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# An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein

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**Biolo, Gianni, Kevin D. Tipton, Samuel Klein, and Robert R. Wolfe.** An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E122–E129, 1997.—Six normal untrained men were studied during the intravenous infusion of a balanced amino acid mixture (~0.15 $g \cdot kg^{-1} \cdot h^{-1}$ for 3 h) at rest and after a leg resistance exercise routine to test the influence of exercise on the regulation of muscle protein kinetics by hyperaminoacidemia. Leg muscle protein kinetics and transport of selected amino acids (alanine, phenylalanine, leucine, and lysine) were isotopically determined using a model based on arteriovenous blood samples and muscle biopsy. The intravenous amino acid infusion resulted in comparable increases in arterial amino acid concentrations at rest and after exercise, whereas leg blood flow was 64 ± 5% greater after exercise than at rest. During hyperaminoacidemia, the increases in amino acid transport above basal were 30–100% greater after exercise than at rest. Increases in muscle protein synthesis were also greater after exercise than at rest (291 ± 42% vs. 141 ± 45%). Muscle protein breakdown was not significantly affected by hyperaminoacidemia either at rest or after exercise. We conclude that the stimulatory effect of exogenous amino acids on muscle protein synthesis is enhanced by prior exercise, perhaps in part because of enhanced blood flow. Our results imply that protein intake immediately after exercise may be more anabolic than when ingested at some later time.

stable isotopes; blood flow; phenylalanine; leucine; lysine; alanine

RESISTANCE EXERCISE leads to muscle hypertrophy because of increased net deposition of contractile proteins (10, 12), but the mechanisms underlying this anabolic response are uncertain. We recently reported that, during recovery from resistance exercise in the fasting state, muscle amino acid transport and protein turnover (i.e., synthesis and degradation) were accelerated. However, net protein balance, although improved, did not shift from negative to positive values (6). On the basis of these previous observations, we hypothesize that increased net muscle protein synthesis after exercise involves an interaction between exercise and nutritional factors.

Several studies suggest that amino acid availability plays an important role in the control of muscle protein kinetics. Intravenous amino acid infusion increases amino acid transport (14) and protein synthesis (2) and, possibly, decreases protein degradation (9) in skeletal muscle. Thus hyperaminoacidemia and postexercise recovery appear to have similar effects on amino acid transport and protein synthesis but opposite effects on protein degradation. Therefore, the present work was designed to evaluate the interactions between resistance exercise and hyperaminoacidemia and their effects on protein kinetics in skeletal muscle. We have infused a mixture of essential and nonessential amino acids in the resting state and after a standardized routine of vigorous resistance exercise. Rates of transport of selected amino acids (phenylalanine, leucine, lysine, and alanine) were measured in skeletal muscle and related to the changes in protein synthesis and degradation.

## METHODS

*Subjects.* Six male volunteers were studied in the postabsorptive state. All subjects were healthy at the time of the study, as shown by physical examination, electrocardiogram, blood count, plasma electrolytes, plasma glucose concentration, as well as liver and renal function. None were engaged in a regular exercise training program for ≥1 yr before the study. The mean age was 29 ± (SE) 5 yr, body weight was 73 ± 5 kg, height was 170 ± 3 cm, leg volume was 10,327 ± 657 ml, and body mass index was 25 ± 1 $kg/cm^2$. All subjects gave informed, written consent before participating in the study, which was approved by the Institutional Review Board of The University of Texas Medical Branch at Galveston, Texas. Each subject was studied twice. On the first occasion, the subject was studied at rest in the postabsorptive state and during amino acid infusion. On the second occasion, the subject was studied immediately after a resistance exercise workout during amino acid infusion. Five to ten days before the exercise study, subjects were familiarized with the exercise protocol, and their 10 and 12 repetition maximum (RM) was determined, 10 and 12 RM representing the maximum weight that a subject can lift for 10 and 12 repetitions, respectively. Their average 10 RM of the knee extensor muscles (both legs) was 62 ± 7 kg.

*Tracer infusions.* L-[1-$^{13}$C]leucine (99% enriched), L-[*ring*-$^{13}C_6$]phenylalanine (99% enriched), and L-[2,3,3,3-$^{2}H_4$]alanine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA); L-[1,2-$^{13}C_2$]leucine (99% enriched), L-[2-$^{15}$N]lysine (99% enriched), L-[1,2-$^{13}C_2$,6,6-$^{2}H_2$]lysine (98% enriched), L-[*ring*-$^{2}H_5$]phenylalanine (98% enriched), and L-[2,3,3,4,4-$^{2}H_5$]glutamine were purchased from Tracer Technologies (Somerville, MA). L[1-$^{13}$C]alanine (99% enriched) was purchased from Isotec (Miamisburg, OH).

*Resting study.* The study protocol is illustrated in Fig. 1. In each case the resting study was performed first. The subjects were admitted to the Clinical Research Center of The University of Texas Medical Branch at Galveston on the morning of the study at 0600 after an overnight fast. Polyethylene catheters were inserted into the left antecubital vein for infusion of labeled amino acids and into the right femoral artery and vein for blood sampling. The femoral arterial catheter was also used for the primed-continuous infusion of indocyanine green (Becton-Dickinson Microbiology Systems, Cockeysville, MD). Systemic concentrations of indocyanine green were measured in the right arterialized wrist vein that was cannulated with a 20-gauge polyethylene catheter.

