantly higher aspartate and alanine (Fig. 3) concentrations by the onset of exercise. There were no further differences between trials in these amino acids; however, alanine was significantly elevated at 5 min for both trials. There were no significant differences between trials in glutamine (Fig. 3), glutamate, serine, asparagine, taurine, arginine, tyrosine, methionine, tryptophan, ornithine, hydroxyproline, and 3-methylhistidine. Glutamate was significantly decreased by 5 min and remained significantly depressed throughout exercise in

Table 4. *Some intramuscular amino acid concentrations*

AA	Trial	Time, min		
		0	5	60
Asp	C	1.37 ± 0.22	1.45 ± 0.24	1.85 ± 0.48
	B	1.80 ± 0.18†	2.02 ± 0.35	1.64 ± 0.20
Glu	C	13.22 ± 1.13	7.38 ± 0.64‡	7.64 ± 1.13‡
	B	13.15 ± 0.45	7.48 ± 0.58‡	8.39 ± 0.43‡
Ser	C	2.10 ± 0.18	2.00 ± 0.15	2.98 ± 0.21‡
	B	2.00 ± 0.12	2.39 ± 0.32	2.27 ± 0.27‡
Gly	C	5.82 ± 1.46	6.46 ± 2.12	6.15 ± 0.44
	B	4.54 ± 0.14	4.97 ± 0.28	4.61 ± 0.23†
His	C	1.51 ± 0.14	1.78 ± 0.19	2.23 ± 0.10
	B	1.64 ± 0.14	1.76 ± 0.19	1.69 ± 0.05†
Thr	C	0.92 ± 0.07	1.03 ± 0.22	1.99 ± 0.14‡
	B	0.95 ± 0.16	1.26 ± 0.18	1.28 ± 0.19†‡
Pro	C	5.25 ± 0.41	4.93 ± 0.38	5.73 ± 0.21
	B	5.34 ± 0.29	5.13 ± 0.13	4.75 ± 0.33†
Met	C	0.70 ± 0.06	0.65 ± 0.05	0.68 ± 0.05
	B	0.89 ± 0.06	0.78 ± 0.04	0.77 ± 0.13
Phe	C	0.42 ± 0.03	0.48 ± 0.05	0.58 ± 0.02‡
	B	0.41 ± 0.02	0.45 ± 0.02	0.46 ± 0.02†‡
Trp	C	0.15 ± 0.01	0.17 ± 0.01	0.20 ± 0.02‡
	B	0.14 ± 0.01	0.16 ± 0.02	0.18 ± 0.02‡
Lys	C	2.98 ± 0.32	3.15 ± 0.29	3.88 ± 0.36
	B	2.73 ± 0.16	2.85 ± 0.18	2.76 ± 0.05†
Hyp	C	0.35 ± 0.07	0.32 ± 0.05	0.37 ± 0.07
	B	0.47 ± 0.08	0.38 ± 0.05	0.40 ± 0.09
3-MH	C	0.51 ± 0.12	0.52 ± 0.12	0.53 ± 0.10
	B	0.61 ± 0.13	0.63 ± 0.18	0.52 ± 0.10
TAA	C	155.1 ± 10.9	152.1 ± 14.4	170.3 ± 10.6
	B	167.6 ± 9.1	159.7 ± 11.7	149.1 ± 16.4
TAA-BCAA	C	152.0 ± 10.7	148.6 ± 14.3	166.6 ± 10.8
	B	164.0 ± 9.1	154.8 ± 11.7	144.6 ± 16.2

Values are means ± SE in mmol/kg dry muscle; $n = 5$ subjects. Ser, serine; Gly, glycine; His, histidine; Pro, proline; Tyr, tyrosine. †Significant difference from control leg and ‡significant difference from 0 min, $P < 0.05$.

both trials. Meanwhile, serine, threonine, phenylalanine, and tryptophan were all significantly elevated by 60 min for both trials. Despite BCAA supplementation and the shifts in some amino acids, the total intramuscular amino acid pools were not significantly altered. This was reflected by the consistent intramuscular TAA and TAA minus BCAA concentrations.

Amino acid flux data. The amino acid flux data are summarized in Table 5. Before the onset of exercise, there was a significant uptake of BCAA by muscle for the BCAA trial compared with control (Fig. 4). After the initiation of exercise the BCAA leg demonstrated a further uptake of BCAA, and this was significantly greater than control at 15, 30, and 60 min. The control leg was characterized by a larger efflux of BCAA early during exercise, and this efflux slowly decreased as exercise progressed. In both trials, the BCAA flux from muscle was significantly different from 0 min at 15, 30, and 45 min. With the larger BCAA uptake for the BCAA trial, it is not surprising that the EAA flux at 0, 15, and 30 min was significantly different from control. Furthermore, the efflux of EAA was significantly greater than that at 0 min at 15, 30, and 45 min for both trials. When the BCAA were subtracted from the EAA, the efflux of EAA minus BCAA was significantly greater than that at 0 min from 5 to 45 min for both trials (Fig. 4). The efflux of EAA minus BCAA for the BCAA trial was consistently lower than for the control trial, and this was significant at 30 and 45 min. The five remaining EAA minus BCAA demonstrated relatively lower effluxes during exercise for the BCAA compared with control trial; however, this was only found to be significant for threonine and tryptophan. When the EAA were subtracted from the TAA, there were no significant differences between trials in the TAA minus BCAA flux. The active muscle demonstrated a significant efflux of amino acids since the TAA and TAA minus BCAA fluxes were significantly greater than that at 0 min throughout exercise for both trials.

Exercise resulted in a significantly different flux from 0 min for all amino acids except tryptophan and 3-methylhistidine. Glutamate, on the other hand, was the only amino acid that was consistently taken up by muscle throughout the experiment. With the onset of exercise, there was a dramatic increase in the efflux of alanine (Fig. 4) and glutamine (Fig. 4) for both trials. The efflux of alanine at 5 and 60 min as well as the efflux of glutamine at 5 and 15 min was significantly greater for the BCAA trial compared with control. There were no significant differences between trials in the flux of aspartate, glutamate, serine, asparagine, glycine, taurine, histidine, arginine, proline, tyrosine, methionine, phenylalanine, ornithine, lysine, hydroxyproline, and 3-methylhistidine.

