

Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans

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Biolo, Gianni, Sergio P. Maggi, Bradley D. Williams, Kevin D. Tipton, and Robert R. Wolfe. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E514–E520, 1995.—The rates of protein synthesis and degradation and of amino acid transport were determined in the leg muscle of untrained postabsorptive normal volunteers at rest and ~3 h after a resistance exercise routine. The methodology involved use of stable isotopic tracers of amino acids, arteriovenous catheterization of the femoral vessels, and biopsy of the vastus lateralis muscle. During postexercise recovery, the rate of intramuscular phenylalanine utilization for protein synthesis increased above the basal value by $108 \pm 18\%$, whereas the rate of release from proteolysis increased by $51 \pm 17\%$. Muscle protein balance improved ($P < 0.05$) after exercise but did not become positive (from -15 ± 12 to -6 ± 3 nmol phenylalanine $\cdot$ min $^{-1}$ $\cdot$ 100 ml leg volume $^{-1}$). After exercise, rates of inward transport of leucine, lysine, and alanine increased ($P < 0.05$) above the basal state from 132 ± 16 to 208 ± 29 , from 122 ± 8 to 260 ± 8 , and from 384 ± 71 to 602 ± 89 nmol $\cdot$ min $^{-1}$ $\cdot$ 100 ml leg $^{-1}$, respectively. Transport of phenylalanine did not change significantly. These results indicate that, during recovery after resistance exercise, muscle protein turnover is increased because of an acceleration of synthesis and degradation. A postexercise acceleration of amino acid transport may contribute to the relatively greater stimulation of protein synthesis.

phenylalanine; protein synthesis; muscle biopsy; arteriovenous balance

whereas an increase in muscle protein breakdown can be presumed after exercise, this has not been directly demonstrated, and thus the magnitude of such a change in breakdown in relation to synthesis is not known. Results of animal studies showed that muscle uptake of the nonmetabolized amino acid analogue α -aminoisobutyric acid was increased after exercise (29). However, the effects of physical exercise on the transport of the naturally occurring amino acids remain to be assessed.

To evaluate, in vivo, the postexercise changes of protein kinetics and amino acid transport in the human skeletal muscle, we have used stable isotopic tracers of amino acids in combination with the arteriovenous catheterization of the femoral vessels and the biopsy of the vastus lateralis muscle (2). This approach allowed the separate assessment of the rates of intracellular amino acid appearance from proteolysis and from transport from plasma as well as the rate of protein synthesis. Amino acid and protein kinetics were assessed ~3 h after completion of a heavy-resistance exercise routine involving leg muscle. Untrained subjects were studied in the postabsorptive state to avoid confounding variables such as hormonal and substrate changes and physiological consequences of training.

METHODS

Subjects

Five male volunteers (age, 24 ± 2 yr; weight, 72 ± 5 kg; height, 169 ± 3 cm; body mass index, 24.9 ± 1.5 kg/m 2) participated in the study. All subjects were healthy at the time of the study, none was taking medication, and none had a family history of diabetes. Each subject had a normal physical examination, electrocardiogram, blood count, plasma electrolytes, and liver and renal function. Leg volume ($10,252 \pm 800$ ml) was estimated using an anthropometric approach (2). No subject was engaged in a regular exercise-training program for ≥ 1 yr before the study. The study design, purpose, and possible risks were carefully explained to each volunteer before written consent was obtained. The experimental protocol was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston. Each subject was studied twice. For ≥ 1 mo before each study, the subjects consumed a weight-maintaining diet containing 15–20% protein. On one occasion the subject was studied at rest, and on the other occasion the subject was studied immediately after a resistance workout. Five to 10 days before the first study, subjects were familiarized with the exercise protocol, and their 10 and 12 repetition maximum (RM) was determined. Ten and 12 RM represent the maximum weight that a subject can possibly lift for 10 and 12 repetitions, respectively. Their average 10 RM of the knee extensor muscles (2 legs) was 64 ± 6 kg.

PHYSICAL ACTIVITY is necessary to maintain normal muscle mass and strength and prevent muscle atrophy. Furthermore the increase in skeletal muscle size with resistance exercise training represents a fundamental biologic adaptation to an increased workload (16, 17). From a dynamic point of view, such muscle hypertrophy results from changes in the rates of protein synthesis and/or breakdown. In addition, an acceleration of the rates of amino acid transport into muscle cells may contribute to muscle anabolism by increasing amino acid availability for protein synthesis. Studies suggest that muscle protein accretion occurs in the recovery phase after exercise rather than during the actual exercise period (6, 21). The leucine tracer incorporation technique has shown that the rate of muscle protein synthesis in humans is increased after exercise (6, 7) and remains elevated for ≥ 24 h (7). In these studies, muscle protein breakdown was not directly measured. However, the increase in protein synthesis was so large that if it were not accompanied by a concomitant increment in protein breakdown, exercise training would result in a greater increase in muscle size than actually occurs (17). Thus,

Isotopes

L-[1-¹³C]leucine (99% enriched), L-[ring-¹³C₆]phenylalanine (99% enriched), and L-[2,3,3,3-²H₄]alanine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA); L-[1,2-¹³C₂]leucine (99% enriched), L-[2-¹⁵N]lysine (99% enriched), L-[1,2-¹³C₂,6,6-²H₂]lysine (98% enriched), and L-[ring-²H₅]phenylalanine (98% enriched) were purchased from Tracer Technologies (Somerville, MA). L-[1-¹³C]alanine (99% enriched) was purchased from Isotec (Miamisburg, OH).