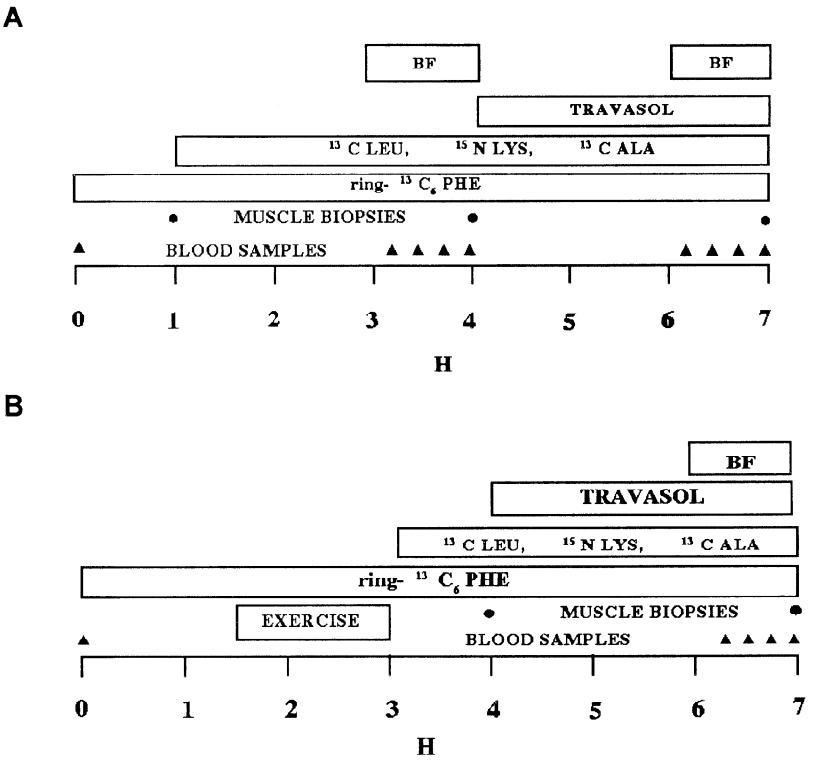


Fig. 1. Protocol for studying muscle amino acid kinetics in resting postabsorptive state in hyperaminoacidemia at rest (*A*) and during hyperaminoacidemia after exercise (*B*). BF, blood flow; $^{13}$C Leu, L-[1-$^{13}$C]leucine; $^{15}$N Lys, L-[2-$^{15}$N]lysine; $^{13}$C Ala, L-[1-$^{13}$C]alanine; *ring*-$^{13}C_6$ Phe, L-[*ring*-$^{13}C_6$]phenylalanine.

After a blood sample for measurement of background amino acid enrichment and indocyanine green concentration was obtained, a primed-continuous infusion of L-[*ring*-$^{13}C_6$]phenylalanine was started, followed at 60 min by L-[1-$^{13}$C]leucine, L-[2-$^{15}$N]lysine, and L-[1-$^{13}$C]alanine. Tracer infusions were maintained throughout the experiment. The following tracer infusion rates (IR) and priming doses (PD) were used: L-[*ring*-$^{13}C_6$]phenylalanine, IR = 0.05 $\mu$mol·kg$^{-1}$·min$^{-1}$, PD = 2 $\mu$mol/kg; L-[1-$^{13}$C]leucine, IR = 0.08 $\mu$mol·kg$^{-1}$·min$^{-1}$, PD = 4.8 $\mu$mol/kg; L-[2-$^{15}$N]lysine, IR = 0.08 $\mu$mol·kg$^{-1}$·min$^{-1}$, PD = 7.2 $\mu$mol/kg; L-[1-$^{13}$C]alanine, IR = 0.35 $\mu$mol·kg$^{-1}$·min$^{-1}$, PD = 35 $\mu$mol/kg. This experimental protocol was designed to assess simultaneously the kinetics of intracellular free amino acids and the fractional synthetic rate (FSR) of protein by the incorporation of L-[*ring*-$^{13}C_6$]phenylalanine in skeletal muscle (5). At 60 min the first muscle biopsy was taken to measure isotopic carbon enrichment of bound and free phenylalanine in muscle. The biopsy was taken from the lateral portion of the right vastus lateralis muscle, ~20 cm above the knee, using a 4-mm Bergström biopsy needle (Stille, Stockholm, Sweden) (3). Approximately 30–50 mg of muscle tissue were obtained with each biopsy. This procedure yields a sample of mixed skeletal muscle. Blood and visible fat and connective tissue were quickly removed from the specimen, and the tissue was immediately frozen in liquid nitrogen and stored at −80°C for later analysis.

To measure leg blood flow, a primed-continuous infusion of indocyanine green dye was started into the femoral artery at 180 min and maintained until 210 min (13). Between 195 and 210 min, blood samples were taken every 5 min (four total) from the femoral vein and the arterialized wrist vein to measure plasma indocyanine green concentration. At 210 min, after the intra-arterial dye infusion was stopped, blood samples were taken every 10 min from the femoral artery and vein until 240 min (four samples total) to measure whole blood concentration and enrichment of free amino acids. At 240 min (end of basal period), the second muscle biopsy was taken to measure concentration and enrichment of free amino acids and enrichment of protein-bound phenylalanine in muscle.

After the basal period, a primed-continuous infusion of unlabeled amino acids was started into the left antecubital vein and was maintained for 3 h until the end of the study. A commercial amino acid mixture (10% Travasol, Clintec Nutrition, Deerfield, IL) and a freshly prepared glutamine solution (Kyowa, Tokyo, Japan) were separately infused at the rate of 1.35 ml·h$^{-1}$·kg$^{-1}$ (prime 0.45 ml) and 0.45 ml·h$^{-1}$·kg$^{-1}$ (prime 0.15 ml/kg), respectively. The concentrations (mg/ml and $\mu$mol/ml, respectively) of the amino acid solution (as reported by the manufacturer) were: leucine 7.3, 55.6; isoleucine 6, 45.7; lysine 5.8, 39.7; valine 5.8, 49.5; phenylalanine 5.6, 33.9; histidine 4.8, 30.9; threonine 4.2, 35.3; methionine 4, 26.8; tryptophan 1.8, 8.8; alanine 20.7, 232.3; arginine 11.5, 66.0; glycine 10.3, 137.2; proline 6.8, 59.1; serine 5, 47.6; tyrosine 0.4, 2.2; total amino acids 100 mg/ml, total nitrogen 16.5 mg/ml. The glutamine solution was prepared on the morning of the study at a concentration of 30 mg/ml. Between 360 and 420 min the measurement of leg blood flow was repeated, and blood samples were taken as described for the basal period. At 420 min, before the tracer and amino acid infusions were stopped, the third muscle biopsy was taken from the vastus lateralis muscle of the right leg.