TAA uptake/release data. Summed over the 60 min of exercise, the total release of BCAA was significantly lower for the BCAA trial compared with control (Fig. 5). This pattern was also observed for the total release of EAA. When the BCAA were subtracted from the EAA, the total release of the EAA minus BCAA was significantly lower for the BCAA trial (Fig. 5). Of the five remaining EAA minus BCAA, the total release of threonine, methionine, and tryptophan were significantly lower for the BCAA trial. Although there were no significant differences between trials in the total release of phenylalanine and lysine, these amino acids demonstrated consistently lower total releases for the BCAA trial compared with control. In contrast, when the BCAA were subtracted from the TAA, there were no significant differences between trials in the TAA minus BCAA release. The release of alanine from 0 to 5 min and from 45 to 60 min was significantly greater for the BCAA trial compared with control (Fig. 5). However, summed over the 60 min of exercise, there was no significant difference between trials in the total release of alanine. Similarly, the release of glutamine from 0 to 5 and from 5 to 15 min was significantly greater for the BCAA trial compared with control (Fig. 5). When the release of glutamine was summed over the 60 min of exercise, there was no significant difference between trials, despite consistently larger releases for the BCAA

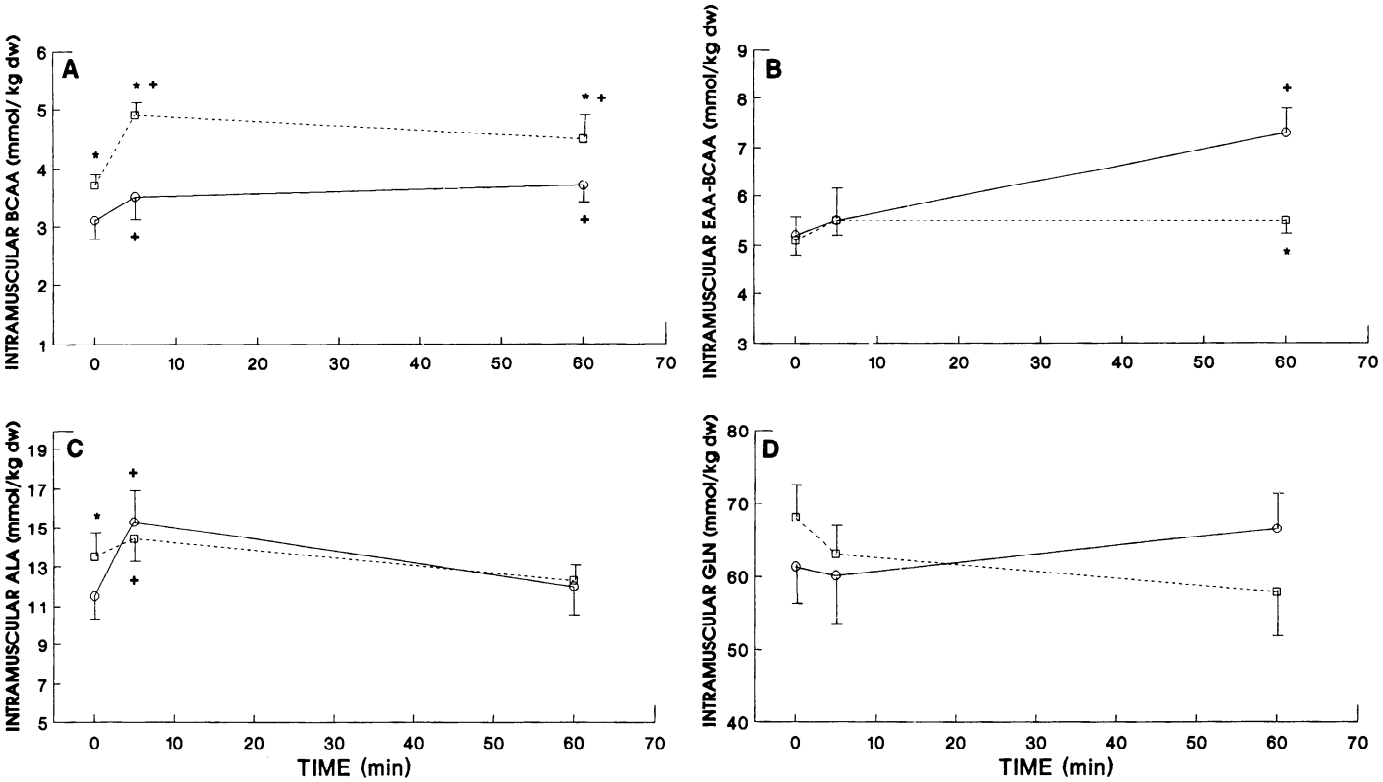

Fig. 3. Summary of intramuscular BCAA (*A*), EAA − BCAA (*B*), alanine (Ala; *C*), and glutamine (Gln; *D*) levels for the experiment. BCAA supplementation results in elevated intramuscular BCAA and lower EAA − BCAA levels during exercise compared with control. □, BCAA leg; ○, control leg. *Significant difference from control leg; + significant difference from 0 min, $P < 0.05$.

trial. There were no other significant differences between trials in the total release of any other amino acid.

NH_3, alanine, and glutamine balance. The NH_3 balance calculations are summarized in Fig. 6. The increase in intramuscular NH_3 (muscle accumulation) and the total release of NH_3 (muscle flux) over 60 min was not significantly different between trials. When muscle accumulation and muscle flux were summed, the total muscle production was also not significantly different between trials. The deamination of one molecule of AMP to IMP produces one molecule of NH_3, thus the intramuscular increase in IMP shares a 1:1 stoichiometric relationship with NH_3. The increase in intramuscular IMP (i.e., NH_3 production from AMP) was not significantly different between trials. When the NH_3 produced from AMP was subtracted from the total muscle production, the net (non-AMP) production was 1,112 ± 279 and 1,670 ± 245 μmol/kg for the control and BCAA trials, respectively, and was significantly higher for the BCAA trial.

