Insulin Action on Muscle Protein Kinetics and Amino Acid Transport During Recovery After Resistance Exercise

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We have determined the individual and combined effects of insulin and prior exercise on leg muscle protein synthesis and degradation, amino acid transport, glucose uptake, and alanine metabolism. Normal volunteers were studied in the postabsorptive state at rest and about 3 h after a heavy leg resistance exercise routine. The leg arteriovenous balance technique was used in combination with stable isotopic tracers of amino acids and biopsies of the vastus lateralis muscle. Insulin was infused into a femoral artery to increase the leg insulin concentrations to high physiologic levels without substantively affecting the whole-body level. Protein synthesis and degradation were determined as rates of intramuscular phenylalanine utilization and appearance, and muscle fractional synthetic rate (FSR) was also determined. Leg blood flow was greater after exercise than at rest ($P < 0.05$). Insulin accelerated blood flow at rest but not after exercise ($P < 0.05$). The rates of protein synthesis and degradation were greater during the postexercise recovery (65 ± 10 and 74 ± 10 nmol · min^{-1} · 100 ml^{-1} leg volume, respectively) than at rest (30 ± 7 and 46 ± 8 nmol · min^{-1} · 100 ml^{-1} leg volume, respectively; $P < 0.05$). Insulin infusion increased protein synthesis at rest (51 ± 4 nmol · min^{-1} · 100 ml^{-1} leg volume) but not during the postexercise recovery (64 ± 9 nmol · min^{-1} · 100 ml^{-1} leg volume; $P < 0.05$). Insulin infusion at rest did not change the rate of protein degradation (48 ± 3 nmol · min^{-1} · 100 ml^{-1} leg volume). In contrast, insulin infusion after exercise significantly decreased the rate of protein degradation (52 ± 9 nmol · min^{-1} · 100 ml^{-1} leg volume). The insulin stimulatory effects on inward alanine transport and glucose uptake were three times greater during the postexercise recovery than at rest ($P < 0.05$). In contrast, the insulin effects on phenylalanine, leucine, and lysine transport were similar at rest and after exercise. In conclusion, the ability of insulin to stimulate glucose uptake and alanine transport and to suppress protein degradation in skeletal muscle is increased after resistance exercise. Decreased amino acid availability may limit the stimulatory effect of insulin on muscle protein synthesis after exercise. *Diabetes* 48:949–957, 1999

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BF, blood flow; FSR, fractional synthetic rate; GCMS, gas-chromatography mass spectrometry; HPLC, high-performance liquid chromatography; IR, infusion rate; IRMS, isotope-ratio mass spectrometer; NAP, nitrogen-acetyl-*N*-propyl ester; NB, net balance; PD, priming doses; RM, repetition maximum.

Insulin and physical exercise are well-known regulators of both protein (1,2) and glucose (3,4) metabolism. Evidence indicates that the ability of insulin to stimulate glucose uptake in skeletal muscle is enhanced after exercise (5). The aim of this study was to determine if prior exercise augments insulin-sensitive processes involving amino acid and protein metabolism, including protein synthesis, protein breakdown, amino acid transport, and de novo synthesis of nonessential amino acids.

It is well known that physical activity, particularly resistance exercise, increases skeletal muscle mass (6,7). Studies suggest that muscle protein accretion occurs in the recovery phase after exercise rather than during the actual exercise period (8,9). After exercise, the rates of protein synthesis and breakdown are simultaneously accelerated (10). In this condition, the anabolic efficacy of amino acid administration on muscle protein deposition is increased (11). It is not known if the anabolic effect of insulin is also increased after exercise.

The mechanisms by which insulin can affect muscle protein kinetics and amino acid transport have been widely investigated in the last few years. Physiologic hyperinsulinemia studied in the setting of constant systemic amino acid concentrations increased muscle protein synthesis and did not significantly change protein breakdown in skeletal muscle of resting normal volunteers (12). Other studies have shown a potential suppressive effect of insulin on muscle protein breakdown (13–16). Insulin also stimulates the transmembrane transport of selected amino acids; however, this effect does not appear to be a primary mediator of the anabolic insulin action on muscle (17,18). Insulin also stimulates the de novo synthesis of alanine in skeletal muscle (12) by increasing the rate of glycolysis and pyruvate availability.

