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Research Communications

Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers

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The present study was performed to test the hypothesis that orally administered essential amino acids, in combination with carbohydrate, will stimulate net muscle protein synthesis in resting human muscle in vivo. Four volunteers ingested 500 mL of a solution containing 13.4 g of essential amino acids and 35 g sucrose (EAA). Blood samples were taken from femoral arterial and venous catheters over a 2-hour period following the ingestion of EAA to measure arteriovenous concentrations of amino acids across the muscle. Two muscle biopsies were taken during the study, one before administration of the drink and one approximately 2 hours after consumption of EAA. Serum insulin increased from normal physiologic levels at baseline (9.2 ± 0.8 μU/mL) and peaked (48 ± 7.1 μU/mL) 30 minutes after EAA ingestion. Arterial essential amino acid concentrations increased approximately 100 to 400% above basal levels between 10 and 30 minutes following drink ingestion. Net nitrogen (N) balance changed from negative (−495 ± 128 nmol/mL) prior to consumption of EAA to a peak positive value (416 ± 140 nmol/mL) within 10 minutes of ingestion of the drink. EAA resulted in an estimated positive net N uptake of 307.3 mg N above basal levels over the 2-hour period. Muscle amino acid concentrations were similar prior to and 2 hours following ingestion of EAA. We conclude that ingestion of a solution composed of carbohydrates to stimulate insulin release and a small amount of essential amino acids to increase amino acid availability for protein synthesis is an effective stimulator of muscle protein anabolism. (J. Nutr. Biochem. 10: 89–95, 1999)

Keywords: oral amino acids; essential amino acids; nitrogen uptake

Introduction

Amino acid availability is an important determinant of muscle protein metabolism. Muscle protein synthesis requires a pool of free amino acids within the muscle cell as substrate. Protein breakdown and amino acid transport into the muscle contribute the free amino acids for this pool.[1] Therefore, the rate of muscle protein synthesis is related to the levels of muscle protein breakdown and/or inward amino acid transport.[1–3]

Administration of exogenous amino acids is one method of increasing amino acid availability for protein synthesis. Previously, we showed that intravenous infusion of amino acids increases inward amino acid transport at rest and after resistance exercise,[4] thus increasing intracellular amino acid concentrations and availability for protein synthesis. Accordingly, muscle protein synthesis has been shown to increase during intravenous infusion of a balanced mixture of amino acids at rest[4,5] and after exercise,[4] with no corresponding increase in protein breakdown.[4,6] Therefore, provision of exogenous amino acids appears to increase muscle protein anabolism.

Although it is clear that infusion of amino acids will increase the anabolic potential of muscle, infusion is not a practical means of administering amino acids for most population groups. Oral supplementation of amino acids is a more commonly used method of delivery. However, it is

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not clear whether oral administration of amino acids has the same anabolic effect on muscle as intravenous infusion. Further, palatability of the supplement may be enhanced by minimizing the total quantity of amino acids. Because nonessential amino acids should be readily available in plasma and the intracellular muscle space, we hypothesize that only essential amino acids are necessary in any supplement designed to stimulate muscle protein anabolism. The total ingested amount of amino acids necessary to elicit an anabolic response can thus be reduced to a minimum by eliminating the nonessential amino acids.

Carbohydrates are typically included, for palatability as well as metabolic reasons, in any supplement or meal designed to increase muscle protein anabolism. The anabolic response of muscle protein metabolism to elevated levels of insulin is well documented. Amino acid release from muscle protein is decreased during hyperinsulinemia, with no apparent change in amino acid uptake into muscle.[7–9] The lack of a change in amino acid uptake with hyperinsulinemia is likely due to hypoaminoacidemia that develops in response to insulin.[10] Thus, a simultaneous intake of amino acids and carbohydrate may result in the optimal anabolic response by muscle. In the present study we determined the response of net muscle protein synthesis in subjects given a mixture of essential amino acids and carbohydrates.

This study was performed to determine whether orally administered essential amino acids in combination with carbohydrate will stimulate net muscle protein synthesis in resting human muscle in vivo.

Methods and materials

Subjects

Four healthy volunteers, two males and two females, participated in the study in the postabsorptive state. The mean ± SEM age was 24.2 ± 2.4 years, body weight was 72.2 ± 11.6 kg, height was 1.76 ± 0.05 m, and body mass index was 23 ± 3 kg/m^2. Leg volume, measured using an anthropometric approach,[2] was 10.821 ± 1.295 L. All subjects were in good health as determined by medical history, physical examination, routine blood and urine tests, and electrocardiogram. The study design, purpose, and possible risks were carefully explained to each volunteer before obtaining written consent. The experimental protocol was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston (UTMB; Galveston, TX).

Study design

Each subject was studied after an overnight fast during an overnight stay at the General Clinical Research Center of UTMB. At approximately 7:00 AM two 8-cm polyethylene catheters were inserted into the femoral artery and femoral vein for blood sampling across the leg tissues.