The NH_3 produced during an exercise bout exits the muscle in predominantly three forms (NH_3, alanine, and glutamine). When the net release of NH_3 and total release of glutamine were summed, the total release of NH_3 plus glutamine was 2,441 ± 543 and 3,581 ± 415 μmol/kg for the control and BCAA trials, respectively, and was significantly different between trials (Fig. 6). When all three of these parameters were summed, the total release of NH_3 plus glutamine plus alanine was 3,515 ± 784 and 4,887 ± 485 μmol/kg for the control and BCAA trials, respectively (Fig. 6). This was not found to be significant despite a 1,372 ± 720 μmol/kg larger release of NH_3 plus glutamine plus alanine for the BCAA trial compared with the control trial.

DISCUSSION

The purpose of this study was to examine the effects of an oral dose of BCAA on amino acid and NH_3 metabolism in the quadriceps muscle mass during one-legged exercise. The major findings were that, immediately after the initiation of exercise, the efflux of alanine and glutamine was significantly higher and the efflux of lactate significantly lower for the BCAA trial compared with control. The BCAA trial was characterized by a significant uptake of BCAA, and this was reflected in a significant increase in the intramuscular BCAA concentrations. There were no significant differences between trials in NH_3 efflux early in the exercise bout. However, when the NH_3 produced from IMP accumulation was subtracted, the BCAA trial demonstrated a significantly greater net muscle NH_3 production. An interesting finding was that BCAA supplementation resulted in significantly lower arterial and intramuscular EAA minus BCAA concentrations during exercise. It was further found that the muscle efflux of these amino acids was also significantly lower during exercise.

Due to the invasiveness of this study, it was necessary to perform both trials on each subject in the same day. As a result, it was necessary to perform the control trial first. To try to minimize any possible effects from the