*Exercise study.* The assessment of the effects of amino acid infusion on muscle protein kinetics was repeated in the same subjects after exercise 1–4 wk after the resting study. A polyethylene catheter was inserted into the left antecubital vein for drawing background blood samples and starting the primed-continuous infusion of labeled phenylalanine. Subsequently, the subjects started a 1-h exercise protocol that consisted of an intense lower-body resistance training session. After a 10-min warm-up of light (<100 W) cycling on a cycle ergometer, the following exercises were performed: incline leg press (five sets of 10 repetitions, 12 RM) and Nautilus duo-squat, leg curls, and leg extensions (four sets of 8 repetitions, 10 RM). Each set was completed in ~30 s with a

2-min rest between sets. On completion of the exercise routine (i.e., at ~60 min from the beginning of the study), the arterial and venous catheters were inserted as described for the resting study and a muscle biopsy was taken from the vastus lateralis muscle of the right leg. Then, intravenous infusions of labeled (L-[1-$^{13}$C]leucine, L-[2-$^{15}$N]lysine, and L-[1-$^{13}$C]alanine) and unlabeled (Travasol and glutamine) amino acid solutions were started as described for the resting study. Leg blood flow was measured between 195 and 210 min, and then blood samples were taken every 10 min from the femoral artery and vein until 240 min. At 240 min, before tracer and amino acid infusions were stopped, the second muscle biopsy was taken.

*Analysis.* Enrichments of phenylalanine, leucine, lysine, and alanine were measured in the femoral arterial and venous whole blood by gas chromatography-mass spectrometry (GC-MS) (Hewlett-Packard 5985, Palo Alto, CA) as nitrogen-acetyl-*n*-propyl esters (20). Glutamine enrichment was measured by GC-MS as the *t*-butyldimethylsylil derivative (19). Isotopic enrichments were expressed as a tracer-to-tracee ratio (tracer/tracee) (18). Concentrations of free amino acids were determined using an internal standard solution containing L-[*ring*-$^{2}$H$_5$]phenylalanine, L-[2,3,3,3-$^{2}$H$_4$]alanine, L-[1,2-$^{13}$C$_2$]leucine, L-[1,2-$^{13}$C$_2$,6,6-$^{2}$H$_2$]lysine, and L-[2,3,3,4,4-$^{2}$H$_5$]glutamine. This solution was added to a known volume of blood as described in Ref. 5. Concentrations were calculated from the internal standard enrichments measured by GC-MS. The concentration of indocyanine green in serum was measured spectrophotometrically at 805 nm. Leg plasma flow was calculated from steady-state values of dye concentration in the femoral and arterialized wrist veins, as described previously (5). Leg blood flow was calculated from the plasma flow and the hematocrit.

Each tissue sample was weighed, and muscle protein was precipitated with 0.5 ml of 10% trichloroacetic acid. An internal standard solution containing L-[*ring*-$^{2}$H$_5$]phenylalanine, L-[2,3,3,3-$^{2}$H$_4$]alanine, L-[1,2-$^{13}$C$_2$]leucine, L-[1,2-$^{13}$C$_2$,6,6-$^{2}$H$_2$]lysine, and L-[2,3,3,4,4-$^{2}$H$_5$]glutamine was added and thoroughly mixed. The tissue was then homogenized and centrifuged, and the supernatant was collected to measure the enrichment and concentration of the free amino acids by GC-MS as described for the blood samples. Measured values of enrichment and concentrations relative to the total tissue water were corrected according to Bergström et al. (3) to obtain the intracellular values. For measurement of protein-bound phenylalanine, the pellet was washed and dried. Then, proteins were hydrolyzed at 110°C for 36 h with 6 N constantly boiling HCl. The protein hydrolysate was then passed through columns of acid-washed celite to remove carbon particles. The purified amino acids were dried under vacuum using a speed-vac (Savant Instruments, Farmingdale, NY). Each sample was reconstituted in 150 $\mu$l of 0.2 M sodium phosphate buffer (pH 3.2). Phenylalanine was isolated by high-performance liquid chromatography (HPLC; LKB, Bromma, Sweden) as described. Purity of the phenylalanine fraction was verified using a different HPLC system with a fluorometric detector (LKB). The samples containing pure phenylalanine were dried under nitrogen and placed in tin containers and combusted using a carbon/nitrogen analyzer (Nitrogen Analyzer 1500, Carlo Erba, Serono, Italy). The resulting $CO_2$ gas was automatically injected into an isotope ratio mass spectrometer (IRMS; VG Isogas, VG Instruments, Middlewich, UK) for determination of the $^{13}$C-to-$^{12}$C isotope ratio in protein-bound phenylalanine.