In this study, we have investigated the interaction between the effects of insulin and exercise on the rates of muscle protein synthesis and breakdown and amino acid transport in untrained normal volunteers. Kinetic parameters were determined in the basal resting state and during recovery after a heavy resistance exercise routine in the postabsorptive state and during physiologic hyperinsulinemia. Measurements were performed in the exercised muscles using stable isotopic tracers of amino acids and combination of the arteriovenous catheterization and muscle biopsy techniques (18).

RESEARCH DESIGN AND METHODS

Subjects. Five healthy male volunteers were studied in the postabsorptive state. Mean (± SE) age was 29 ± 5 years, body weight was 73 ± 5 kg, height was 170 ±

3 cm, and BMI was 25 ± 1 kg/cm^2. Leg volume, estimated using an anthropometric approach (19), was 10,327 ± 657 ml. 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. No subject had been engaged in a regular exercise training program for at least 1 year before the study. 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. For at least 1 month before each study, the subjects consumed a weight-maintaining diet containing 15–20% protein. Each subject was studied three times, once at rest and twice immediately after leg resistance workouts. In the resting study, the subject was studied in the basal postabsorptive state and during hyperinsulinemia. In the two postexercise studies, the subject was studied in the postabsorptive state or during hyperinsulinemia. The sequence of the three studies was randomized, with an interval of 2–3 weeks between each study. Five to ten days before the first study, subjects were familiarized with the exercise protocol, and their 10- and 12-repetition maximums (RMs) (the maximum weight a subject can possibly lift for 10 and 12 repetitions) were determined. The average 10 RM of the knee extensor muscles (two legs) was 64 ± 6 kg. In each subject, the entire study protocol was completed within 5–6 weeks.

Isotopes. L-[1-^{13}C]Leucine (99% enriched), L-[ring-$^{13}C_6$]phenylalanine (99% enriched), and L-[2,3,3,3-$^2H_4$]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-$^2H_2$]lysine (98% enriched), and L-[ring-$^2H_5$]phenylalanine (98% enriched) were purchased from Tracer Technologies (Somerville, MA). L-[1-^{13}C]Alanine (99% enriched) was purchased from Isotec (Miamisburg, OH).

Resting: basal postabsorptive state and hyperinsulinemia. The subjects were admitted to the Clinical Research Center of the University of Texas Medical Branch at Galveston at 6:00 A.M. after an overnight fast. An 18-gauge polyethylene catheter was inserted into the left antecubital vein for infusion of labeled amino acids. Using flexible guide wires, two 8-cm-long polyethylene catheters (Cook, Bloomington, IN) were inserted for blood sampling, one in the right femoral artery and one in the vein. The femoral artery catheter was also used for continuous infusion of insulin and primed-continuous infusion of indocyanine green (Becton Dickinson Microbiology Systems, Cockeysville, MA). Systemic concentrations of insulin and indocyanine green were measured in the right arterialized wrist vein, which was cannulated with a 20-gauge polyethylene catheter and maintained at approximately 65°C. Catheters were inserted using lidocaine. Patency of catheters was maintained by saline infusion.

After obtaining a blood sample for measurement of background amino acid enrichment and indocyanine green concentration, the infusion protocol was initiated. First, 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, 0.05 µmol · kg^{-1} · min^{-1} (IR) and 2 µmol/kg (PD); L-[1-^{13}C]leucine, 0.08 µmol · kg^{-1} · min^{-1} and 4.8 µmol/kg; L-[2-^{15}N]lysine, 0.08 µmol · kg^{-1} · min^{-1} and 7.2 µmol/kg; L-[1-^{13}C]alanine, 0.35 µmol · kg^{-1} · min^{-1} and 35 µmol/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-$^{13}C_6$]phenylalanine (10–12,18). Isotopic steady states in the free amino acid pools in blood and muscle were required to calculate intracellular amino acid kinetics at the end of the basal and insulin periods, that is, between 180 and 240 min and between 360 and 420 min. Measurement of FSR by the incorporation of L-[ring-$^{13}C_6$]phenylalanine required steady-state enrichment of the precursor during the incorporation period, that is, between 60 and 240 min and between 240 and 420 min (10–12,18).