Table 5. *Amino acid flux data*

AA	Trial	Time, min						
		−45	0	5	15	30	45	60
Asp	C		−0.1 ± 0.1	−2.5 ± 1.5‡	0.3 ± 0.5	−1.0 ± 1.5	0.5 ± 0.4	0.0 ± 0.4
	B	0.0 ± 0.1	0.3 ± 0.3	−2.1 ± 0.7‡	−1.1 ± 0.6	−0.1 ± 0.2	−0.6 ± 0.8	−0.8 ± 0.4
Glu	C		4.3 ± 0.9	5.6 ± 1.0	5.2 ± 1.6	6.8 ± 1.8‡	7.5 ± 1.9	6.3 ± 1.2
	B	3.9 ± 0.6	3.9 ± 1.1	6.6 ± 1.2	7.7 ± 2.7	8.4 ± 1.3‡	5.7 ± 2.7	1.7 ± 1.5
Ser	C		−0.6 ± 0.2	−9.3 ± 7.2‡	−3.7 ± 3.8	−6.0 ± 3.9	−0.7 ± 3.2	−1.1 ± 1.3‡
	B	−0.2 ± 0.7	1.5 ± 1.6	−11.3 ± 2.3‡	−4.4 ± 2.5	−1.5 ± 1.8	−3.7 ± 3.2	−5.4 ± 2.4‡
Asn	C		−1.4 ± 0.4	−5.0 ± 1.9‡	−0.8 ± 2.4	−5.1 ± 1.6‡	−3.4 ± 1.5‡	−1.9 ± 1.5
	B	−0.9 ± 0.3	−0.3 ± 0.2†	−3.5 ± 0.9‡	−4.5 ± 0.6	−2.3 ± 0.7‡	−2.7 ± 0.7‡	−1.4 ± 0.7
Gly	C		−4.1 ± 0.9	−19.5 ± 6.3‡	−11.2 ± 4.4‡	−18.7 ± 5.5‡	−5.3 ± 2.9‡	−12.2 ± 4.5‡
	B	−5.0 ± 1.1	−0.6 ± 1.0*†	−23.1 ± 2.7‡	−18.3 ± 2.5‡	−12.8 ± 5.5‡	−9.5 ± 1.6‡	−11.5 ± 1.9‡
Tau	C		0.0 ± 0.2	−5.5 ± 1.4‡	−3.1 ± 1.1‡	−2.6 ± 1.0‡	−2.8 ± 0.9	−2.9 ± 0.3‡
	B	−0.5 ± 0.3	0.2 ± 0.2	−2.9 ± 1.0‡	−4.1 ± 0.7‡	−2.1 ± 1.3‡	−0.9 ± 2.7	−4.0 ± 2.7‡
His	C		−1.3 ± 0.3	−5.0 ± 2.3‡	−3.2 ± 1.9‡	−7.4 ± 2.7‡	−6.3 ± 1.4‡	−5.2 ± 1.3‡
	B	−1.1 ± 0.2	−0.6 ± 0.5	−6.6 ± 0.8‡	−4.1 ± 0.8‡	−2.3 ± 1.3‡	−4.3 ± 0.9‡	−5.7 ± 2.4‡
Thr	C		−2.6 ± 0.4	−5.7 ± 1.5‡	−6.2 ± 0.9‡	−7.8 ± 2.7‡	−7.9 ± 1.7‡	−2.9 ± 2.6
	B	−1.9 ± 0.3	−1.2 ± 0.4†	−4.3 ± 2.1‡	−1.8 ± 1.1†‡	−2.0 ± 1.0†‡	−2.0 ± 1.0†‡	−5.8 ± 0.8
Arg	C		−0.9 ± 0.4	−5.2 ± 1.5‡	−5.9 ± 3.4‡	−2.1 ± 1.2	−2.4 ± 2.2	0.5 ± 1.4
	B	−0.8 ± 0.3	−0.6 ± 0.3	−5.6 ± 1.4‡	−5.5 ± 0.8‡	−1.8 ± 2.2	−2.2 ± 0.8	−3.4 ± 1.7
Pro	C		−2.3 ± 0.6	−14.7 ± 10.4	−13.5 ± 6.9	−15.0 ± 6.6‡	−5.2 ± 2.4	−8.5 ± 3.2‡
	B	−3.4 ± 0.9	−2.0 ± 1.0	−15.1 ± 6.9	−6.3 ± 4.0	−10.6 ± 5.3‡	−6.9 ± 3.8	−7.6 ± 4.3‡
Tyr	C		−1.0 ± 0.4	−4.6 ± 1.9‡	−2.7 ± 1.5‡	−3.6 ± 1.3‡	−2.1 ± 0.5‡	−1.6 ± 0.9
	B	−0.8 ± 0.2	−0.3 ± 0.1	−4.4 ± 1.3‡	−2.6 ± 0.5‡	−2.7 ± 0.8‡	−1.9 ± 0.5‡	−0.8 ± 0.3
Val	C		−1.5 ± 0.5	−12.9 ± 3.1	−8.0 ± 3.6‡	−9.9 ± 3.0‡	−8.6 ± 2.2‡	−5.5 ± 3.7
	B	−1.2 ± 0.5	7.7 ± 2.9*†	5.9 ± 13.5	−2.8 ± 3.7‡	−1.4 ± 3.0†	−9.0 ± 5.1‡	1.7 ± 5.2
Met	C		−0.7 ± 0.4	−2.3 ± 0.7	−0.4 ± 0.5	−2.6 ± 0.8‡	−1.8 ± 0.6	−1.6 ± 1.4
	B	−0.5 ± 0.1	−0.3 ± 0.2	−0.4 ± 1.0	−0.1 ± 0.5	−0.9 ± 0.6‡	−1.1 ± 0.8	−0.1 ± 0.6
Ile	C		−0.5 ± 0.3	−3.9 ± 2.4	−5.4 ± 1.3‡	−1.7 ± 1.1	−0.3 ± 2.0	−1.0 ± 0.8
	B	−0.5 ± 0.1	4.0 ± 1.4*†	0.5 ± 3.8	0.8 ± 2.7†‡	2.7 ± 1.8†	1.5 ± 2.7	1.3 ± 1.7
Leu	C		−1.1 ± 0.3	−6.4 ± 2.2	−5.4 ± 1.5‡	−4.2 ± 1.6‡	−2.9 ± 0.5	−0.3 ± 1.1
	B	−0.8 ± 0.3	6.4 ± 1.9*†	1.5 ± 6.9	3.1 ± 3.9†‡	0.4 ± 2.2†‡	−2.6 ± 1.7	1.6 ± 3.0
Phe	C		−0.6 ± 0.2	−3.6 ± 1.2‡	−1.8 ± 1.1	−1.8 ± 1.0	−1.3 ± 0.4‡	−0.4 ± 0.6
	B	−0.8 ± 0.2	−0.2 ± 0.1	−2.5 ± 1.1‡	−1.2 ± 0.5	−1.4 ± 0.7	−1.0 ± 0.3‡	−0.4 ± 0.5
Trp	C		0.0 ± 0.1	−0.9 ± 0.5	−0.2 ± 0.2	−1.3 ± 0.8	−1.8 ± 1.0	−0.1 ± 1.3
	B	−0.1 ± 0.1	−0.4 ± 0.5	0.2 ± 0.2	0.6 ± 0.2†	−0.2 ± 0.4	0.2 ± 0.6†	−1.0 ± 1.0
Orn	C		−0.7 ± 0.2	−7.9 ± 2.6‡	−3.2 ± 1.8	−1.0 ± 2.6	−2.5 ± 2.3	−2.1 ± 1.7‡
	B	−1.1 ± 0.8	0.2 ± 0.4	−6.3 ± 0.8‡	−3.4 ± 2.2	−1.2 ± 0.8	−3.1 ± 1.6	−2.3 ± 0.5‡
Lys	C		−1.7 ± 0.7	−6.6 ± 1.6‡	−10.2 ± 4.1‡	−6.4 ± 2.6‡	−8.2 ± 0.4‡	−5.7 ± 1.6
	B	−1.4 ± 0.6	−0.4 ± 0.5	−7.6 ± 1.6‡	−6.3 ± 1.5‡	−5.0 ± 2.5‡	−6.2 ± 1.8‡	−4.1 ± 3.7
Hyp	C		−0.2 ± 0.1	−0.6 ± 0.6	−0.4 ± 0.6	−0.7 ± 0.3	−0.3 ± 0.2	−0.8 ± 0.3‡
	B	−0.3 ± 0.1	0.2 ± 0.1*†	0.0 ± 0.6	0.1 ± 0.4	0.3 ± 0.7	−0.6 ± 0.4	−0.3 ± 0.2‡
3-MH	C		−0.1 ± 0.1	−0.2 ± 0.2	−0.1 ± 0.3	0.5 ± 0.5	−0.5 ± 0.2	−0.1 ± 0.2
	B	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	−0.9 ± 0.1	0.0 ± 0.2	−0.6 ± 0.5	0.2 ± 0.2
TAA	C		−40 ± 11	−163 ± 28‡	−125 ± 40‡	−159 ± 39‡	−90 ± 23‡	−73 ± 20‡
	B	−40 ± 12	4 ± 8*†	−172 ± 26‡	−138 ± 24‡	−79 ± 21‡	−111 ± 32‡	−100 ± 32‡
EAA	C		−9 ± 3	−42 ± 9	−38 ± 10‡	−36 ± 8‡	−33 ± 5‡	−18 ± 12
	B	−7 ± 2	16 ± 6*†	−7 ± 27	−8 ± 9†‡	−8 ± 9†‡	−20 ± 12‡	−7 ± 11
TAA-BCAA	C		−37 ± 10	−140 ± 25‡	−107 ± 35‡	−144 ± 37‡	−79 ± 21‡	−66 ± 15‡
	B	−38 ± 11	−14 ± 3	−181 ± 9‡	−139 ± 22‡	−81 ± 16‡	−101 ± 24‡	−105 ± 250‡

Values are means ± SE in $\mu mol \cdot min^{-1} \cdot kg^{-1}$; $n = 5$ subjects. *Significant difference between −45 and 0 min; †significant difference from control leg; and ‡significant difference from 0 min, $P < 0.05$.

previous exercise bout, ~90–105 min were allowed between the two successive exercise trials. From the blood metabolite data the only parameters that had not returned to basal levels by the onset of the BCAA trial were arterial FFA and glycerol. Despite these higher levels, there were no significant differences between trials in FFA uptake. Furthermore, there were no significant differences between trials in O_2 consumption, glucose uptake, intramuscular lactate, or glycogen utilization. It therefore appears that the effects of the previous exercise were minimal and had no significant bearing on the major findings of the study.