A blood sample was obtained for baseline amino acid and insulin levels, and a muscle biopsy was taken to measure baseline intracellular amino acid concentrations. The biopsy was taken from the lateral portion of the vastus lateralis muscle, approximately 20 cm above the knee. Using sterile technique, the skin and subcutaneous tissue were anesthetized and an incision of approximately 6 mm was made. A 5-mm Bergström biopsy needle (Depuy, Warsaw, IN USA) was advanced 3 to 5 cm through the fascia deep into the middle of the muscle with the cutting window closed. With suction applied, the cutting cylinder was opened and then closed twice. A sample of approximately 30 to 50 mg of mixed muscle tissue was obtained with each biopsy. Each sample was blotted dry, and blood, visible fat, and connective tissue were quickly removed. The tissue was immediately frozen and stored at −80°C until later processing and analysis.

Following baseline blood and muscle sampling, a bolus 500 mL solution of 13.4 g of essential amino acids and 35 g of sucrose (EAA) was consumed as quickly as possible. Arteriovenous (AV) blood samples were obtained 10, 30, 45, 60, 90, and 120 minutes following ingestion of the solution. Blood samples were obtained from the artery 30, 60, 90, and 120 minutes for insulin levels. A second muscle biopsy was obtained 120 minutes following ingestion of EAA.

EAA solution

The 500-mL EAA solution consumed by each subject contained 35 g of sucrose and 13.4 g of essential amino acids. The amino acid composition (g and mol, respectively) of the drink was: histidine 1.6, 0.0103; isoleucine 1.1, 0.0084; leucine 2.4, 0.0183; lysine 1.6, 0.0109; methionine 0.5, 0.0034; phenylalanine 2.0, 0.0121; threonine 2.1, 0.0176; and valine 2.1, 0.0184. Sucrose and amino acids were mixed in 500 mL of distilled, deionized water and an artificial sweetener (Crystal Light, Kraft, White Plains, NY USA) was added to increase palatability.

Analysis

Arterial insulin levels were determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA USA). Standards, controls, and samples were added to the appropriate antibody-coated tubes. After the addition of ^{125}I-labeled hormone, tubes were incubated for 24 hours at room temperature, the liquid aspirated, and the tubes placed in a gamma counter to determine counts per minute. Intraassay coefficient of variation (CV) was 1.64%.

Free amino acid concentrations in blood were determined from plasma samples by high performance liquid chromatography (HPLC). Fifty microliters of plasma were diluted 1:20 with distilled, deionized water, and 20 μL of a norvaline internal standard was added. The sample was deproteinized by ultrafiltration using hydrophilic membranes with a molecular weight cutoff of 5,000 D and centrifuged for 2 hours at 5,000 g. The resulting supernatant was analyzed for free amino acid concentration by HPLC and pre-column derivatization with ortho-pthalaldehyde (OPA) and 3 mecaptoproprionic acid.

Muscle tissue samples were analyzed for free intracellular amino acid concentrations. Each sample was cut into 10-mg pieces, placed in 400 μL of a 5% perchloric acid (PCA) solution mixed with 20 μL of norvaline internal standard, and allowed to stand on dry ice for approximately 1 hour. The sample was then homogenized for approximately 5 minutes, centrifuged, the supernatant transferred to a tube containing 70 μL of 5 N KOH, and recentrifuged. One hundred microliters of the supernatant were then deproteinized by ultrafiltration, as were the plasma samples described above, and analyzed for amino acid concentration by HPLC. The muscle was weighed prior to processing and again following 24 hours in a drying oven at 60°C. The difference in weight was used to calculate muscle water.

The HPLC separation of the amino acids was performed on a Waters 2960 system (Waters Corp., Milford, MA USA) with a Zorbax SB-C_{18} column (3.0 × 150 mm inner diameter, 3.5 μm particle size) and a multiple step gradient elution from 100% solvent A (0.05 M CH_3COONa, 0.05 M Na_2HPO_4, pH 7.2: CH_3OH:THF, 96:2:2) to 100% solvent B (CH_3OH:H_2O, 65:35). Detection was by a Waters 420-AC fluorescence detector (Waters Corp.) at excitation and emission wavelengths of 338 and 455 nm, respectively.