Resting Study

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 6 A.M., after an overnight fast. An 18-gauge polyethylene catheter was inserted into the left antecubital vein for infusion of labeled amino acids. With use of flexible guide wires, two 8-cm-long polyethylene catheters (Cook, Bloomington, IN) were inserted 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 and maintained at ~65°C. Catheters were inserted using lidocaine. Patency of catheters was maintained by saline infusion.

After a blood sample was obtained for measurement of background amino acid enrichment and indocyanine green concentration, a primed-continuous infusion of L-[ring-¹³C₆]phenylalanine was started, followed at 60 min by L-[1-¹³C]leucine, L-[2-¹⁵N]lysine, and L-[1-¹³C]alanine. Tracer infusions were maintained throughout the experiment. The following tracer infusion rates (IR) and priming doses (PD) were used: L-[ring-¹³C₆]phenylalanine: IR = 0.05 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, PD = 2 $\mu\text{mol}/\text{kg}$; L-[1-¹³C]leucine: IR = 0.08 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, PD = 4.8 $\mu\text{mol}/\text{kg}$; L-[2-¹⁵N]lysine: IR = 0.08 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, PD = 7.2 $\mu\text{mol}/\text{kg}$; L-[1-¹³C]alanine: IR = 0.35 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, PD = 35 $\mu\text{mol}/\text{kg}$. This experimental protocol was designed to simultaneously assess in skeletal muscle the kinetics of intracellular free amino acids and the fractional synthetic rate (FSR) of protein by the incorporation of L-[ring-¹³C₆]phenylalanine (2). Isotopic steady state in the free amino acid pools in blood and muscle was required to calculate intracellular amino acid kinetics at the end of the basal and postexercise period, i.e., between 200 and 240 min. Measurement of FSR by the incorporation of L-[ring-¹³C₆]phenylalanine required steady-state enrichment of the precursor during the incorporation period, i.e., between 60 and 240 min.

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, with use of a 4-mm Bergström biopsy needle (Stille, Stockholm, Sweden) (2). 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 at 180 min, a primed-continuous infusion of indocyanine green dye was started into the femoral artery and maintained until 240 min (2). Between 200 and 240 min, blood samples were taken every 20 min from the femoral vein, an arterialized wrist vein, and the femoral artery. To allow sampling from the femoral artery, the dye infusion was

stopped for <10 s and then quickly resumed. Arterial samples were always taken after samples from the femoral and wrist veins to avoid interference with blood flow measurement. 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.

Exercise Study

The assessment of muscle protein kinetics and amino acid transport was repeated 1–4 wk after the resting study in the same subjects after exercise. An 18-gauge 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 the exercise protocol that consisted of an intense lower body resistance-training session. Before the resistance routine, the subject executed a 10-min warmup of light (<100-W) cycling on a cycle ergometer. Then the following resistance exercise routine was completed in ~40 min: 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 2–3 min of rest between sets. After completion of the exercise routine, the femoral arterial and venous catheters and the right wrist vein catheter were inserted, the first muscle biopsy was performed, L-[1-¹³C]leucine, L-[2-¹⁵N]lysine, and L-[1-¹³C]alanine infusions were started, and the protocol was continued as described for the resting study.

Analysis

Blood samples from the femoral artery and vein for determination of amino acid enrichment and concentration were immediately precipitated in preweighed tubes containing 15% sulfosalicylic acid. Simultaneously, a known amount of an internal standard mixture containing L-[ring-²H₅]phenylalanine, L-[1,2-¹³C₂]leucine, L-[1,2-¹³C₂,6,6-²H₂]lysine, and L-[2,3,3,3-²H₄]alanine was added to the tube and thoroughly mixed (2). The sulfosalicylic extract was frozen for later analysis. To determine the enrichment of the infused tracers and of the internal standards of free phenylalanine, leucine, lysine, and alanine in the whole blood, the nitrogen-acetyl-*n*-propyl esters (NAP) were prepared as described previously (2, 27). Blood samples from the femoral and arterialized wrist veins were collected to measure indocyanine green concentration in serum, as described elsewhere (2). Leg plasma flow was calculated from steady-state values of dye concentration in the femoral and arterialized wrist veins (2). Leg blood flow was calculated from 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 the same isotopes used for the blood samples, but in different proportion, was added to the tissue and thoroughly mixed (2). The tissue was then homogenized and centrifuged, and the supernatant was collected to produce the NAP derivatives of intracellular free amino acids (2, 25). The pellet was washed with absolute ethanol, and the precipitated proteins were then hydrolyzed with 6 N constant boiling HCl. Phenylalanine was isolated from the amino acid mixture by high-performance liquid chromatography (HPLC; LKB, Bromma, Sweden). The samples containing pure phenylalanine were combusted using a carbon/nitrogen analyzer (Nitrogen Analyzer 1500, Carlo Erba, Saronno, Italy). The resulting CO₂ gas was automatically injected into an isotope-ratio mass spectrometer (IRMS; VG Isogas, VG Instruments, Middlewich, UK) for determination of the ¹³C-to-¹²C ratio in protein-bound phenylalanine.