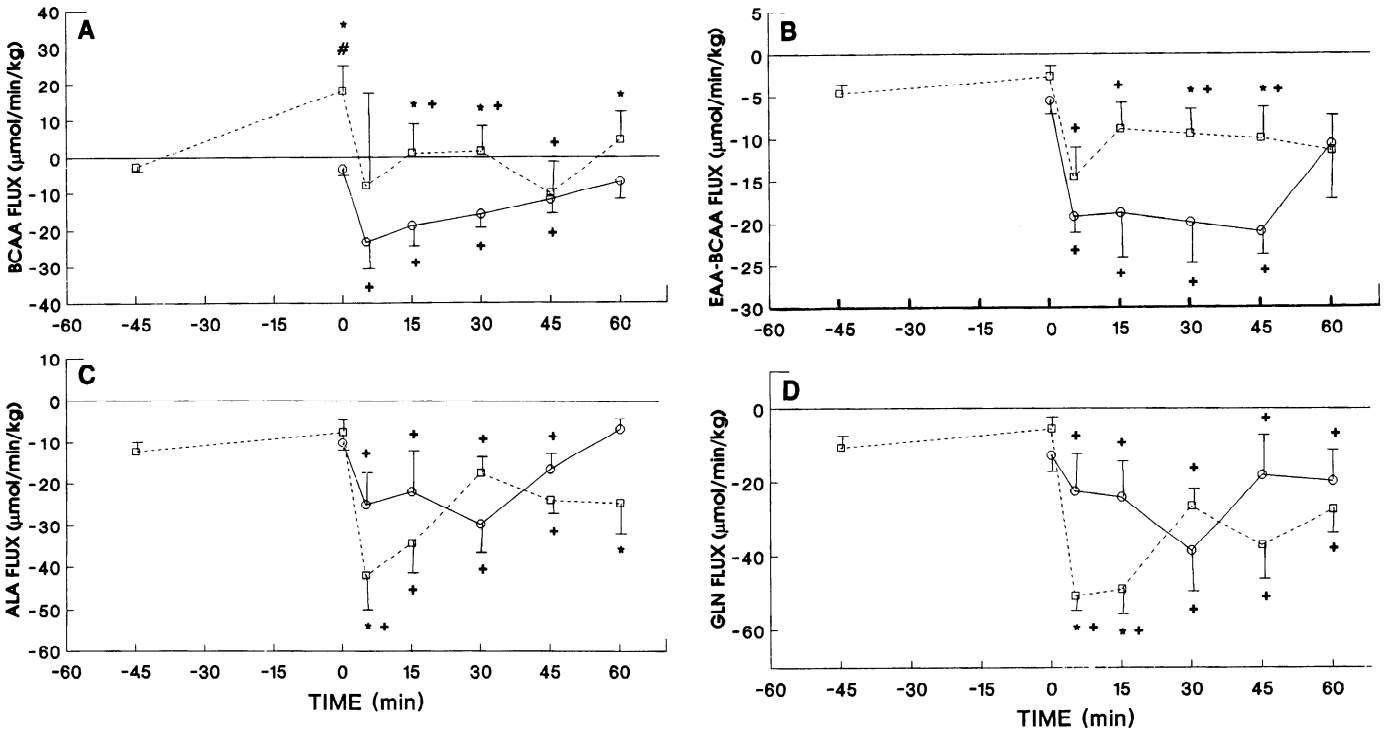

Fig. 4. Summary of BCAA (*A*), EAA − BCAA (*B*), Ala (*C*), and Gln (*D*) flux data for the experiment. Positive value indicates uptake; negative value indicates efflux. There was uptake of BCAA before and during exercise for the BCAA leg. In contrast, dramaticaly lower efflux of EAA − BCAA was observed during the same period compared with control. □, BCAA leg; ○, control leg. *Significant difference from control; # significant difference between −45 amd 0 min; + significant difference from 0 min, $P < 0.05$.

The sources of the NH_3 produced from skeletal muscle during a prolonged submaximal exercise bout are not completely clear. It is well established that a considerable amount of NH_3 can be produced during a high-intensity exercise bout from the deamination of AMP to IMP as one of the steps of the PNC (9). On the other hand, a considerable amount of evidence suggests that the deamination of the BCAA can produce a substantial