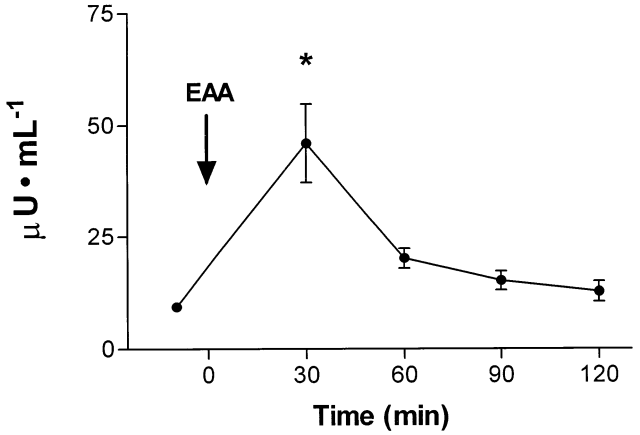

Figure 1 Plasma insulin concentrations at baseline (−10 minutes) and for 120 minutes following ingestion of the essential amino acid solution. Data are means ± SE, $N = 4$. *Significantly different from baseline ($P < 0.05$).

Statistical analysis

Data are expressed as mean ± SE. Results across time were compared by repeated measures analysis of variance using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA USA) with significance indicated by a *P*-value of less than 0.05. When the overall *P*-value was significant, Dunnett's post-hoc test was used to determine individual differences between basal and each time point following ingestion of EAA.

Results

Plasma insulin concentrations

Insulin concentrations in plasma were significantly ($P < 0.05$) increased by fivefold 30 minutes following ingestion of the drink (*Figure 1*). By 60 minutes following EAA ingestion, the plasma insulin values were not significantly different from basal levels.

Plasma amino acid concentrations and AV differences

Within 10 minutes of ingestion of the drink, arterial plasma concentrations of the ingested essential amino acids were increased significantly (*Table 1*). The cumulative arterial concentration of the essential amino acids peaked to 215% above the basal level at 30 minutes. Individually, the arterial concentrations of methionine, isoleucine, and leucine

Table 1 Arterial and venous concentrations and arteriovenous difference (A-V) of amino acids ingested in EAA solution

		Time from drink (min)						
AA		−10	10	30	45	60	90	120
His	A	55 ± 14	83 ± 16	164 ± 29†	169 ± 43†	141 ± 33†	107 ± 16	106 ± 25
	V	77 ± 10	88 ± 7	158 ± 12†	158 ± 2†	139 ± 24†	105 ± 11	99 ± 14
	A-V	−22 ± 5	−5 ± 10	6 ± 23	11 ± 22	2 ± 17	2 ± 8	6 ± 11
Thr	A	54 ± 20	144 ± 35	254 ± 53†	266 ± 81†	210 ± 39*	195 ± 28*	183 ± 30*
	V	85 ± 14	109 ± 14	196 ± 21†	187 ± 29†	184 ± 28†	168 ± 20†	166 ± 26†
	A-V	−31 ± 7	35 ± 24	56 ± 40	78 ± 56	26 ± 26	27 ± 17	16 ± 8
Val	A	186 ± 19	486 ± 24†	559 ± 19†	546 ± 54†	475 ± 25†	436 ± 33†	364 ± 50†
	V	191 ± 22	304 ± 13†	450 ± 21†	419 ± 28†	400 ± 41†	351 ± 21†	335 ± 51†
	A-V	−5 ± 5	182 ± 27†	109 ± 12†	127 ± 30†	75 ± 21*	85 ± 22*	29 ± 8
Met	A	25 ± 3	78 ± 5†	74 ± 7†	65 ± 8†	58 ± 6†	52 ± 7†	40 ± 6*
	V	28 ± 3	53 ± 4†	69 ± 7†	62 ± 8†	56 ± 7†	52 ± 6†	45 ± 6†
	A-V	−2 ± 1	25 ± 5†	6 ± 4	3 ± 6	2 ± 1	0 ± 8	−6 ± 4
Phe	A	52 ± 1	179 ± 20†	199 ± 4†	174 ± 5†	158 ± 10†	151 ± 18†	124 ± 20†
	V	56 ± 1	105 ± 7†	164 ± 7†	149 ± 4†	146 ± 11†	133 ± 10†	126 ± 18†
	A-V	−3 ± 2	74 ± 16†	35 ± 7†	25 ± 5*	12 ± 6	18 ± 8	−2 ± 3
Ile	A	59 ± 7	212 ± 6†	205 ± 9†	171 ± 15†	166 ± 29	111 ± 14	84 ± 17
	V	62 ± 7	121 ± 7†	162 ± 10†	136 ± 11†	117 ± 14†	89 ± 8*	78 ± 17
	A-V	−3 ± 3	90 ± 9†	43 ± 4	34 ± 6	49 ± 30*	23 ± 9	7 ± 3
Leu	A	118 ± 12	473 ± 19†	450 ± 15†	368 ± 23†	312 ± 24†	264 ± 32†	211 ± 44*
	V	122 ± 12	258 ± 17†	353 ± 20†	302 ± 21†	266 ± 32†	211 ± 19†	188 ± 38*
	A-V	−4 ± 6	215 ± 21†	96 ± 11†	66 ± 10†	47 ± 11*	53 ± 19*	23 ± 11*
Lys	A	161 ± 36	300 ± 39*	378 ± 79*	311 ± 80*	306 ± 87*	283 ± 80*	196 ± 40
	V	145 ± 29	200 ± 33	286 ± 50†	263 ± 55†	248 ± 53†	212 ± 40*	182 ± 33
	A-V	15 ± 18	100 ± 12	92 ± 39	47 ± 57	58 ± 41	72 ± 50	14 ± 18
Ess AA	A	710 ± 97	1,954 ± 60†	2,283 ± 179†	2,069 ± 244†	1,826 ± 184†	1,599 ± 175†	1,308 ± 212*
	V	766 ± 95	1,235 ± 63†	1,832 ± 63†	1,671 ± 169†	1,554 ± 195†	1,314 ± 109†	1,216 ± 181*
	A-V	−45 ± 21	740 ± 108†	406 ± 109†	350 ± 105†	276 ± 74*	370 ± 76*	119 ± 46
EssAA-BCAA	A	347 ± 60	783 ± 71†	1,069 ± 142†	984 ± 158†	873 ± 128†	787 ± 106†	648 ± 108
	V	392 ± 54	552 ± 48	867 ± 85†	814 ± 112†	772 ± 113†	663 ± 68†	615 ± 84†
	A-V	−49 ± 15	202 ± 35†	171 ± 81†	141 ± 62†	93 ± 24†	102 ± 71†	44 ± 41