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, about 20 cm above the knee, using a 4-mm Bergström biopsy needle (Stille, Stockholm, Sweden) (20). Approximately 30–50 mg of muscle tissue was 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 in the basal state, a primed-continuous infusion of indocyanine green dye (IR, 0.5 mg/min; PD, 5 mg) was started at 165 min into the femoral artery and maintained until 240 min (20,21). Between 180 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 seconds and then quickly resumed. Arterial samples were always taken after samples from the femoral and wrist veins to avoid interference with the blood flow measurement. At 240 min (end of basal period), the second muscle biopsy was taken. After the basal period, insulin was infused directly into the femoral artery at 0.15 mU · min^{-1} · 100 ml^{-1} leg volume for 3 h. Blood glucose was maintained at the basal euglycemic levels by means of appropriate 5% dextrose infusion. 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 stopping tracer and insulin infusions, the third muscle biopsy was taken from the vastus lateralis muscle of the right leg.

Postexercise recovery: basal postabsorptive state. At 6:00 A.M., after an overnight fast, 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 subjects warmed up for 10 min with easy (<100 watts) cycling on a cycle ergometer. Then the following resistance exercise routine was completed in about 40 min: incline leg press (five sets of 10 repetitions, 12 RM), Nautilus duo-squat, leg curls, and leg extensions (four sets of eight repetitions each, 10 RM). Each set was completed in approximately 30 s with a 2- to 3-min rest between sets. After completion of the exercise routine, the femoral arterial and venous catheters as well as the right wrist vein catheter were inserted; the first muscle biopsy was performed; L-[1-^{13}C]leucine, L-[2-^{15}N]lysine, and L-[1-^{13}C]alanine infusions were started; and the protocol continued as described for the resting study.

Postexercise recovery: hyperinsulinemia. As described above, the subjects started the exercise protocol after catheter insertion, blood drawing, and isotope administration. After completion of the exercise routine, the femoral arterial and venous catheters as well as the right wrist vein catheter were inserted, and the first muscle biopsy was performed. Then, intravenous infusions of L-[1-^{13}C]leucine, L-[2-^{15}N]lysine, and L-[1-^{13}C]alanine and the intraarterial insulin infusion were started as described. Blood glucose was maintained at basal euglycemic levels by means of appropriate 5% dextrose infusion. Between 180 and 240 min, the measurement of leg blood flow was repeated, and blood samples were taken as described for the basal period. At 240 min, before stopping tracer and insulin infusions, the third muscle biopsy was taken from the vastus lateralis muscle of the right leg.

Analysis. Concentrations of selected amino acids (phenylalanine, leucine, lysine, and alanine) and isotopic enrichment of infused tracers were measured in whole blood samples taken from the femoral artery and vein as described (10–12,18,22). The stable isotopes L-[ring-$^2H_5$]phenylalanine, L-[1,2-$^{13}C_2$]leucine, L-[1,2-$^{13}C_2$,6,6-$^2H_2$]lysine, and L-[2,3,3,3-$^2H_4$]alanine were added to the tubes as internal standards (10–12,18). 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 (10–12,18,22). Blood samples from the femoral and arterialized wrist veins were collected to measure indocyanine green concentration in serum, as previously described (21,23). Leg plasma flow was calculated from steady-state values of dye concentration in the femoral and arterialized wrist veins (21,23). 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 as those used for the blood samples, but in different proportion, was added to the tissue and thoroughly mixed (10–12,18). The tissue was then homogenized and centrifuged, and the supernatant was collected to produce the NAP derivatives of intracellular free amino acids (10–12,18,22). 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, Serono, Italy). The resulting CO_2 gas was automatically injected into an isotope-ratio mass spectrometer (IRMS) (VG Isogas; VG Instruments, Middlewich, England) for determination of the $^{13}C/^{12}C$ isotope ratio in protein-bound phenylalanine.

The isotopic enrichment of free amino acids in blood and muscle samples was determined by gas-chromatography mass spectrometry (GCMS) (model 5985; Hewlett-Packard, Palo Alto, CA) by chemical ionization and selected ion monitoring (10–12,18). Data were expressed as tracer/tracee ratio, with correction for the contribution of isotopomers of small weight to the apparent enrichment of isotopomers with a greater mass (10–12,18). Enrichment of L-[ring-$^{13}C_6$]phenylalanine was further corrected using a factor, 0.93, to account for an overestimation of enrichment due to the different isotopomer distribution of the tracer and the naturally occurring phenylalanine (10–12,18). Concentrations of free amino acids in blood and total muscle water were calculated as described (10–12,18).