*Calculations.* The kinetics of free amino acids in leg muscle have been described by the model shown in Fig. 2 (5). Amino acids enter and leave the leg via the femoral artery ($F_{in}$) and the femoral vein ($F_{out}$), respectively. Free amino acid pools in arterial (A) and venous (V) blood and in muscle (M) are connected by arrows indicating the unidirectional amino acid flow between compartments. $F_{M,A}$ and $F_{V,M}$ refer to the rates of net amino acid movement from artery to muscle and from muscle to femoral vein, i.e., inward and outward transmembrane transport, respectively. $F_{M,O}$ for the essential amino acids phenylalanine, leucine, and lysine defines the rate of intracellular amino acid appearance from protein breakdown. In the case of alanine, $F_{M,O}$ represents the sum of release from protein breakdown and de novo synthesis from pyruvate. Because phenylalanine and lysine are not oxidized in muscle (16), $F_{O,M}$ for these amino acids refers to the rate of utilization for protein synthesis. In the case of leucine, $F_{O,M}$ represents utilization for protein synthesis plus oxidation. Each kinetic parameter is defined as follows (see Ref. 5 for derivation of the equations)

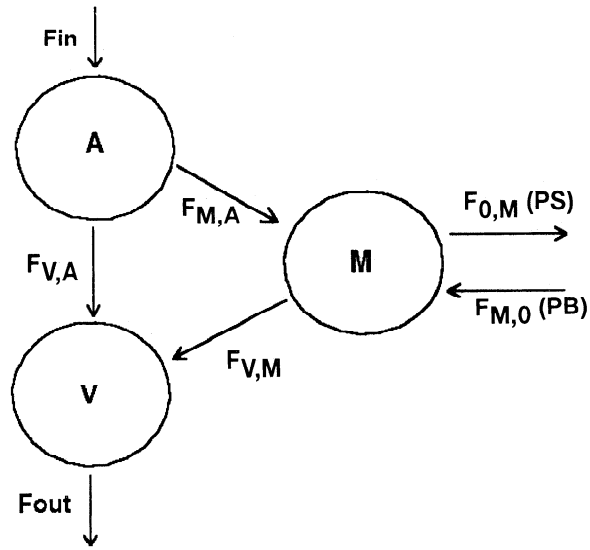


Fig. 2. Three-compartmental model of leg muscle amino acid kinetics. Free amino acid pools in femoral artery (A), femoral vein (V), and muscle (M) are connected by arrows indicating unidirectional amino acid flow between compartments. Amino acids enter leg via femoral artery ($F_{in}$) and leave leg via femoral vein ($F_{out}$). $F_{V,A}$, direct amino acid flow from artery to vein without entering intracellular fluid; $F_{M,A}$ and $F_{V,M}$, inward and outward amino acid transport from artery to muscle and from muscle to vein, respectively; $F_{M,O}$, intracellular amino acid appearance from endogenous sources (i.e., proteolysis and de novo synthesis, if any; PB, protein breakdown); $F_{O,M}$, rate of disappearance of intracellular amino acids [i.e., protein synthesis (PS) and other fates, if any].

$$F_{in} = C_A \cdot BF \quad (1)$$

$$F_{out} = C_V \cdot BF \quad (2)$$

$$NB = (C_A - C_V) \cdot BF \quad (3)$$

$$F_{M,A} = [(E_M - E_V)/(E_A - E_M)] \cdot C_V + C_A \cdot BF \quad (4)$$

$$F_{V,M} = [(E_M - E_V)/(E_A - E_M)] \cdot C_V + C_V \cdot BF \quad (5)$$

$$F_{V,A} = F_{in} - F_{M,A} \quad (6)$$

$$F_{M,O} = F_{M,A} \cdot (E_A/E_M - 1) \quad (7)$$

$$F_{O,M} = F_{M,O} + NB \quad (8)$$

where $C_A$ and $C_V$ are free amino acid concentrations in the femoral artery and vein, respectively; $E_A$, $E_V$, and $E_M$ are

amino acid enrichments in femoral artery, femoral vein, and muscle, respectively; NB is net amino acid balance across the leg; and BF is leg blood flow.

*Model assumptions.* The calculation of intracellular amino acid utilization ($F_{O,M}$) and appearance ($F_{M,O}$) assumes there is not de novo tracer production in the leg. There is no problem with this assumption for the tracers used in this study. The calculation of protein synthesis from the phenylalanine and leucine tracers assumes that these amino acids are not oxidized in muscle. The calculation relying on the net plasma balance measurements and the muscle biopsy data assumes that muscle accounts for the leg metabolism of amino acids. It is assumed that tissue enrichment and concentration measurements are representative of the intracellular space and that the intracellular free amino acid pool is homogeneous. Finally, we assume that the free amino acid pool is the precursor for protein synthesis. These assumptions have been discussed in detail in Refs. 4 and 5.

Muscle FSR in the basal postabsorptive period and during amino acid infusion at rest and after exercise was calculated by dividing the increment in enrichment in the product, i.e., protein-bound L-[*ring*-$^{13}C_6$]phenylalanine tracer/tracee, by the enrichment of the precursor, i.e., free intracellular L-[*ring*-$^{13}C_6$]phenylalanine tracer/tracee. Increments in protein-bound L-[*ring*-$^{13}C_6$]phenylalanine enrichment between two biopsies ($\Delta E_P$) were obtained from the IRMS measurements of the $^{13}C$-to-$^{12}C$ isotope ratio in the protein-bound phenylalanine in each biopsy

$$\Delta E_P = [^{13}C/^{12}C_{(2)} - {}^{13}C/^{12}C_{(1)}] \cdot 1.5 \quad (9)$$

where the subscripts 1 and 2 denote values in two subsequent biopsies (i.e., the first and second or the second and third biopsies in the resting study and the first and second biopsy in the exercise study). Differences between the $^{13}C$-to-$^{12}C$ isotope ratio in distinct biopsies were multiplied by the factor 1.5 to obtain the increment of tracer/tracee of the protein-bound L-[*ring*-$^{13}C_6$]phenylalanine from one biopsy to another. The factor 1.5 arises because in the L-[*ring*-$^{13}C_6$]phenylalanine molecule six of a total of nine carbon atoms are labeled. Then, the FSR was calculated as follows (6)

$$FSR = \frac{\Delta E_P}{[E_{M(1)} + E_{M(2)}]/2] \cdot T} \cdot 60 \cdot 100 \quad (10)$$

$E_{M(1)}$ and $E_{M(2)}$ are the L-[*ring*-$^{13}C_6$]phenylalanine enrichments in the free muscle pool in the two subsequent biopsies. Average values between $E_{M(1)}$ and $E_{M(2)}$ were used as precursor enrichments for muscle protein synthesis. $T$ indicates time intervals (min) between two biopsies. The factors 60 (min/h) and 100 are needed to express the FSR in percent per hour.