Values are means ± SE; n = 4 subjects in nmol · mL^{-1}.
*Significantly different from −10 min (basal) value, $p < 0.05$.
†Significantly different from −10 min (basal) value, $p < 0.01$.
AA, amino acid; His, histidine; Thr, threonine; Val, valine, Met, methionine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; Lys, lysine. Ess AA, sum of essential amino acids in EAA solution; EssAA–BCAA, sum of the essential amino acids minus the branched-chain amino acids.

peaked by 10 minutes postingestion (209%, 259%, and 302% greater than basal levels, respectively). Branched-chain amino acids (BCAA) are the only essential amino acids that are oxidized in muscle.

The levels of essential amino acids minus the BCAA significantly increased ($P < 0.01$) by 10 minutes postingestion and peaked by 30 minutes to approximately 300% above baseline. Arterial concentrations of lysine, phenylalanine, and valine peaked to 135%, 280%, and 200%, respectively, above preingestion levels 30 minutes following the ingestion. Histidine and threonine concentrations peaked 45 minutes following ingestion at 209% and 392%, respectively, above resting levels.

Venous concentrations of all essential amino acids were also dramatically increased after ingestion of the drink, but the peak values were not as great as those in the arterial plasma (*Table 1*). The cumulative peak value for the essential amino acids was 139% above the basal level at 30 minutes postingestion. Thus, the cumulative AV difference for the essential amino acids switched from negative to positive (i.e., from release to uptake) within 10 minutes following ingestion of EAA. The concentration of essential amino acids minus BCAA changed significantly by 30 minutes and peaked at approximately 220%. Individually, all essential amino acids except lysine had negative AV differences prior to drink ingestion. AV differences of all essential amino acids changed to positive values; however, the changes in histidine, threonine, and lysine were not statistically significant ($P = 0.49$, 0.08, and 0.06, respectively). The AV difference of the essential amino acids minus the BCAA was significantly ($P < 0.01$) increased to positive levels within 10 minutes of EAA ingestion and stayed elevated above baseline for 90 minutes. Because these amino acids cannot be oxidized in muscle, the change to positive is indicative of net protein synthesis.

Table 2 shows the arterial, venous, and AV differences of the nonessential amino acids and tryptophan not provided in the ingested EAA solution and the cumulative total concentration and AV difference of all measured amino acids. Arterial concentration of the cumulative nonessential amino acids was increased 45 minutes after ingestion of the EAA and peaked at 29% above basal levels 60 minutes postingestion. Individually, arterial concentrations of only alanine and tyrosine were greater following EAA ingestion than at baseline. Alanine concentration was increased 30 minutes after ingestion of EAA and peaked to 175% greater than basal values at 45 minutes. The increase in arterial alanine concentration accounted for nearly all of the increase in the cumulative nonessential amino acid concentration.

There was no significant change in the venous concentration or the AV difference of cumulative nonessential amino acids (*Table 2*). Individually, the only statistically significant changes in venous amino acid concentrations were an increase in alanine and tyrosine and a decrease in glycine. Although the mean value for the AV difference of alanine tended to increase and reached a positive value of 1 ± 25 at 45 minutes, there was no statistically significant change in the AV difference of alanine or any other individual nonessential amino acid except glycine. The drop in the venous concentration of glycine resulted in a significant increase in AV difference, but positive values were never reached.