Calculations. The kinetics of free amino acids in leg muscle have been determined according to the model described (18). Briefly, amino acids enter and leave the leg via the femoral artery and femoral vein, respectively. The unidirectional flow of free amino acids from artery to intramuscular compartment is determined by the model as rate of inward amino acid transport. The rate of intracellular appearance for the essential amino acids phenylalanine, leucine, and lysine defines the

rate of release from protein breakdown. In the case of alanine, the rate of intracellular appearance represents the sum of release from protein breakdown and de novo synthesis from pyruvate. Since phenylalanine and lysine are not oxidized in muscle (24), the rate of intracellular utilization for these amino acids refers to the rate of utilization for protein synthesis. In the case of leucine, this figure represents utilization for protein synthesis plus oxidation. In the case of alanine, intracellular utilization represents utilization for protein synthesis plus rate of nonprotein utilization. Each kinetic parameter is defined as follows (see Biolo et al. [18] for the derivation of the equations):

$$NB = (C_A - C_V) \times BF \quad (1)$$

$$\text{Inward transport} = \{[(E_M - E_V)/(E_A - E_M)] \times C_V + C_A\} \times BF \quad (2)$$

$$\text{Intracellular utilization} = [(C_A \times E_A - C_V \times E_V) \times BF]/E_M \quad (3)$$

$$\text{Intracellular appearance} = \{[(C_A \times E_A - C_V \times E_V) \times BF]/E_M\} - NB \quad (4)$$

where NB is net balance; C_A and C_V are free amino acid concentrations in the femoral artery and vein, respectively; BF is leg blood flow; and E_M, E_V, and E_A are amino acid enrichments in the vastus lateralis muscle, femoral artery, and femoral vein, respectively.

We have assumed that amino acids are released from proteolysis and incorporated into protein in proportion to their relative content in muscle protein. Therefore, the rates of alanine appearance from protein breakdown and utilization for protein synthesis can be calculated from the value of intracellular appearance and utilization of phenylalanine and the molar ratio alanine/phenylalanine in muscle protein (18). The rates of alanine de novo synthesis and nonprotein utilization were then calculated from the rates of total intracellular alanine utilization and appearance, respectively, as follows:

$$\text{Nonprotein alanine utilization} = \text{intracellular alanine utilization} - (\text{intracellular phenylalanine utilization} \times 2.80) \quad (5)$$

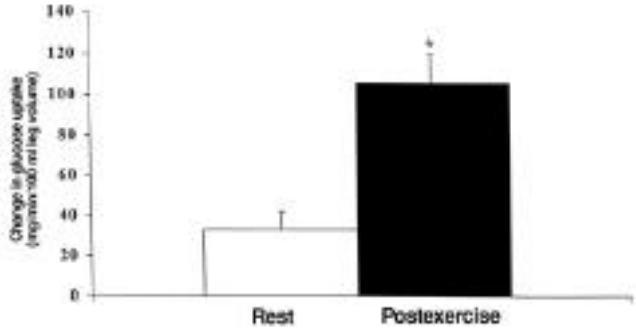

FIG. 1. Insulin-mediated changes of glucose uptake across the leg at rest and in the postexercise recovery. Values are changes from basal (i.e., rest and postexercise without insulin, respectively). *$P < 0.05$ postexercise vs. rest.

$$\text{De novo alanine synthesis} = \text{intracellular alanine appearance} - (\text{intracellular phenylalanine appearance} \times 2.80) \quad (6)$$

where 2.80 is the molar ratio alanine/phenylalanine in human muscle protein (18). The vastus lateralis muscle has been considered to be representative of the total leg muscle.