*Statistical analysis.* Data are expressed as means ± SE. Results in the basal state and during hyperaminoacidemia at rest and after exercise were compared using analysis of variance with randomized block design followed by Fisher's test. During hyperaminoacidemia at rest or after exercise, percent changes from basal of kinetic parameters were compared with the Student's paired $t$-test. The relationship between values of leg blood flow and muscle FSR was assessed using linear regression analysis. Data of pooled observations from six individuals, investigated in the basal resting state and during hyperaminoacidemia at rest and after exercise ($n = 18$), were used. $P \leq 0.05$ was taken as significant for between-group comparisons, and $P < 0.01$ was considered significant for the correlation data.

## RESULTS

Amino acid concentrations and enrichments in the femoral artery and vein were in steady state in the basal period and in the last 30 min of amino acid infusion at rest and after exercise, as reflected by values from the multiple samples taken over those times (data not shown). In Tables 1 and 2, the average values are reported for free amino acid concentrations and enrichments in the femoral artery and vein and in muscle. Amino acid infusion at rest and after exercise resulted in comparable increments in amino acid concentrations in the femoral artery. Arterial phenylalanine, leucine, and alanine increased ~200%, whereas lysine and glutamine increased ~90% and ~30%, respectively. In skeletal muscle, intracellular concentrations of free phenylalanine and leucine increased by ~100%, alanine concentrations increased by 60%, but lysine and glutamine concentrations did not change significantly. After amino acid infusion at rest and after exercise, the enrichment of free amino acids significantly decreased in the femoral artery and vein as well as in muscle (Table 2). Amino acid enrichments in blood and muscle were not significantly different during hyperaminoacidemia at rest and after exercise.

Plasma insulin concentration in the basal postabsorptive state was 10 ± 2 μU/ml. After amino acid infusion, insulin concentration increased both at rest and after exercise to 20 ± 9 and 19 ± 2 μU/ml, respectively. At rest, leg blood flow was 2.58 ± 0.42 ml·min$^{-1}$·100 ml leg volume$^{-1}$. The resting blood flow value was significantly increased by 25% to 3.22 ± 0.45 ml·min$^{-1}$·100 ml leg volume$^{-1}$ by amino acid infusion. After exercise, amino acid infusion resulted in leg blood flow (5.10 ± 0.80 ml·min$^{-1}$·100 ml leg volume$^{-1}$) that was signifi-

Table 1. *Effects of amino acid infusion at rest or during postexercise recovery on free amino acid concentration*

| | Femoral Artery | Femoral Vein | Muscle |
|---|---|---|---|
| Phenylalanine | | | |
| Basal | 46 ± 4 | 52 ± 3 | 83 ± 5 |
| AA at rest | 154 ± 18* | 149 ± 17* | 171 ± 18* |
| AA postexercise | 163 ± 15* | 157 ± 15* | 168 ± 11* |
| Leucine | | | |
| Basal | 111 ± 5 | 118 + 6 | 203 + 11 |
| AA at rest | 334 ± 7* | 304 ± 10* | 339 ± 34* |
| AA postexercise | 359 ± 21* | 319 ± 21* | 343 ± 19* |
| Lysine | | | |
| Basal | 231 ± 19 | 243 ± 19 | 747 ± 37 |
| AA at rest | 399 ± 24* | 387 ± 24* | 716 ± 70 |
| AA postexercise | 421 ± 21* | 399 ± 23* | 804 ± 83 |
| Alanine | | | |
| Basal | 201 ± 19 | 245 ± 20 | 1,683 ± 208 |
| AA at rest | 644 ± 63* | 641 ± 58* | 2,319 ± 347 |
| AA postexercise | 764 ± 62* | 735 ± 60* | 2,534 ± 308* |
| Glutamine | | | |
| Basal | 530 ± 30 | 583 ± 34 | 13,314 ± 1,174 |
| AA at rest | 651 ± 27* | 720 ± 18* | 10,992 ± 1,018 |
| AA postexercise | 754 ± 31* | 785 ± 32* | 13,991 ± 590 |

Values are amino acid (AA) concns (means ± SE) expressed as nmol/ml whole blood or intracellular water. *$P < 0.05$, AA infusion (at rest or recovery) vs. Basal.

Table 2. *Effects of amino acid infusion at rest or during postexercise recovery on amino acid enrichment*

| | Femoral Artery | Femoral Vein | Muscle |
|---|---|---|---|
| Phenylalanine | | | |
| Basal | 0.0738 ± 0.0038 | 0.0567 ± 0.0032 | 0.0474 ± 0.0016 |
| AA at rest | 0.0278 ± 0.0015* | 0.0268 ± 0.0012* | 0.0252 ± 0.0011* |
| AA postexercise | 0.0278 ± 0.0015* | 0.0263 ± 0.0015* | 0.0245 ± 0.0015* |
| Leucine | | | |
| Basal | 0.0764 ± 0.0030 | 0.0596 ± 0.0031 | 0.0521 ± 0.0027 |
| AA at rest | 0.0383 ± 0.0011* | 0.0353 ± 0.0010* | 0.0301 ± 0.0013* |
| AA postexercise | 0.0381 ± 0.0011* | 0.0359 ± 0.0011* | 0.0289 ± 0.0027* |
| Lysine | | | |
| Basal | 0.0772 ± 0.0030 | 0.0642 ± 0.0029 | 0.0336 ± 0.0030 |
| AA at rest | 0.0453 ± 0.0033* | 0.0420 ± 0.0031* | 0.0251 ± 0.0026* |
| AA postexercise | 0.0414 ± 0.0010* | 0.0385 ± 0.0011* | 0.0250 ± 0.0012* |
| Alanine | | | |
| Basal | 0.0752 ± 0.0041 | 0.0456 ± 0.0028 | 0.0126 ± 0.0014 |
| AA at rest | 0.0293 ± 0.0014* | 0.0236 ± 0.0013* | 0.0091 ± 0.0020* |
| AA postexercise | 0.0254 ± 0.0021* | 0.0219 ± 0.0018* | 0.0083 ± 0.0013* |

Enrichments are means ± SE expressed as tracer-to-tracee ratio. *$P < 0.05$, AA (at rest or recovery) vs. Basal.

cantly increased by 100% over basal and by 58% over amino acid infusion at rest.