The cumulative total plasma concentration of amino acids increased significantly in both the vein and artery (*Table 2*). Total amino acid concentration peaked at 30 minutes in both the artery and the vein. Arterial concentration peaked 101% above basal in the artery and 57% above basal in the vein. Thus, the AV difference of the total amino acids switched from negative preingestion to positive 10 minutes after EAA ingestion.

Amino N concentrations and AV differences

Figure 2 shows the AV difference of total, essential, and nonessential amino N from 10 minutes prior to EAA ingestion to 120 minutes post-EAA ingestion. Following ingestion of EAA, there was no statistically significant change in nonessential amino N from basal. Essential amino N and thus total amino N changed significantly from negative values to positive within 10 minutes of EAA ingestion and remained positive until 120 minutes post-EAA ingestion.

Muscle nonessential and essential amino acid concentrations

The muscle free nonessential amino acid and essential amino acid concentrations are shown in *Figure 3*. There was no change in the muscle free nonessential amino acid concentration from prior to ingestion of EAA to 120 minutes following ingestion. The essential amino acid concentration was significantly ($P < 0.05$) increased by 25% from preingestion to 120 minutes postingestion of EAA. The total sum of all amino acids was not significantly ($P = 0.53$) changed from baseline at 120 minutes postingestion (19,769 and 17,983 nmol/mL intracellular water, repectively).

Discussion

The purpose of the present study was to determine if a solution composed of only essential amino acids plus carbohydrate (EAA) would effectively stimulate muscle protein anabolism. We found that within 30 minutes of ingestion of the EAA solution, insulin levels were increased and there was a dramatic increase in plasma concentrations of essential and total amino acids. AV differences of essential and total, but not nonessential, amino acid N changed from negative to positive, indicating a change from net release to net uptake of the essential and the total amino N across the leg. Because there was no significant change in the total intracellular amino acid concentration, this corresponded to a significant stimulation of net muscle protein synthesis. Further support for a stimulation of net protein synthesis comes from the fact that the AV difference of essential amino acids minus BCAA changes from negative to positive (*Table 1*). BCAA are the only essential amino acids that can be oxidized in muscle. Therefore, even if we assume that 100% of the BCAA were oxidized in the muscle, the net uptake of the essential amino acids minus the BCAA, combined with no increase in the intracellular

Table 2 Arterial and venous concentrations and arteriovenous difference of nonessential amino acids and tryptophan ingested in EAA solution