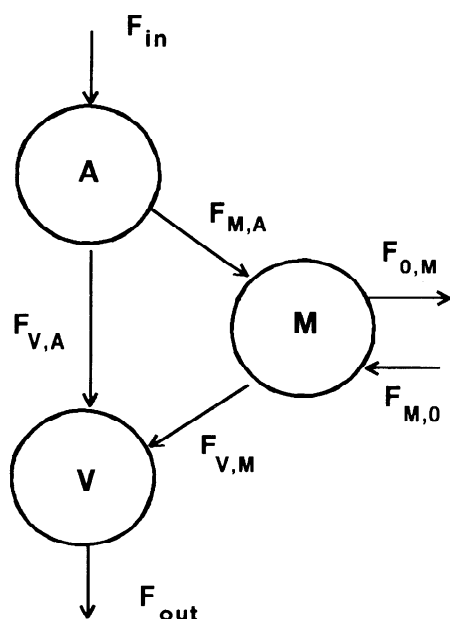


Fig. 1. Three-compartmental model of leg muscle amino acid kinetics. Free amino acid pools in femoral artery (A), femoral vein (V), and muscle (M) are connected by arrows indicating unidirectional amino acid flow between each compartment. Amino acids enter leg via femoral artery (F_{in}) and leave leg 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); $F_{O,M}$, rate of disappearance of intracellular amino acids (i.e., protein synthesis and other fates, if any).

The isotopic enrichment of free amino acids in blood and muscle samples was determined by gas chromatographic mass spectrometry (GCMS; model 5985, Hewlett-Packard, Palo Alto, CA) by chemical ionization and selected ion monitoring (2, 25). Data are expressed as tracer-to-tracee ratio, with correction for the contribution of isotopomers of small weight to the apparent enrichment of isotopomers with a greater mass (2, 25). Enrichment of L-[ring- $^{13}\text{C}_6$]phenylalanine was further corrected using a factor of 0.93 to account for an overestimation of enrichment due to the different isotopomer distribution of the tracer and the naturally occurring phenylalanine (25).

Concentrations (C, nmol/ml) of free amino acids in blood and total muscle water were calculated as follows (2, 25)

$$C = Q_{IS}/(V \cdot E_{IS}) \quad (1)$$

where Q_{IS} (nmol) is the amount of internal standard added to the sample, V is the volume of blood or muscle water, and E_{IS} is the internal standard tracer-to-tracee ratio in blood or muscle water as measured by GCMS. Measured values of enrichment and concentrations relative to total tissue water were corrected according to Biolo et al. (2) to obtain intracellular values.

Calculations. The kinetics of free amino acids in leg muscle have been described by the model shown in Fig. 1 (2). Amino acids enter and leave the leg via the femoral artery (F_{in}) and 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 each compartment. $F_{M,A}$ 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 (2), $F_{O,M}$ 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. 7 for derivation of equations)

$$F_{in} = C_A \cdot BF \quad (2)$$

$$F_{out} = C_V \cdot BF \quad (3)$$

$$NB = (C_A - C_V) \cdot BF \quad (4)$$

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

$$F_{V,M} = \{[(E_M - E_V)/(E_A - E_M)] \cdot C_V + C_V\} \cdot BF \quad (6)$$

$$F_{V,A} = F_{in} - F_{M,A} \quad (7)$$

$$F_{M,O} = F_{M,A} \cdot (E_A/E_M - 1) \quad (8)$$

$$F_{O,M} = F_{M,O} + NB \quad (9)$$

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 the femoral artery, femoral vein, and vastus lateralis muscle, respectively, BF is leg blood flow, and NB is net amino acid balance across the leg. The vastus lateralis muscle has been considered as representative of the total leg muscle, because its relative content of slow- and fast-twitch fibers is similar to that in the total leg muscle mass (23).

Muscle FSR in the basal period and during insulin infusion was calculated by dividing the increment in enrichment in the product, i.e., protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine tracer-to-tracee ratio, by the enrichment of the precursor, i.e., free intracellular L-[ring- $^{13}\text{C}_6$]phenylalanine tracer-to-tracee ratio (2). Delta increments of protein-bound L-[ring- $^{13}\text{C}_6$]phenylalanine enrichment during the incorporation period (i.e., between 60 and 240 min; ΔE_p) were obtained from the IRMS measurements of the protein-bound phenylalanine enrichment in the first and second biopsy, as described previously (2).