After amino acid infusion at rest and during the postexercise recovery, the rate of leg arterial amino acid delivery from the systemic circulation ($F_{in}$) significantly increased above the basal values (Table 3). Increments in amino acid delivery were proportional to the changes in arterial amino acid concentrations and leg blood flow. Phenylalanine, leucine, and alanine delivery all increased by ~300% at rest and by ~600% after exercise during AA infusion. Lysine delivery increased by ~120 and ~280% at rest and after exercise, respectively. The effects of amino acid infusion at rest and after exercise on the rates of amino acid transport ($F_{M,A}$) are shown in Table 3. Rates of amino acid transport significantly increased after amino acid infusion both at rest and after exercise. However, rates of transport were 40–100% greater during hyperaminoacidemia after exercise than during hyperaminoacidemia at rest.

The rates of intracellular phenylalanine and lysine utilization for protein synthesis ($F_{O,M}$) significantly increased from basal both at rest and after exercise (Fig. 3). However, values for amino acid utilization for protein synthesis were significantly greater ($P < 0.05$) during hyperaminoacidemia after exercise than during hyperaminoacidemia at rest. The FSR data agreed well with the model-derived values for $F_{O,M}$. In the basal postabsorptive state, the FSR for muscle protein was 0.0657 ± 0.0067%/h. This value increased ($P < 0.05$) to 0.1002 ± 0.0104%/h and to 0.1442 ± 0.0093%/h during hyperaminoacidemia at rest and after exercise, respectively. Breakdown of muscle protein during hyperaminoacidemia, determined as rate of intracellular appearance ($F_{M,O}$) of the essential amino acids phenylalanine, leucine, and lysine, was not significantly different from the basal value either at rest or after exercise (Table 3).

The relationships between FSR and a variety of factors were assessed by linear regression analysis. FSR was significantly correlated ($P < 0.01$) with the

Table 3. *Effects of amino acid infusion at rest or during postexercise recovery on parameters of leg muscle amino acid kinetics*

| | $F_{in}$ Arterial Delivery | $F_{out}$ Venous Outflow | $F_{M,A}$ Inward Transport | $F_{V,M}$ Outward Transport | $F_{O,M}$ Disposal | $F_{M,O}$ Appearance | NB Net Balance |
|---|---|---|---|---|---|---|---|
| Phenylalanine | | | | | | | |
| Basal | 117 ± 16 | 132 ± 17 | 71 ± 9 | 86 ± 9 | 22 ± 3 | 38 ± 4 | −16 ± 2 |
| AA at rest | 481 ± 43* | 466 ± 52* | 308 ± 57* | 293 ± 57* | 49 ± 5* | 34 ± 4 | 15 ± 2* |
| AA postexercise | 841 ± 119*† | 808 ± 116*† | 396 ± 98* | 363 ± 96 | 83 ± 7*† | 50 ± 5 | 33 ± 4*† |
| Leucine | | | | | | | |
| Basal | 293 ± 50 | 311 ± 54 | 211 ± 46 | 229 ± 50 | 69 ± 10 | 88 ± 12 | −18 ± 4 |
| AA at rest | 1,079 ± 143* | 989 ± 144* | 449 ± 57* | 359 ± 63 | 209 ± 17* | 119 ± 18 | 90 ± 11* |
| AA postexercise | 1,873 ± 295*† | 1,674 ± 275*† | 647 ± 107*† | 448 ± 93 | 391 ± 51*† | 192 ± 46 | 199 ± 21*† |
| Lysine | | | | | | | |
| Basal | 601 ± 93 | 630 ± 93 | 147 ± 14 | 177 ± 15 | 180 ± 38 | 209 ± 38 | −29 ± 3 |
| AA at rest | 1,293 ± 165* | 1,256 ± 165* | 245 ± 20* | 221 ± 21 | 239 ± 33* | 202 ± 33 | 36 ± 6* |
| AA postexercise | 2,180 ± 283*† | 2,071 ± 279*† | 470 ± 43*† | 408 ± 75* | 417 ± 49*† | 308 ± 44 | 109 ± 15*† |
| Alanine | | | | | | | |
| Basal | 500 ± 53 | 605 ± 52 | 176 ± 21 | 282 ± 20 | 812 ± 143 | 918 ± 145 | −105 ± 7 |
| AA at rest | 1,982 ± 167* | 1,973 ± 164* | 587 ± 89* | 579 ± 74* | 1,655 ± 438 | 1,647 ± 432 | 8 ± 21* |
| AA postexercise | 3,857 ± 467*† | 3,714 ± 450*† | 950 ± 136*† | 806 ± 125* | 2,483 ± 739 | 2,339 ± 732 | 144 ± 23*† |

Values are means ± SE in nmol·min$^{-1}$·100 ml leg volume$^{-1}$. *$P < 0.05$, AA vs. Basal; †$P < 0.05$ AA postexercise vs. AA at rest. $F_{in}$, amino acid inflow into the leg from systemic circulation via femoral artery; $F_{out}$, amino acid outflow from leg via femoral vein; $F_{M,A}$, inward amino acid transport from femoral artery into free muscle amino acid pool; $F_{V,M}$, outward amino acid transport from intracellular pool into femoral vein; $F_{V,A}$, direct amino acid flow from artery to vein without entering intracellular fluid; $F_{O,M}$, intracellular amino acid utilization (i.e., protein synthesis for phenylalanine and lysine, protein synthesis plus other fates for leucine and alanine); $F_{M,O}$, intracellular amino acid appearance (i.e., proteolysis for phenylalanine, leucine and lysine, proteolysis plus de novo synthesis for alanine); NB, net amino acid balance across the leg (negative numbers indicate net release).