AA		Time from drink (min)						
		−10	10	30	45	60	90	120
Ala	A	133 ± 41	214 ± 47	340 ± 47†	366 ± 63†	359 ± 53†	338 ± 49†	310 ± 38†
	V	233 ± 38	263 ± 36	358 ± 38†	365 ± 51†	381 ± 44†	372 ± 48†	371 ± 33†
	A-V	−100 ± 22	−49 ± 27	−19 ± 33	1 ± 25	−22 ± 26	−35 ± 29	−61 ± 29
Glu	A	75 ± 14	77 ± 8	64 ± 6	67 ± 10	61 ± 3	58 ± 5	62 ± 13
	V	19 ± 3	16 ± 2	19 ± 5	18 ± 2	16 ± 3	14 ± 2	19 ± 7
	A-V	56 ± 15	61 ± 7	45 ± 3	54 ± 7	45 ± 3	44 ± 3	43 ± 6
Gln	A	499 ± 21	517 ± 23	547 ± 23	561 ± 23	588 ± 21	588 ± 12	566 ± 16
	V	629 ± 86	662 ± 85	664 ± 75	633 ± 82	665 ± 81	629 ± 87	619 ± 79
	A-V	−130 ± 25	−145 ± 40	−117 ± 13	−72 ± 17	−77 ± 16	−41 ± 33	−53 ± 40
Gly	A	112 ± 65	117 ± 61	124 ± 54	110 ± 36	137 ± 47	151 ± 44	174 ± 37
	V	234 ± 51	232 ± 51	197 ± 42	176 ± 39*	181 ± 39*	176 ± 43*	204 ± 33
	A-V	−122 ± 21	−115 ± 24	−73 ± 15	−66 ± 14*	−44 ± 9†	−26 ± 12†	−30 ± 4†
Asp	A	4 ± 0.3	4 ± 0.2	3 ± 0.4	3 ± 0.7	4 ± 0.6	4 ± 0.5	4 ± 0.4
	V	2 ± 0.3	2 ± 0.1	2 ± 0.1	2 ± 0.3	2 ± 0.1	2 ± 0.4	2 ± 0.4
	A-V	2 ± 0.4	2 ± 0	1 ± 0.3	1 ± 0.8	2 ± 0.5	3 ± 0.5	2 ± 0.4
Asn	A	47 ± 6	39 ± 6	39 ± 7	43 ± 13	36 ± 7	31 ± 6	31 ± 7
	V	37 ± 8	41 ± 7	42 ± 8	38 ± 8	39 ± 7	33 ± 8	33 ± 7
	A-V	10 ± 7	−2 ± 3	−3 ± 2	5 ± 6	−2 ± 1	−2 ± 3	−2 ± 2
Ser	A	65 ± 6	63 ± 6	62 ± 7	59 ± 13	54 ± 7	43 ± 6	41 ± 7
	V	36 ± 14	44 ± 16	48 ± 17	43 ± 15	39 ± 13	36 ± 12	38 ± 9
	A-V	28 ± 13	19 ± 10	13 ± 9	15 ± 9	15 ± 10	7 ± 5	3 ± 10
Cit	A	24 ± 5	21 ± 3	20 ± 3	20 ± 3	24 ± 5	21 ± 3	22 ± 2
	V	25 ± 2	23 ± 3	21 ± 2	21 ± 2	21 ± 3	19 ± 3	21 ± 3
	A-V	−2 ± 3	−2 ± 1	−1 ± 1	−1 ± 1	3 ± 2	2 ± 1	1 ± 1
Arg	A	68 ± 17	75 ± 14	72 ± 13	62 ± 9	67 ± 14	61 ± 13	62 ± 13
	V	74 ± 15	79 ± 15	78 ± 15	73 ± 15	73 ± 16	64 ± 15	62 ± 13*
	A-V	−5 ± 6	−4 ± 6	−6 ± 2	−11 ± 8	−6 ± 2	−4 ± 5	0 ± 7
Tyr	A	50 ± 5	69 ± 6†	76 ± 7†	77 ± 8†	75 ± 6†	69 ± 5†	59 ± 5
	V	58 ± 8	66 ± 7	74 ± 8†	73 ± 8†	73 ± 7†	65 ± 6	63 ± 6
	A-V	−8 ± 3	3 ± 2*	2 ± 1*	3 ± 2*	1 ± 3	4 ± 4*	−4 ± 2
Trp	A	30 ± 2	32 ± 3	26 ± 2	23 ± 3	21 ± 1*	18 ± 2†	15 ± 4†
	V	28 ± 1	31 ± 2	26 ± 1	23 ± 4	21 ± 1*	16 ± 1†	15 ± 2†
	A-V	1 ± 2	1 ± 2	0 ± 1	−1 ± 3	1 ± 0	2 ± 3	1 ± 2
Noness AA	A	1,105 ± 238	1227 ± 232	1,372 ± 219	1,392 ± 224*	1,426 ± 222*	1,381 ± 201*	1,345 ± 176
	V	1,375 ± 212	1,459 ± 210	1,530 ± 188	1,462 ± 209	1,509 ± 198	1,426 ± 214	1,447 ± 173
	A-V	−269 ± 86	−238 ± 112	−167 ± 69	−72 ± 41	−97 ± 57	21 ± 68	−86 ± 100
Total AA	A	1,814 ± 327	3,182 ± 275†	3,655 ± 377†	3,461 ± 447†	3,253 ± 346†	2,980 ± 270†	2,653 ± 334*
	V	2,141 ± 299	2,694 ± 261*	3,362 ± 281†	3,133 ± 354†	3,064 ± 331†	2,740 ± 255*	2,663 ± 281*
	A-V	−326 ± 76	488 ± 93†	293 ± 135†	327 ± 112†	189 ± 91*	240 ± 178†	−10 ± 112

Values are means ± SE; n = 4 subjects in nmol · mL^{-1}.
*Significantly different from −10 min (basal) value, $p < 0.05$.
†Significantly different from −10 min (basal) value, $p < 0.01$.
AA, amino acid; A, arterial concentration; V, venous concentration; A-V, arteriovenous difference; Ala, alanine; Glu, glutamate; Gln, glutamine; Gly, glycine; Asp, aspartate; Asn, asparagine; Ser, serine; Cit, citrulline; Arg, arginine; Tyr, tyrosine; Trp, tryptophan; Noness AA, sum of nonessential amino acids plus tryptophan not in EAA solution; Total AA, sum of all measured amino acids.

pool, supports the assertion that there was net protein synthesis.

These results indicate that an orally administered solution containing carbohydrates and 13.4 g of essential amino acids stimulates net uptake of amino acids across the leg in resting volunteers. It is not surprising that nonessential amino acid concentrations did not change, because none were ingested. The maintenance of resting concentrations while net uptake of essential amino acids were stimulated indicates that de novo synthesis of nonessential amino acids can keep pace with an accelerated rate of incorporation into protein. Thus, it is not necessary to include nonessential amino acids in a solution composed of amino acids and carbohydrates designed to stimulate muscle anabolism.