Table 1. Amino acid concentrations in basal state and during postexercise recovery

	Femoral Artery	Femoral Vein	Muscle
Phenylalanine			
Basal	43 ± 3	49 ± 3	130 ± 16
Postexercise	40 ± 4	41 ± 4	77 ± 13
Leucine			
Basal	114 ± 6	119 ± 9	219 ± 9
Postexercise	120 ± 6	117 ± 6	272 ± 46
Lysine			
Basal	188 ± 10	200 ± 11	910 ± 48
Postexercise	172 ± 7	175 ± 7	867 ± 46
Alanine			
Basal	278 ± 30	324 ± 31	2,388 ± 117
Postexercise	250 ± 24	276 ± 25	3,741 ± 789

Values (means ± SE; nmol/ml whole blood or intracellular water) relative to femoral artery and vein are averages of measurements at steady state in postabsorptive resting state or 140–180 min after exercise. Values in muscle are relative to a single biopsy taken at rest or 180 min after exercise.

Table 2. Amino acid enrichments in basal state and during postexercise recovery

	Femoral Artery	Femoral Vein	Muscle
Phenylalanine			
Basal	0.0797 ± 0.0031	0.0607 ± 0.0028	0.0489 ± 0.0037
Postexercise	0.0758 ± 0.0026*	0.0648 ± 0.0029*	0.0340 ± 0.0028*
Leucine			
Basal	0.0763 ± 0.0024	0.0575 ± 0.0031	0.0334 ± 0.0038
Postexercise	0.0700 ± 0.0045	0.0588 ± 0.0025	0.0312 ± 0.0050
Lysine			
Basal	0.0800 ± 0.0039	0.0653 ± 0.0044	0.0281 ± 0.0030
Postexercise	0.0759 ± 0.0050	0.0632 ± 0.0033	0.0335 ± 0.0030
Alanine			
Basal	0.0607 ± 0.0036	0.0354 ± 0.0024	0.0140 ± 0.0013
Postexercise	0.0625 ± 0.0094	0.0383 ± 0.0029	0.0130 ± 0.0015

Values (means ± SE; tracer-to-tracee ratios) relative to femoral artery and vein are averages of measurements at steady state in postabsorptive resting state or 140–180 min after exercise. Values in muscle are relative to a single biopsy taken at rest or 180 min after exercise. * $P < 0.05$ vs. basal.

Then FSRs were calculated as follows (2)

$$\text{FSR} = \frac{\Delta E_p \cdot 1.5}{\{[E_{M(1)} + E_{M(2)}]/2\} \cdot T} \cdot 60 \cdot 100 \quad (10)$$

where $E_{M(1)}$ and $E_{M(2)}$ are the L -[ring- $^{13}\text{C}_6$]phenylalanine enrichments in the free muscle pool in the first and second biopsy, respectively. Average values between $E_{M(1)}$ and $E_{M(2)}$ were used as precursor enrichments for muscle protein synthesis. T indicates the time interval (min) between first and second biopsy. The factors 60 (min/h) and 100 are needed to express the FSR in percent per hour. Whole body amino acid rates of appearance were calculated by dividing the tracer infusion rates by the enrichments in the arterial blood.

Statistical analysis. Values are means ± SE. Results in the resting basal state and during the postexercise recovery period were compared with Student's paired t -test. $P \leq 0.05$ was taken as indicating a significant difference.

RESULTS

In the last 40 min of the resting and the exercise study, amino acid enrichments and concentrations in the femoral artery and vein were in near steady-state conditions. In Tables 1 and 2, whole blood and intramus-

cular values of free amino acid concentrations and enrichments in the basal resting state and during postexercise recovery are reported. Amino acid concentrations in all sampled pools did not change significantly after exercise. Arterial phenylalanine enrichment was lower after exercise than in the basal resting state. Consequently, whole body phenylalanine rate of appearance slightly but significantly increased after exercise from 0.63 ± 0.02 to $0.66 \pm 0.02 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($P < 0.05$). The arterial enrichment of the other amino acid did not change significantly after exercise.

In the resting state, leg blood flow was $2.92 \pm 0.52 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$ and increased ($P < 0.05$) after exercise to $5.56 \pm 0.52 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$. Consequently the rates of arterial delivery of amino acids from the systemic circulation to the leg (F_{in}) increased ($P < 0.05$) after exercise by ~80% to ~110%, depending on the individual amino acid (phenylalanine, from 129 ± 22 to $226 \pm 33 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$; leucine, from 337 ± 52 to $668 \pm 70 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$; lysine, from 557 ± 80 to $951 \pm 78 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$; alanine, from 820 ± 141 to $1,404 \pm 222 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$). Figure 2 shows the values of inward transport from artery to muscle cells of individual amino acids in the basal state and after exercise. Transport rates of leucine, lysine, and alanine significantly ($P < 0.05$) increased after exercise by ~60% to ~120%, depending on the individual amino acid, whereas transport of phenylalanine did not change significantly.