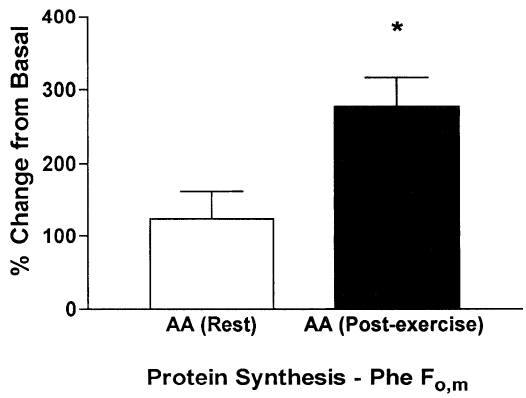


Fig. 3. Rates of muscle protein synthesis ($F_{O,M}$) during hyperaminoacidemia at rest and after exercise, expressed as percent change from basal. *Significantly different from hyperaminoacidemia at rest, $P < 0.05$.

rate of inflow into the leg ($F_{in}$) for each amino acid, and also to the rate of inward transport ($F_{M,A}$) of each amino acid. These relationships all reflected a high correlation between blood flow and FSR ($r = 0.72$, $P < 0.01$, Fig. 4), as blood flow is used in calculating $F_{in}$ and $F_{M,A}$. The relationship between blood flow and FSR is striking because of the independence of the values used to calculate these two factors.

## DISCUSSION

In this study we examined the response of muscle protein kinetics to hyperaminoacidemia at rest and ~3 h after a heavy resistance exercise routine in untrained volunteers. We found that the stimulatory effect of hyperaminoacidemia on net muscle protein synthesis was enhanced by previous performance of physical exercise. Muscle anabolism during hyperaminoacidemia occurred entirely because of a stimulation of protein synthesis, because protein breakdown was not significantly different from basal at rest or after exercise. These results suggest that increased availability of free amino acids to skeletal muscle immediately after

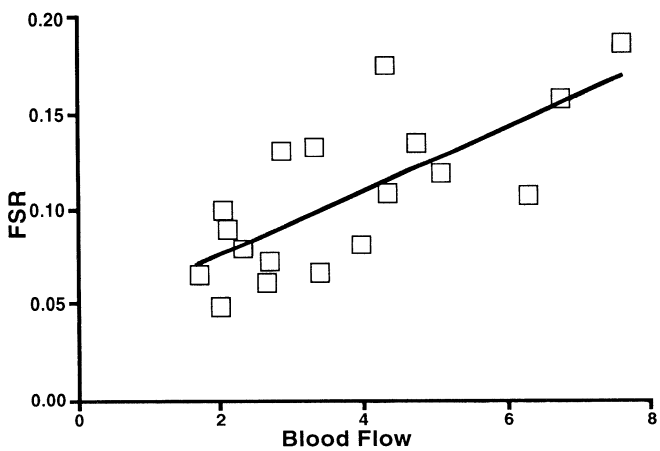


Fig. 4. Relationship between rates of leg blood flow ($x$) and muscle fractional protein synthesis (FSR, $y$). Observations are from 6 subjects investigated in basal state and during hyperaminoacidemia at rest and after exercise ($n = 18$).

performance of physical exercise can maximize the anabolic effect of nutrition and/or exercise training.

A recent report from our laboratory (6) showed that, after resistance exercise in the postabsorptive state, muscle protein synthesis was accelerated by ~100%. In the present study, amino acid infusion at rest increased protein synthesis by ~150%, whereas hyperaminoacidemia after exercise increased muscle protein synthesis by >200%. Thus physical exercise and hyperaminoacidemia have additive effects on protein synthesis. Previously, when subjects were studied in the fasted state (6), we reported an increase in protein degradation that was not seen in the present study when amino acids were infused. Taken together, the results suggest that the amino acid infusion limited the increase in protein breakdown that normally follows resistance exercise. Consequently, it seems that exogenous amino acids enhance muscle anabolism after exercise by increasing muscle protein synthesis and decreasing breakdown.

The regulatory role of intracellular amino acid concentration on protein breakdown has been recognized in in vitro studies for many years (17). An inhibitory effect of hyperaminoacidemia on protein breakdown is a logical mechanism by which to explain the lack of increase in protein breakdown after exercise in this study, since the intracellular amino acid concentration was increased by the amino acid infusion. On the other hand, hyperaminoacidemia had no effect on protein breakdown at rest. These results are consistent with the notion that a fall in the intracellular amino acid concentration below the normal postabsorptive value stimulates protein breakdown to restore normal concentrations but that an increase in the concentration above normal by exogenous infusion has no further inhibitory effect on protein breakdown. Extending this concept to the current study, we propose that exercise stimulated muscle protein synthesis, which caused intracellular amino acids to be incorporated into protein at an increased rate. This caused a transient drop in concentration, which in turn caused an acceleration of protein breakdown to restore the normal intramuscular amino acid concentrations. We propose that hyperaminoacidemia prevented this scenario by maintaining amino acid concentrations above the basal values. We did not directly assess this possibility in this study, as we did not start measuring the intracellular amino acid concentration until ~2 h into recovery from resistance exercise.