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-$^{13}C_6$]phenylalanine tracer/tracee ratio, by the enrichment of the precursor, i.e., free intracellular L-[ring-$^{13}C_6$]phenylalanine tracer/tracee ratio (18). Delta increments of protein-bound L-[ring $^{13}C_6$]phenylalanine enrichment during the 3-h incorpo-

TABLE 1
Free amino acid concentration in femoral artery and vein and skeletal muscle

	Femoral artery (nmol/ml whole blood)	Femoral vein (nmol/ml whole blood)	Muscle free pool (nmol/ml intracellular water)
Phenylalanine			
Rest			
Basal	43 ± 3	49 ± 3	130 ± 16
Insulin	41 ± 2	40 ± 2*	108 ± 15*
Postexercise			
Basal	40 ± 8	42 ± 4	77 ± 13
Insulin	40 ± 4	38 ± 4†	54 ± 6†
Leucine			
Rest			
Basal	113 ± 7	120 ± 9	219 ± 9
Insulin	98 ± 6*	92 ± 6*	138 ± 9*
Postexercise			
Basal	119 ± 7	116 ± 6	272 ± 46
Insulin	85 ± 7*†	79 ± 7*†	169 ± 16*
Lysine			
Rest			
Basal	190 ± 10	198 ± 11	1,016 ± 114
Insulin	181 ± 9	179 ± 11*	727 ± 67*
Postexercise			
Basal	173 ± 6	176 ± 7	805 ± 69
Insulin	178 ± 9	172 ± 9	632 ± 70†
Alanine			
Rest			
Basal	293 ± 21	333 ± 29	2,386 ± 117
Insulin	274 ± 24	295 ± 26*	2,616 ± 260
Postexercise			
Basal	253 ± 24	274 ± 27	3,742 ± 789‡
Insulin	331 ± 34	318 ± 31	3,061 ± 629*

Data are means ± SE. *$P < 0.05$ insulin vs. basal; †$P < 0.05$, postexercise insulin vs. rest basal; ‡$P < 0.05$, postexercise basal vs. rest basal.

ration periods (ΔE_P) were obtained from the IRMS measurements of the protein-bound phenylalanine enrichment in the first and second biopsy as described (9). Then, FSRs were calculated as follows (18):

$$FSR = \Delta E_P/\{[(E_{M(1)} + E_{M(2)})/2] \times T\} \times 60 \times 100 \quad (7)$$

where $E_{M(1)}$ and $E_{M(2)}$ are the L-[ring-$^{13}C_6$]phenylalanine enrichments in the free muscle pool in the biopsies at the beginning and at the end of the incorporation period, 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 used to express the FSR in percent per hour.

Statistical analysis. Data were expressed as mean ± SE. Results in the four different experimental conditions—resting postabsorptive state, resting hyperinsulinemia, postabsorptive state after exercise, and hyperinsulinemia after exercise—were compared with analysis of variance with randomized block design followed by Duncan test. The insulin effects at rest and after exercise were compared using the Student's paired t test. A P value ≤0.05 was taken as indicating a significant difference.

RESULTS

Plasma insulin concentration in the basal resting state was 60 ± 12 pmol/l. After intraarterial insulin infusion, the hormone concentration in the femoral vein increased at rest and after exercise to 444 ± 56 and 360 ± 48 pmol/l, respectively (P < 0.05). Insulin infusion also caused insulin concentrations to increase slightly in the systemic circulation (measured in the arterialized wrist vein) to 115 ± 19 and 121 ± 21 pmol/l at rest and after exercise, respectively (P < 0.05). After exercise, without insulin infusion, plasma insulin concentration (54 ± 11 pmol/l) did not change with respect to the basal resting value. Plasma glucose concentrations were similar both in the basal resting state and after exercise and were maintained at approximately the same level during insulin infusions at rest and after exercise by intravenous glucose infusion at variable rates. In the last hour of the insulin infusion studies at rest and after exercise, the glucose infusion rate was stable and averaged 1.61 ± 0.52 and 2.12 ± 0.41 mg · min^{-1} · kg^{-1}, respectively.

In the basal postabsorptive state, the rate of glucose uptake across the leg was similar at rest (8 ± 2 mg · min^{-1} · 100 ml^{-1} leg volume) and after exercise (8 ± 5 mg · min^{-1} · 100 ml^{-1} leg volume). Insulin infusion significantly increased glucose uptake at rest (40 ± 9 mg · min^{-1} · 100 ml^{-1} leg volume) and after exercise (114 ± 17 mg · min^{-1} · 100 ml^{-1} leg volume) (P < 0.05). The rate of glucose uptake was significantly greater during hyperinsulinemia after exercise than during hyperinsulinemia at rest (P < 0.05). Figure 1 shows the insulin-mediated changes from the basal postabsorptive state of leg muscle glucose uptake at rest and after exercise. During the postexercise recovery, the insulin-mediated increase of glucose uptake across the leg was about three times greater than that at rest (P < 0.05).