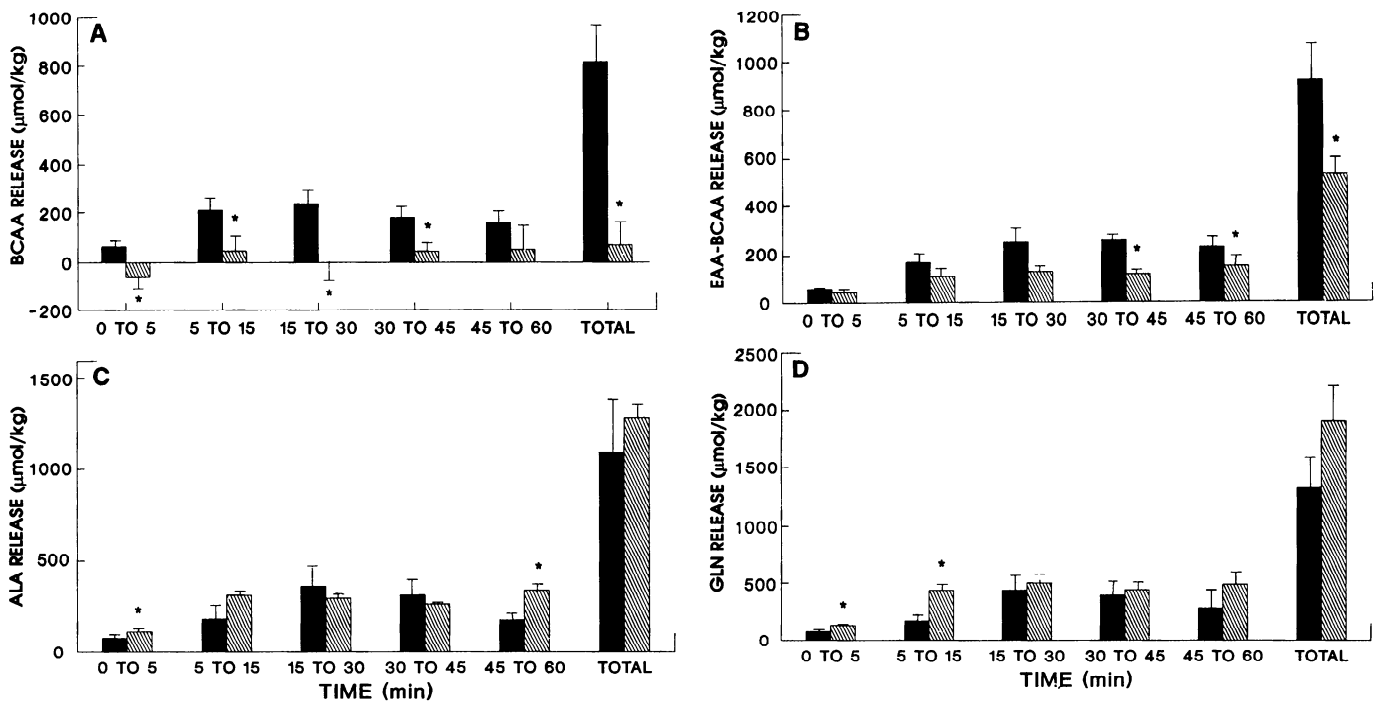

Fig. 5. Summary of BCAA (*A*), EAA − BCAA (*B*), Ala (*C*), and Gln (*D*) release data for the experiment. Release of these parameters was calculated over various time segments and then summed for the entire experiment (Total). Because most of these amino acids were released during the experiment, a positive value indicates release, and a negative value indicates uptake. Hatched bars, BCAA leg; closed bars, control leg. *Significant difference from control leg.

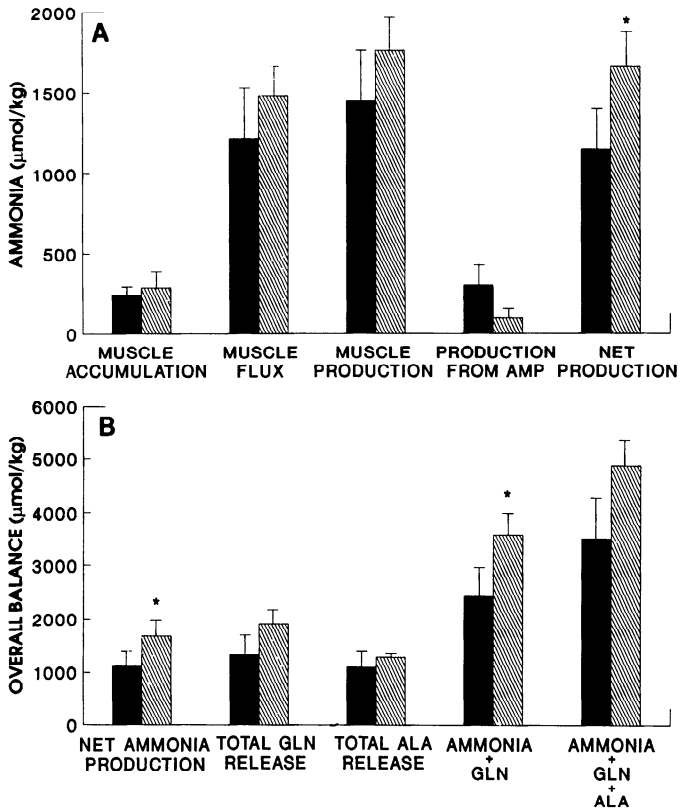

Fig. 6. Summary of the estimate for net muscle NH_3 production (*A*) and overall NH_3, Gln, and Ala balance (*B*). See RESULTS for explanation of calculation for net muscle NH_3 production. Overall, NH_3 + Gln and NH_3 + Gln + Ala balance was estimated by summing net NH_3 production (*A*) with total release of these other amino acids. Hatched bars, BCAA leg; closed bars, control leg. *Significant difference from control leg, $P < 0.05$.

quantity of NH_3 during a prolonged submaximal exercise bout (21, 22, 29). A previous study by MacLean and Graham (21) demonstrated that the same oral dose of BCAA resulted in significantly higher venous plasma NH_3 and glutamine levels during 60 min of two-legged cycling exercise. However, the authors were unable to precisely determine the site and source of the increased NH_3 production. Therefore, in the present study, the authors used the isolated one-legged extensor model and the same BCAA dose to further distinguish between the two potential sources of NH_3 production in skeletal muscle.