During the amino acid infusion, the transport of phenylalanine, leucine, lysine, and alanine increased from basal by factors of 2 to 3 at rest and by factors of 3 to 6 after exercise. Furthermore, the rates of inward amino acid transport were significantly correlated with the rate of muscle protein synthesis, measured either by the direct incorporation technique (FSR) or by the arteriovenous balance technique (phenylalanine $F_{O,M}$). This correlation was due entirely to the correlation between blood flow and FSR (Fig. 4) or protein synthesis. Thus, in this study, the postexercise increases in amino acid transport may have mediated the response of muscle protein synthesis by increasing intracellular availability of free amino acids. Alternatively, a stimu-

lation of synthesis may in turn have caused a more rapid rate of transport. In either case, there clearly is a link between inward transport and synthesis. In contrast to the changes in the rates of transport, the values of intracellular amino acid concentrations were not different during hyperaminoacidemia at rest and after exercise. These results suggest that the rate of amino acid transport into cells is a better indicator of the availability of free amino acids for protein synthesis than the intracellular concentrations.

Hyperaminoacidemia presumably accelerates the rates of amino acid transport into cells by increasing substrate availability for membrane transport systems. Animal studies have shown that, within a physiological range of extracellular amino acid concentrations, transmembrane transport in muscle is not normally saturated and increases proportionally with the concentrations of amino acid in the perfusate (11).

In contrast to the situation of hyperaminoacidemia, the mechanism of the exercise-mediated acceleration of amino acid transport is less apparent. The increase in blood flow after exercise is a possible mechanism. Performance of physical exercise leads to the opening of previously underperfused or nonperfused capillaries, thus shortening the distance for amino acids to diffuse from the blood to the cell membranes. Amino acid infusions at rest and after exercise resulted in similar increases in arterial amino acid concentrations. However, during hyperaminoacidemia after exercise, the values of $F_{in}$ were ~80% greater than during hyperaminoacidemia at rest because, as indicated by the present results, blood flow after exercise was accelerated.

Over the last decade, evidence has accumulated supporting the hypothesis that blood flow is a major regulator of glucose uptake in skeletal muscle (1). The results of our study suggest that variations in blood flow may also affect muscle protein metabolism by increasing transport of free amino acids into cells, which in turn stimulates protein synthesis. This notion is supported by the high correlation between blood flow and FSR (Fig. 4).

On the other hand, an alternative explanation must also be considered. This is because, regardless of the substrate (e.g., glucose or amino acids), it would be expected that blood flow would increase transport by decreasing the concentration gradient between the blood and the interstitial fluid, from which uptake occurs, as a consequence of decreased diffusion distance. Whereas this mechanism seems logical in the case of glucose, which moves into the cell down its concentration gradient, it is harder to imagine how decreasing the gradient between interstitial fluid and blood amino acid concentration would facilitate inward amino acid transport. An increase in blood flow would be expected to increase the interstitial concentrations of amino acids if those concentrations were initially lower than the corresponding blood concentrations. However, this is not likely to be the case. The interstitial fluid concentrations of substrates generally fall somewhere between the intracellular concentrations and the concentrations in the venous blood leaving the tissue. In the case of amino acids, specific transport mechanisms maintain the intracellular concentrations in muscle higher than the corresponding femoral vein concentrations (Table 1), so the interstitial fluid concentrations are likely also to be greater than the venous blood concentrations. Evidence from an experiment using dialysis probes in human subjects supports this notion (15). Consequently, we must also consider the possibility that correlation between blood flow and FSR is due to the increased demand for energy resulting from the direct stimulation of synthesis by exercise. The role of blood flow per se can only be directly assessed by changing the rate of blood flow while amino acid concentrations remain constant.

The rate of infusion of amino acids we used provided a total caloric intake of ~475 kJ. We must therefore consider the possibility that the stimulation of muscle protein synthesis may have reflected, in part, the response to energy intake, rather than a specific effect of the amino acids. The positive effect of energy intake on N balance is well known (8). However, it is unlikely that much of the effect of hyperaminoacidemia was due to the energy intake alone. First, the total caloric intake was only about 1/20th of the total daily caloric requirement, and provision of this amount of energy in the form of either glucose or fat would have little or no effect on muscle metabolism. Second, not all of the amino acids can be oxidized by muscle, so the overall energy content of the amino acid mixture overestimates the extra energy made available to the muscle. Third, if we consider the specific cases of lysine and phenylalanine as examples of essential amino acids that cannot be oxidized in muscle, the ratios of their rates of incorporation into protein ($F_{O,M}$) to the total rates of intracellular appearance of amino acid ($F_{M,A}+F_{M,O}$) were similar in the basal state, during hyperaminoacidemia at rest, and during hyperaminoacidemia after exercise. This relation indicated ~20% efficiency of protein synthesis in the case of phenylalanine and 55% in the case of lysine. These data are consistent with the notion that the increase in protein synthesis during hyperaminoacidemia could not have occurred in the absence of an increased availability of amino acids as precursors for synthesis.

From a practical standpoint, it is pertinent to relate our findings to the question of how exercise affects protein and/or amino acid requirements and exercise. On one hand, our results explain the general finding that N balance can be maintained on a lower protein intake if one performs resistance exercise (7), since the same amino acid infusion had a more positive effect on protein balance after exercise than at rest. On the other hand, the enhanced stimulatory effect of amino acids on muscle protein synthesis immediately after exercise suggests that a higher than normal protein intake will effectively stimulate net protein balance in individuals involved in resistance training. Our results also indicate that the timing of the food intake in relation to the time at which exercise is performed may be important in determining the effect of exercise on whole body N balance.

In summary, the results of our study demonstrate that net protein synthesis during amino acid administration can be doubled by previous performance of heavy resistance exercise. Moreover, the data suggest a link between the stimulation of protein synthesis after exercise and an acceleration in amino acid transport. The greater rate of transport after exercise may be due to the increase in blood flow.

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