From the present results it is not possible to determine whether the change from net release to net uptake of amino N was due to an increase in protein synthesis, a decrease in protein breakdown, or a combination of the two. Previously, we have shown that an infusion of mixed amino acids increased protein synthesis with no change in protein breakdown.[4] Others have demonstrated an increase in protein synthesis[5,11,12] and an amelioration of protein breakdown[6,12] with amino acid infusions. Giordano et al.[11] concluded that muscle protein synthesis responds in a dose-dependent manner to hyperaminoacidemia. That is, increased amino acid levels in the physiologic range decreased muscle protein breakdown, whereas higher levels of hyperaminoacidemia (two times the basal level) increased muscle protein synthesis. In our study, total amino acid levels were increased twofold within 30 minutes of EAA ingestion (*Table 2*). Thus, it is possible that muscle protein synthesis played a role in the switch from net catabolism to

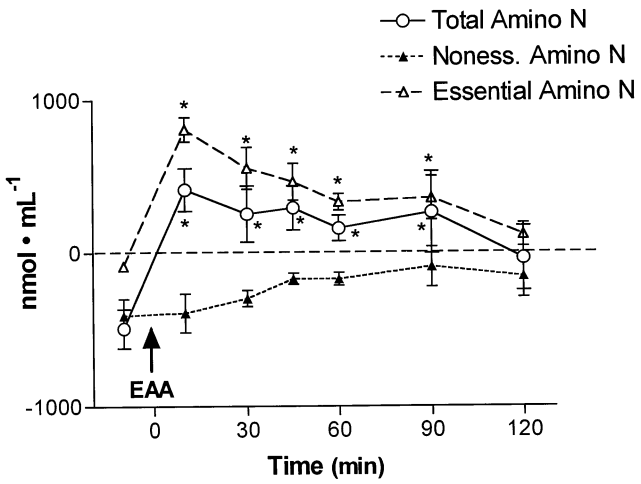

Figure 2 Arteriovenous difference of amino N for 120 minutes following ingestion of essential amino acid solution. Data are means ± SE. *Significantly different from basal level ($P < 0.05$).

net anabolism in the muscle. Further, when hyperaminoacidemia was associated with increased insulin levels, as seen in the present study, protein synthesis increased and protein breakdown decreased.[13] Although it is not possible to determine the mechanism of the change from the current data on the basis of previous work, it is likely that the net response was due primarily to an increase in synthesis.

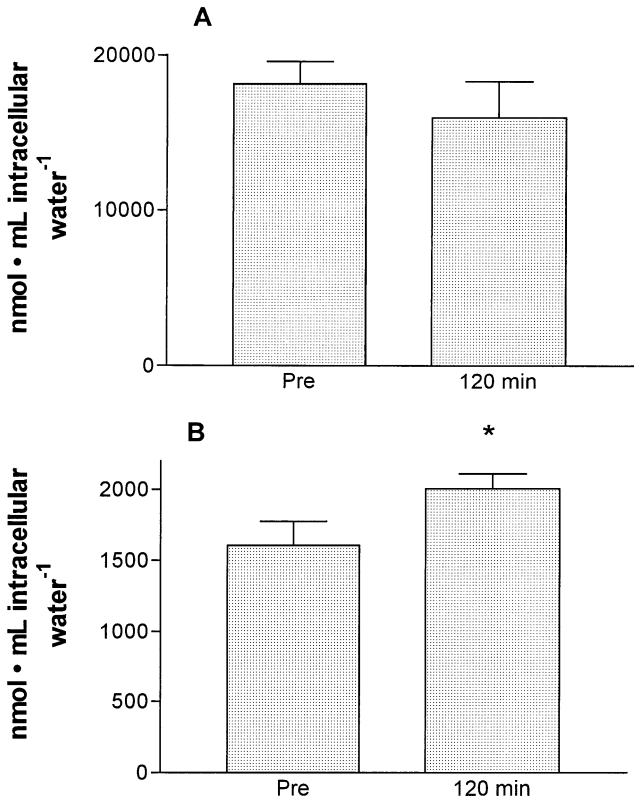

Figure 3 Muscle intracellular nonessential (*Figure 3A*) and essential (*Figure 3B*) amino acid concentration prior to (Pre) and 120 minutes following ingestion of essential amino acid solution. *Significantly different from Pre ($P < 0.05$).

Table 3 N uptake across the leg following ingestion of amino acid solution

Subject no.	Uptake across leg mg N	Ratio of uptake/ingested
1	278.2	0.15
2	387.1	0.21
3	301.1	0.16
4	262.8	0.14
Mean SE	307.3	0.17
	27.7	0.02

However, it is also possible that a decrease in breakdown also played a role.