The reactions of the PNC are as follows: *1*) AMP + $H_2O \rightarrow$ IMP + NH_3, catalyzed by AMP deaminase; *2*) IMP + GTP + aspartate $\rightarrow$ adenylosuccinate (S-AMP) + GDP + P_i, catalyzed by S-AMP synthetase; and *3*) S-AMP $\rightarrow$ AMP + fumarate, catalyzed by S-AMP lyase. The first reaction is nonreversible under physiological conditions and is considered the first half or deamination arm of the PNC. The last two reactions involve the salvaging of the adenine nucleotides by reamination of IMP to AMP using aspartate as an NH_3 donor. The primary function of the PNC is the maintenance of the energy state of the cell (20). During intense muscle contraction when the ATP-to-ADP ratio becomes compromised, the removal of AMP via AMP deamination allows the near-equilibrium adenylate kinase reaction to move in the direction of ATP production (2 ADP $\leftrightarrow$ AMP + ATP). It is evident that the reactions of the PNC may not be of significant importance during a prolonged submaximal exercise bout, since the ATP-to-ADP ratio and energy state of the cell are easily maintained. This is reflected in the present study since there were no significant shifts or differences between trials in ATP, ADP, AMP, or TAN.

Several researchers have attributed all of the NH_3 produced during a submaximal exercise bout to the reactions of the PNC (5). In the present study 1,407 and 1,760 μmol/kg NH_3 (not including glutamine or alanine) were produced by the control and BCAA trials, respectively. Both trials demonstrated a significant increase in IMP after 60 min of exercise and was similar in magnitude to that previously observed in humans (25). The increase in IMP shares a 1:1 stoichiometric relationship with NH_3. Therefore, the NH_3 produced from AMP deamination was 295 ± 140 and 94 ± 50 μmol/kg for the control and BCAA trials, respectively. Furthermore, when the NH_3 produced from IMP accumulation was subtracted from the total amount of NH_3 produced, the BCAA trial (1,670 ± 245 μmol/kg) demonstrated significantly more net muscle NH_3 production than the control trial (1,112 ± 279 μmol/kg). Given the minor role that IMP accumulation plays in total muscle NH_3 production, it is evident that a substantial portion of the NH_3 produced not only during the control trial but also during the BCAA trial must come from another source.

It is possible that the reactions of the PNC may be acting in concert where AMP deamination is followed by IMP reamination. This would result in NH_3 production with no measurable change in the intramuscular AMP concentrations. Researchers have investigated this possibility in rodents and have reported that the PNC does not act as a cycle in metabolically active fibers (23). That is, AMP deamination occurs during muscle contraction, but IMP reamination does not occur until the muscle fiber is in a state of recovery. During a prolonged submaximal exercise bout, it is difficult to explain why PNC cycling would be active or necessary. Previous studies (5, 10) and the present study have demonstrated that NH_3 production is not only continuous but increases as exercise progresses. Furthermore, the synthesis of glutamine from glutamate requires free NH_3, and thus the glutamine synthesized in the present study would represent a doubling of the NH_3 produced in either trial (a calculation usually overlooked). Therefore, for the PNC to produce the NH_3 plus glutamine formed by either trial, it would need to be cycling at a substantial rate. Furthermore, the PNC cycling would have to be increasing as exercise progresses and increasing at an even greater rate during the BCAA trial. Therefore, it is difficult to suggest what would be activating the cycle to a greater extent as exercise progresses and what would be triggering a greater degree of cycling during the BCAA trial.

Another convincing argument against PNC cycling as the only contributor to NH_3 production during prolonged exercise is the utilization of aspartate as the NH_3 donor for IMP reamination. In the present study, 2,441

and 3,581 μmol/kg NH_3 plus glutamine were produced by the control and BCAA trials, respectively. Because one aspartate is needed to form one NH_3, it would require ~11.1 and 16.8 mmol aspartate/kg dry muscle to form all of the NH_3 produced by the control and BCAA trials, respectively. The intramuscular aspartate levels in the present study were not significantly altered with exercise and are eightfold lower than what is needed for PNC cycling. Furthermore, there was no substantial exchange of aspartate with the circulation, as indicated by the total release of only 24 ± 22 and 45 ± 17 μmol/kg wet wt for the control and BCAA trials, respectively. Similarly, it is unlikely that the aspartate was produced from endogenous protein breakdown, since there were no significant shifts in the intramuscular TAA pools and since only 6.49 ± 1.57 and 5.98 ± 0.96 mmol/kg wet wt TAA were released over the 60 min of exercise for the control and BCAA trials, respectively. Therefore, it seems virtually impossible that enough aspartate would be available for the PNC to produce the NH_3 observed for the control trial, let alone the BCAA trial. From the above discussion, it is reasonable to suggest that any significant PNC cycling was unlikely or at the very least minimal and constant during the exercise bouts. Thus the potential contribution to NH_3 production from PNC cycling in a net sense was not significant under these circumstances. Therefore, the role of the PNC in NH_3 production in this study was most likely limited to the NH_3 produced through IMP accumulation.

There are six amino acids that can be oxidized by skeletal muscle (aspartate, glutamate, alanine and the three BCAA, isoleucine, leucine, and valine). Of these, the BCAA are the amino acids primarily oxidized in human skeletal muscle (24). The first step in the catabolism of BCAA involves the removal of the NH_3 group by transamination with 2-oxoglutarate (2-OG) to form glutamate and branched-chain keto acid (BCKA) catalyzed by BCAA aminotransferase. Glutamate can then be oxidatively deaminated by glutamate dehydrogenase, releasing the NH_3 and reforming 2-OG. Both of these reactions are near equilibrium and, when coupled, form a transdeamination reaction that has been suggested to be the primary pathway for BCAA deamination in skeletal muscle (24). The second step in BCAA catabolism involves the decarboxylation of the BCKA by branched-chain keto acid dehydrogenase (BCKADH). This step is nonreversible and constitutes the rate-limiting step in BCAA oxidation.

It is well established that amino acid oxidation increases during exercise (6, 11, 32). Furthermore, both rodent and human studies have shown that the BCKADH complex is significantly activated by exercise (16, 28). In the present study, the control leg effluxed a total of 816 μmol/kg BCAA while demonstrating an intramuscular increase of 700 μmol/kg dry muscle (~150 μmol/kg wet wt). Furthermore, the remaining EAA (EAA − BCAA) also demonstrated a significant efflux and increase in intramuscular concentrations. This suggests that protein catabolism was occurring and that a considerable amount of BCAA could have been available for deamination and oxidation. In fact, the muscle efflux of BCAA was only 300 μmol/kg less than what would be required to produce all of the NH_3 for the control trial. Therefore, it is possible that a substantial portion of the NH_3 produced during the control trial was from the metabolism of BCAA.