Figure 3 shows the calculated values of muscle protein kinetics at rest and after exercise. Values are expressed as the rate of intracellular phenylalanine utilization for protein synthesis ($F_{0,M}$) and appearance from protein breakdown ($F_{M,0}$; $\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$). After exercise, protein synthesis was approximately double the resting value. Protein breakdown also increased after exercise, but only ~50% with respect to the resting value. Consequently protein balance improved (became less negative) but did not shift from release to net uptake. These observations are also confirmed by the data obtained with the other essential amino acids (Table 3). In fact, the rates of intracellular appearance

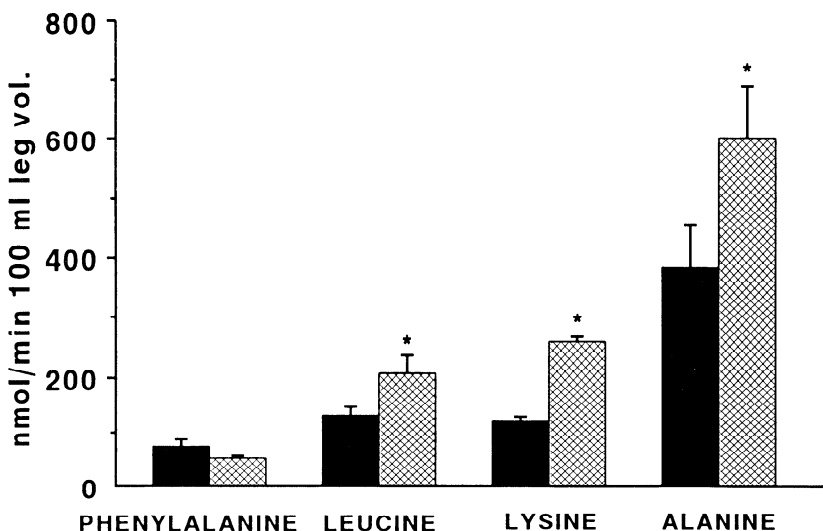


Fig. 2. Rates of inward amino acid transport from femoral artery into muscle cells (F_{MA}) at rest (filled bars) and ~3 h after exercise (cross-hatched bars). * $P < 0.05$, postexercise vs. rest.

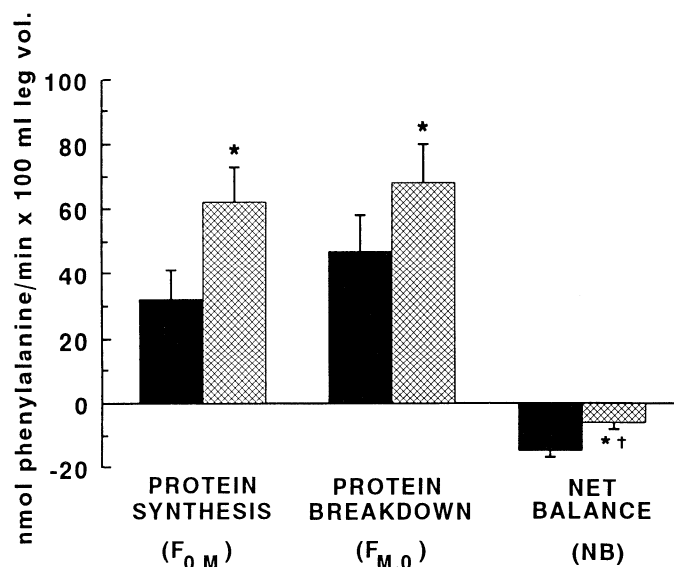


Fig. 3. Model-derived values of muscle protein synthesis, breakdown, and net protein balance at rest (filled bars) and ~3 h after exercise (crosshatched bars), expressed as rate of intracellular phenylalanine utilization for protein synthesis ($F_{0,M}$) and release from protein breakdown ($F_{M,0}$). * $P < 0.05$, postexercise vs. rest; † not significantly different from zero.

from proteolysis ($F_{M,0}$) of leucine and lysine increased after exercise (Table 3). Also lysine incorporation into muscle protein ($F_{0,M}$) increased after exercise. In agreement with the model-derived values of protein kinetics, the values of muscle protein synthesis obtained with the direct incorporation technique (Fig. 4) increased ($P < 0.05$) during the postexercise period.

DISCUSSION

The purpose of this study was to examine the changes of muscle protein kinetics and of amino acid transport ~3 h after a heavy-resistance exercise routine in untrained postabsorptive volunteers. We found that, after exercise, the rates of muscle protein turnover and amino acid transport were increased. Protein synthesis and

Table 3. Parameters of intracellular leucine, lysine, and alanine kinetics in basal state and 3 h after exercise

	$F_{0,M}$	$F_{M,0}$	NB
Leucine			
Basal	151 ± 18	171 ± 17	-20 ± 11
Postexercise	288 ± 63	271 ± 17	17 ± 9*
Lysine			
Basal	202 ± 8	231 ± 13	-29 ± 6
Postexercise	322 ± 23*	333 ± 24*	-11 ± 5*
Alanine			
Basal	1,170 ± 215	1,296 ± 217	-126 ± 8
Postexercise	2,028 ± 209*	2,165 ± 199*	-137 ± 15

Values are means ± SE in $\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$. NB, net amino acid balance across leg (negative numbers indicate net release); $F_{M,0}$, intracellular amino acid appearance (i.e., proteolysis for leucine and lysine, proteolysis plus de novo synthesis for alanine); $F_{0,M}$, intracellular amino acid utilization (i.e., protein synthesis for lysine, protein synthesis plus other fates for leucine and alanine). Phenylalanine data are shown in Fig. 2. * $P < 0.05$ vs. basal.