Inasmuch as it is clear that consumption of the EAA solution resulted in a stimulation of N uptake by muscle, it is not certain the extent to which each of the components, essential amino acids or carbohydrates, contributed to the muscle anabolism. It is unlikely that the increased insulin levels from the carbohydrate ingestion were solely responsible for the anabolic response of muscle, because systemic hyperinsulinemia alone did not improve net balance, and it did not affect muscle protein synthesis or breakdown in the forearm or the leg.[7,14] Further, despite the absence of an effect in muscle, the systemic amino acid concentrations and whole body protein breakdown were reduced during hyperinsulinemia. Thus, insulin seems to have caused these responses by acting in nonmuscle tissues.[7,14] Insulin reduces the plasma amino acid concentration, thereby reducing amino acid availability. We have repeatedly demonstrated that amino acid availability is strongly related to protein synthesis.[1,2,4,15] Previously, we showed that hyperinsulinemia alone decreased the muscle intracellular concentration, thereby reducing the availability of amino acids for protein synthesis.[16] Hence, hyperinsulinemia seems to stimulate the synthetic capacity of muscle, but whether it is reflected in increased muscle protein synthesis depends on an abundant supply of amino acids. In the present study, however, the ingestion of the essential amino acids, along with the increased insulin levels, maintained the muscle intracellular concentrations of amino acids (*Figure 2*), thereby maintaining the availability of amino acids for protein synthesis. The combination of hyperinsulinemia and hyperaminoacidemia that resulted from EAA ingestion was likely responsible for the muscle anabolism.

Table 3 shows the individual values for each volunteer for the estimated uptake across the leg of amino N for 120 minutes following ingestion of the EAA solution, as well as the ratio of leg uptake to ingested amino N. The total amount of amino N ingested in the EAA drink was 1,832.0 mg and the mean value of the calculated amino N uptake was 307.3 mg N. If anything, we likely have underestimated the uptake of amino N across the leg. Although blood flow was not measured, we made this calculation by assuming a uniform resting blood flow of 250 mL · min^{-1} (unpublished observations). Because both hyperaminoacidemia[4] and hyperinsulinemia[16] have been shown to increase blood flow, we underestimated amino N uptake using 250 mL · min^{-1} as a constant value. It is interesting to note that, even with this underestimation, uptake of amino N across the leg

accounts for a large portion (approximately 17%) of the total N ingested in the EAA solution (*Table 3*). Further, if we assume that all amino acid uptake was due to muscle (i.e., that uptake by skin does not change with hyperaminoacidemia)[17] and that leg muscle accounts for 25% of total body muscle, we can then estimate the whole body muscle amino N uptake. Estimated amino N uptake averaged 1,229.2 mg (approximately 67%) of the total amino N ingested. It appears that the provision of amino acids in this form is selectively targeting the muscle.

The response of muscle protein metabolism to ingestion of oral solutions has not been widely studied. Previously, results from our lab[4] and others[5,6] demonstrated that increasing amino acid levels by amino acid infusion could effectively stimulate muscle protein anabolism. There is less evidence for the stimulation of muscle protein anabolism by an oral supplement. Thus, a further goal of the present study was to test the hypothesis that an oral supplement could also effectively stimulate muscle protein anabolism. In our previous study,[4] the concentrations of only five amino acids were measured. The uptake of these five amino acids—leucine, lysine, phenylalanine, alanine, and glutamine—calculated over a 2-hour period was 41.2 mg. We can compare uptake by muscle from amino acid infusion to an oral solution by also calculating the uptake of the same five amino acids from the present study using a constant value of leg blood flow of 250 mL/min. In the present study, the uptake of these five amino acids across the muscle tissue was 162.4 mg, which is fourfold greater than the uptake from amino acid infusion in our previous study.[4] When compared with the amount of amino acids given in each condition, the effectiveness of the EAA solution over the infusion is even more striking. In our previous study,[4] 1,484.9 mg of amino acid N of the five measured amino acids were given over a 2-hour period, whereas in the present study, 732.1 mg of amino acid N of these same five amino acids were ingested over the same time period. Thus, net muscle uptake was only 2.8% of the total amount of amino N from these five amino acids given when infused, but 22.2% when given orally along with carbohydrates. Because both the infusion[4] and the oral supplement dramatically increased plasma amino acid concentrations, and thus amino acid delivery to the muscle, there is no reason to believe that a supplement would increase muscle protein anabolism due to the fact that it is delivered orally. However, the insulin response to the orally ingested supplement (*Figure 1*) was much greater than that in our previous study during the amino acid infusion.[4] Therefore, the greater efficiency of uptake from the EAA solution over the infusion[4] was likely due to the insulin response to the carbohydrates that were included in the EAA solution.

Conclusion

The ingestion of a solution composed of carbohydrates to stimulate insulin release and a small amount of essential amino acids to increase amino acid availability for protein synthesis is an effective stimulator of muscle protein anabolism. Further, an effective reutilization of the endogenously provided nonessential amino acids seems to make it unnecessary to include nonessential amino acids in an orally ingested solution to stimulate muscle anabolism. The EAA solution would appear to be an effective supplement for individuals, such as the elderly or those recovering from chronic bedrest and/or disease, who would benefit from increased muscle protein anabolism.

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