In the present study, an oral dose of 77 mg/kg BCAA was administered to alter the arterial plasma BCAA levels before the onset of exercise. It has been demonstrated that ingested BCAA selectively escape uptake by the liver (30) due to a low BCAA aminotransferase activity in the liver (8). In the present study, the arterial plasma BCAA levels were more than doubled by the onset of exercise and are similar to venous BCAA levels previously observed for the same dose (21). Previous studies have shown that, when arterial BCAA levels were elevated, skeletal muscle was the primary tissue responsible for their removal (7). The BCAA trial demonstrated a significant uptake of BCAA before exercise and overall only released 68 μmol/kg BCAA over 60 min of exercise. This translated into a significant increase in the intramuscular BCAA levels not only before exercise but throughout the experiment. Because the transdeamination reactions are near equilibrium, any increase in the intramuscular BCAA concentration should promote NH_3 production. Similarly, the BCKADH complex has been shown to be sensitive to substrate concentrations (1). Therefore the significantly higher intramuscular BCAA levels during the BCAA trial should promote a greater production of NH_3 and BCKA. This in turn would result in a greater activation of the BCKADH complex and BCKA utilization. This would further serve to remove one of the products of the transdeamination reaction and would help to keep the reactions moving in the direction of NH_3 production. Thus the greater BCAA availability during the BCAA trial most likely resulted in greater BCAA utilization and NH_3 production.

The efflux of glutamine during the BCAA trial was significantly greater than control at 5 and 15 min. The total release of glutamine was again consistently larger for the BCAA for all subjects but was not found to be significant. When the total release of NH_3 and glutamine was summed, the combined release was significantly greater for the BCAA trial compared with control. On the other hand, when the total release of NH_3, glutamine, and alanine was summed, the BCAA trial demonstrated a larger release for each subject but was not significantly different. Despite the lack of significance, it is clear that exercise after BCAA supplementation puts a much greater NH_3 load on the muscle than during the control experiments. Furthermore, the amount of NH_3 (amino nitrogen + NH_3) produced after amino acid supplementation is more than doubled when the amino nitrogen contributions from alanine and glutamine are included in the calculation. Overall, these data strongly suggest that BCAA are a substantial source of NH_3 production during exercise.

The formation of alanine involves the transamination of an NH_3 group from glutamate to pyruvate. By calculating total glucose uptake and glycogen utilization, an estimate of total pyruvate availability can be

made. From this, the percent of the pyruvate that went into lactate and alanine formation can be estimated. In the control trial, 0.6 ± 0.2 and 32.0 ± 11.2% of the pyruvate went into the formation of alanine and lactate, respectively. In the BCAA trial, 0.8 ± 0.1 and 21.3 ± 1.5% of the pyruvate went into the formation of alanine and lactate, respectively, and there were no significant differences between trials. These data suggest that a 77 mg/kg dose of BCAA does not significantly alter the overall distribution of pyruvate into lactate and alanine compared with control. Furthermore, it is evident that very little of the pyruvate made available during exercise goes toward alanine formation.

It is generally accepted that, during prolonged exercise, there is a net breakdown of whole body protein accomplished by a decrease in the rate of protein synthesis and an increase in the rate of protein degradation in the liver (6) as well as a net increase in the rate of noncontractile protein breakdown in muscle (17). In the present study, the control trial demonstrated a significant increase in the efflux of the EAA, BCAA, and EAA minus BCAA during exercise. Despite the elevated efflux, the same trial illustrated a significant increase in the intramuscular EAA, BCAA, and EAA minus BCAA pools after 60 min of exercise. Because the EAA cannot be synthesized to any significant extent by the body, an elevation in their concentration suggests an increase in net muscle protein degradation. From the EAA efflux and intramuscular data, it is clear that a considerable amount of protein degradation was occurring during the control trial. After BCAA supplementation, the efflux of EAA minus BCAA was suppressed throughout the experiment, whereas the arterial EAA minus BCAA levels were significantly lower from 30 to 60 min compared with control. Similar findings have been reported during BCAA infusion in humans at rest (19), and thus it is noteworthy to point out that an oral supplement can produce results analogous to infusion. However, in the present study, exercise was added and intramuscular determinations were also made. The intramuscular EAA minus BCAA levels were significantly lower than control at 60 min. Overall, in the BCAA trial the active muscle released significantly lower quantities of EAA minus BCAA (531 ± 70 μmol/kg) than control (924 ± 148 μmol/kg) over 60 min of exercise. These data strongly suggest that BCAA supplementation before exercise suppresses the rate of net muscle protein breakdown normally observed during exercise of this intensity and duration.

As mentioned above, studies have reported that muscle protein degradation occurs primarily in noncontractile protein while contractile protein degradation is spared or even decreased (6, 17). In the present study, there were no significant elevations in the flux of 3-methylhistidine (a marker of contractile protein degradation) from muscle for either trial. There were also no significant shifts in the intramuscular 3-methylhistidine pools for either trial. These data suggest that the degree of contractile protein degradation was not increased during exercise for either trial. From the EAA minus BCAA data, it is clear that endogenous muscle protein degradation was occurring, and the present data support the contention that it was of noncontractile nature.

In summary, this study clearly shows that BCAA supplementation results in significantly greater muscle NH_3 (less NH_3 formation from IMP accumulation) production during exercise. Furthermore, BCAA supplementation imposes a substantial NH_3 load on muscle, as indicated by the consistently larger total alanine and glutamine releases observed during exercise. The elevated BCAA levels also suppress the degree of net muscle protein degradation that normally occurs during exercise of this magnitude and duration. Overall, these data demonstrated that BCAA can be a significant source of NH_3 during submaximal exercise.

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Address for reprint requests: D. A. MacLean, School of Human Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

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