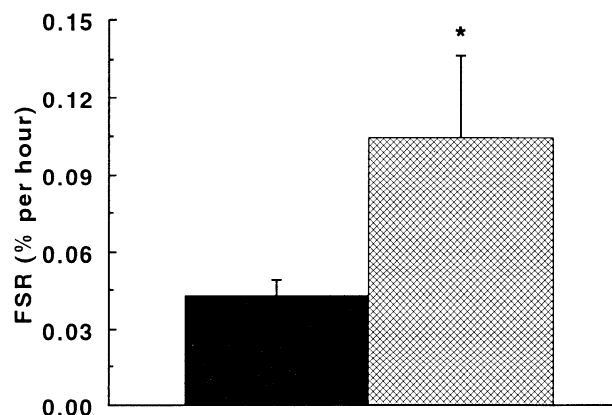


Fig. 4. Fractional rate of muscle protein synthesis (FSR) at rest (filled bar) and after exercise (crosshatched bar) measured by direct incorporation technique. Postexercise value is relative to entire incorporation period, i.e., first 3 h after exercise. * $P < 0.05$, postexercise vs. rest.

breakdown increased simultaneously but to a different extent. Synthesis increased by ~100%, whereas breakdown increased by only ~50%. Consequently, protein balance (synthesis minus breakdown) improved after exercise (becoming not significantly different from zero) but did not shift to a positive value. These results suggest that physical exercise can restrain net muscle protein catabolism but does not directly promote net protein deposition in the postabsorptive state. Thus exercise probably needs to interact with other factors, such as feeding, to promote muscle anabolism.

In this study, the intracellular appearance of leucine, lysine, and alanine was greatly increased after exercise because of a simultaneous acceleration of transmembrane amino acid transport ($F_{M,A}$) and of breakdown of endogenous protein ($F_{M,0}$). Such increased availability of intracellular free amino acids may have caused the postexercise stimulation of protein synthesis. An acute stimulation of protein synthesis mediated by an increased availability of free amino acids suggests a post-transcriptional mechanism, in agreement with the observation that RNA concentrations in muscle cells do not change immediately after exercise (7, 27, 28). The notion that increased amino acid availability can directly regulate protein synthesis is further supported by the fact that the rate of synthesis was enhanced during amino acid infusion (12) or in catabolic patients, in whom a large primary increase of breakdown occurs (3, 23b). In the present study therefore the acceleration of protein breakdown and amino acid transport may have contributed to the increase in protein synthesis. Because of the increase in amino acid transport, the changes in protein degradation have been more than offset by the increased rate of synthesis.

Our new method, which combines the arteriovenous catheterization technique and the muscle biopsy, allows the direct determination of the intracellular amino acid appearance from protein breakdown. The present study represents therefore the first direct assessment of the response of muscle protein breakdown to recovery from exercise in humans. We found that, after exercise, the absolute rate of protein breakdown was accelerated. This catabolic response almost counteracted the in-

crease in protein synthesis. Thus, by relying only on the protein synthetic measurements, a considerable overestimation of the net anabolic effect of the exercise could have resulted. As a result of the simultaneous increases in protein synthesis and breakdown, the overall turnover of muscle protein increased after exercise.

The mechanism responsible for the increased degradation of muscle protein after exercise is unknown. Studies in rats indicate that lysosomal proteinases and cytoplasmic systems are involved in the exercise-induced increase in the rate of muscle protein degradation (9, 10, 15, 19). Furthermore severe exercise, as used in our study, can result in muscle damage and inflammation (11). Such injuries are known to attract phagocytic cells (11), which may account for some of the proteolytic activity detected in our study.

By use of different approaches, other studies showed that physical exercise may increase (10, 20, 21, 24), decrease (4), or not modify (5, 6, 8, 13, 24) the rate of protein breakdown. These discrepancies are likely to be due to the inadequacy of the methodologies used to measure this parameter of protein metabolism in vivo. The urinary excretion of 3-methylhistidine has been used as an index of myofibrillar protein degradation after exercise (24). However, its use has been criticized, because 3-methylhistidine may also be released from skin and intestine (19, 22). The rate of appearance in plasma of an essential amino acid such as leucine has also been used as an index of protein breakdown at the whole body level (23a, 24). However, from this measurement, it is not possible to extrapolate conclusions regarding skeletal muscle, because other tissue may significantly contribute to the systemic amino acid appearance.

The arteriovenous catheterization technique across a limb (forearm or leg) combined with the systemic infusion of the isotopic tracer of an amino acid that is neither synthesized nor oxidized in muscle, i.e., phenylalanine, has been used to evaluate muscle protein metabolism (12). This approach, while preferable to the methods cited above, is still not accurate and underestimates the actual values of muscle protein breakdown by the amount of amino acids released from breakdown that do not appear in plasma, because they are directly utilized for synthesis. In fact, when in our study phenylalanine rate of appearance across the leg was calculated according to Gelfand et al. (12), there was no significant effect of exercise (from 32 ± 5 to 34 ± 5 $\text{nmol} \cdot \text{min}^{-1} \cdot 100$ ml leg^{-1}). Furthermore, in the current study, whole body phenylalanine rate of appearance in plasma increased only slightly after exercise, whereas the whole body leucine and lysine rates of appearance did not significantly change after exercise. Such a small effect of exercise on blood amino acid kinetics, compared with the increase of muscle protein breakdown of $\sim 50\%$ detected in this study, shows the inadequacy of the traditional techniques based on plasma measurements to assess the intracellular muscle protein metabolism.

In this study, we have assessed the postexercise changes of muscle protein synthesis by using two different techniques simultaneously. The results obtained

with both methods were in agreement. Protein synthesis, measured as the rate of amino acid (phenylalanine and lysine) disappearance from the intracellular pool or as the fractional synthesis rate of protein, increased after exercise. Nonetheless these two methods are not equivalent. The arteriovenous method provided an assessment of protein synthesis and breakdown at ~ 3 h after exercise, whereas by the direct tracer incorporation technique, protein synthesis was measured over the entire incorporation period, i.e., between the first biopsy immediately after exercise and the second biopsy 3 h after exercise.

In our study, arterial amino acid concentrations did not change after exercise, but leg blood flow increased by $\sim 90\%$ with respect to the basal resting values. Consequently the rate of amino acid delivery to muscle tissue increased proportionally (F_{in}), if it is assumed that no changes in blood flow distribution to leg tissues occurred. The fact that, after exercise, the rates of leucine, lysine, and alanine transport from the arterial blood to muscle increased by the same magnitude (60–120%) suggests that such increased amino acid delivery to the tissue may have enhanced amino acid transport. The importance of blood flow in determining substrate uptake by tissues has been already shown for glucose (1). Our study suggests that this mechanism may also be important for amino acid and protein metabolism. Thus physical exercise may not have a direct regulatory effect on the membrane transport systems, but its effect may be due to the increased amino acid delivery to muscle tissue secondary to the increased blood flow.

The intracellular availability of amino acids may not be the sole acute regulator of muscle protein synthesis, inasmuch as hormones and other factors may have direct effects. Nonetheless it seems clear that the rates of breakdown and inward amino acid transport are important factors. The importance of variations in inward transport can be appreciated when the difference between the anabolic response to exercise is compared with the catabolic response to critical illness. In both circumstances, the rate of breakdown is increased (3, 23b), but in the case of critical illness, inward transport is relatively impaired, rather than stimulated (3). As a consequence, muscle synthesis is not stimulated to the same extent as breakdown, with net catabolism resulting. Thus the increase in inward transport after exercise appears to be an important response that enables synthesis to increase to a greater extent than breakdown.

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REFERENCES

1. Baron, A. D. Hemodynamic actions of insulin. *Am. J. Physiol.* 267 (Endocrinol. Metab. 30): E187–E202, 1994.
2. Biolo, G., R. Y. D. Fleming, S. P. Maggi, and R. R. Wolfe. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am. J. Physiol.* 268 (Endocrinol. Metab. 31): E75–E84, 1995.

3. Biolo, G., S. P. Maggi, R. Y. D. Fleming, D. N. Herndon, and R. R. Wolfe. Relationship between transmembrane amino acid transport and protein kinetics in muscle tissue of severely burned patients (Abstract). *Clin. Nutr.* 12: 4, 1993.
4. Bylund-Fellenius, A. C., K. M. Ojamaa, K. E. Flaim, J. B. Li, S. J. Wassner, and L. S. Jefferson. Protein synthesis versus energy state in contracting muscles of perfused rat hindlimb. *Am. J. Physiol.* 246 (*Endocrinol. Metab.* 9): E297–E305, 1984.
5. Carraro, F., C. A. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolfe. Whole body and plasma protein synthesis in exercise and recovery in human subjects. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E821–E831, 1990.
6. Carraro, F., C. A. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolfe. Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 259 (*Endocrinol. Metab.* 22): E470–E476, 1990.
7. Chesley, A., J. D. MacDougall, M. A. Tarnopolsky, S. A. Atkinson, and K. Smith. Changes in human muscle protein synthesis after resistance exercise. *J. Appl. Physiol.* 73: 1383–1388, 1992.
8. Devlin, J. T., I. Brodsky, A. Scrimgeour, S. Fuller, and B. M. Bier. Amino acid metabolism after intense exercise. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E249–E255, 1990.
9. Dohm, G. L., G. J. Kasperek, E. B. Tapscott, and G. R. Beecher. Effect of exercise on synthesis and degradation of muscle protein. *Biochem. J.* 188: 255–262, 1980.
10. Dohm, G. L., R. T. Williams, G. J. Kasperek, and A. M. van Rij. Increased excretion of urea and *N*^γ-methylhistidine by rats and humans after a bout of exercise. *J. Appl. Physiol.* 52: 27–33, 1982.
11. Evans, W. J., and J. G. Cannon. The metabolic effects of exercise-induced muscle damage. *Exercise Sport Sci. Rev.* 19: 99–125, 1991.
12. Gelfand, R. A., M. G. Glickman, P. Castellino, R. J. Louard, and R. A. DeFronzo. Measurement of L-[1-¹⁴C]leucine kinetics in splanchnic and leg tissues in humans: effect of infusion. *Diabetes* 37: 1365–1372, 1988.
13. Hickson, J. F., I. Wolinsky, G. P. Rodriguez, J. M. Pivarnik, M. C. Kent, and N. W. Shier. Failure of weight training to affect urinary indices of protein metabolism in men. *Med. Sci. Sports Exercise* 18: 563–567, 1986.
14. Kasperek, G. J., G. R. Conway, D. S. Krayeski, and J. J. Lohne. A reexamination of the effect of exercise on rate of muscle protein degradation. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E1144–E1150, 1992.
15. Kasperek, G. J., and R. D. Snider. Total and myofibrillar protein degradation in isolated soleus muscles after exercise. *Am. J. Physiol.* 257 (*Endocrinol. Metab.* 20): E1–E5, 1989.
16. Lüthi, J. M., H. Howald, H. Claassen, K. Rösler, P. Vock, and H. Hoppeler. Structural changes in skeletal muscle tissue with heavy resistance exercise. *Int. J. Sports Med.* 7: 123–127, 1986.
17. MacDougall, J. D. Morphological changes in human skeletal muscle following strength training and immobilization. In: *Human Muscle Power*, edited by N. L. Jones, N. McCartney, and A. J. McComas. Champaign, IL: Human Kinetics, 1986, p. 269–288.
19. Millward, D. J., P. C. Bates, G. K. Grimble, J. G. Brown, M. Nathan, and M. J. Rennie. Quantitative importance of non-skeletal-muscle of *N*^γ-methylhistidine in urine. *Biochem. J.* 190: 225–228, 1980.
20. Pivarnik, J. M., J. F. Hickson, and I. Wolinsky. Urinary 3-methylhistidine excretion increases with repeated weight training exercise. *Med. Sci. Sports Exercise* 21: 283–287, 1989.
21. Rennie, M. J., R. H. T. Edwards, S. Krywawych, D. Halliday, J. C. Waterlow, and D. J. Millward. Effect of exercise on protein turnover in man. *Clin. Sci. Lond.* 61: 627–639, 1981.
22. Rennie, M. J., and D. J. Millward. 3-Methylhistidine excretion and urinary 3-methylhistidine/creatinine ratio are poor indicators of skeletal muscle protein breakdown. *Clin. Sci. Lond.* 65: 217–225, 1983.
23. Saltin, B., and P. Gollnick. Skeletal muscle adaptability: significance for metabolism and performance. In: *Handbook of Physiology. Skeletal Muscle*. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 10, p. 555–631.
- 23a. Tarnopolsky, M. A., S. A. Atkinson, J. D. MacDougall, B. B. Senor, P. W. R. Lemon, and H. Swarczew. Whole body leucine metabolism during and after resistance exercise in fed humans. *Med. Sci. Sports Exercise* 23: 326–333, 1991.
- 23b. Wolfe, R. R. Nutrition and metabolism in burns. In: *Critical Care: State of the Art*, edited by B. Chernow and W. C. Shoemaker. Fullerton, CA: Soc. Crit. Care Med., 1986, vol. 7, p. 19–62.
24. Wolfe, R. R. Does exercise stimulate protein breakdown in humans? Isotopic approaches to the problem. *Med. Sci. Sports Exercise* 19: 172–178, 1987.
25. Wolfe, R. R. Radioactive and stable isotope tracers in biomedicine. In: *Principles and Practice of Kinetics Analysis*. New York: Wiley-Liss, 1992, p. 417–419.
27. Wong, T. S., and F. W. Booth. Protein metabolism in rat tibialis anterior muscle after stimulated chronic concentric exercise. *J. Appl. Physiol.* 69: 1709–1717, 1990.
28. Wong, T. S., and F. W. Booth. Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *J. Appl. Physiol.* 69: 1718–1724, 1990.
29. Zorzano, A., T. W. Balon, L. P. Garetto, M. N. Goodman, and N. B. Ruderman. Muscle α -aminoisobutyric acid transport after exercise: enhanced stimulation by insulin. *Am. J. Physiol.* 248 (*Endocrinol. Metab.* 11): E546–E552, 1985.