Amino acid concentrations and enrichments in the femoral artery and vein were in steady-state conditions in the last 40 min of each of the four periods. In Tables 1 and 2, average values of free amino acid concentrations and enrichments in the femoral artery and vein and in muscle are reported. Arterial values of leucine concentrations significantly decreased during insulin infusion both at rest and after exercise,

TABLE 2
Free amino acid enrichments in femoral artery and vein and skeletal muscle

	Femoral artery	Femoral vein	Muscle-free pool
Phenylalanine			
Rest			
Basal	0.0800 ± 0.0031	0.0608 ± 0.0031	0.0488 ± 0.0037
Insulin	0.0905 ± 0.0026*	0.0746 ± 0.0033*	0.0546 ± 0.0044
Postexercise			
Basal	0.0764 ± 0.0024†	0.0642 ± 0.0029	0.0318 ± 0.0039†
Insulin	0.0845 ± 0.0023*	0.0760 ± 0.0011‡	0.0397 ± 0.0067
Leucine			
Rest			
Basal	0.0761 ± 0.0024	0.0562 ± 0.0033	0.0335 ± 0.0037
Insulin	0.0886 ± 0.0025*	0.0691 ± 0.0036*	0.0444 ± 0.0038
Postexercise			
Basal	0.0700 ± 0.0047	0.0588 ± 0.0027	0.0288 ± 0.0033
Insulin	0.0813 ± 0.0045*	0.0669 ± 0.0024	0.0338 ± 0.0037
Lysine			
Rest			
Basal	0.0795 ± 0.0040	0.0648 ± 0.0044	0.0282 ± 0.0030
Insulin	0.0935 ± 0.0050*	0.0785 ± 0.0050*	0.0421 ± 0.0032*
Postexercise			
Basal	0.0756 ± 0.0045	0.0620 ± 0.0033	0.0326 ± 0.0020
Insulin	0.0781 ± 0.0046*	0.0662 ± 0.0040	0.0387 ± 0.0031
Alanine			
Rest			
Basal	0.0605 ± 0.0028	0.0347 ± 0.0023	0.0140 ± 0.0013
Insulin	0.0642 ± 0.0043	0.0371 ± 0.0031	0.0154 ± 0.0007
Postexercise			
Basal	0.0595 ± 0.0083	0.0376 ± 0.0050	0.0142 ± 0.0016
Insulin	0.0557 ± 0.0064	0.0294 ± 0.0025§	0.0140 ± 0.0019

Data are means ± SE and are expressed as tracer/tracee ratio. *P < 0.05, insulin vs. basal; †P < 0.05, postexercise basal vs. rest basal; ‡P < 0.05, postexercise insulin vs. rest basal; §P < 0.05, postexercise insulin vs. rest insulin.

whereas concentrations of other amino acids did not change significantly. In the basal postabsorptive state, the intramuscular concentrations of the essential amino acids phenylalanine, leucine, and lysine were not significantly different at rest and after exercise, whereas alanine concentration was greater after exercise than at rest. Intramuscular concentrations of all the essential amino acids tended to decrease after insulin infusion both at rest and during the postexercise recovery. Insulin infusion significantly decreased muscle alanine concentration after exercise but not at rest. Arterial amino acid enrichments tended to be lower after exercise in the basal postabsorptive state than at rest ($P < 0.05$ for phenylalanine enrichment). Insulin infusion at rest and after exercise significantly increased arterial enrichment of the essential amino acids phenylalanine, leucine, and lysine, indicating an insulin-mediated suppression of whole-body protein turnover both at rest and after exercise. Amino acid enrichments in the femoral vein and muscle tissue were lower than in the artery because of the de novo intracellular appearance of unlabeled amino acids across muscle tissue.

Figure 2 shows the regulation of leg blood flow by insulin and prior exercise alone and in combination. During the postexercise recovery in the postabsorptive state, leg blood flow increased by ~75% from the basal resting value. Insulin infusion increased leg blood flow by ~40% at rest. However, the hormone infusion did not significantly change leg blood flow after exercise. The insulin-mediated change in leg blood flow was greater at rest (1.17 ± 0.46 ml · min^{-1} · 100 ml^{-1} leg volume) than after exercise (0.50 ± 0.36 ml · min^{-1} · ml^{-1} leg volume; $P < 0.01$).

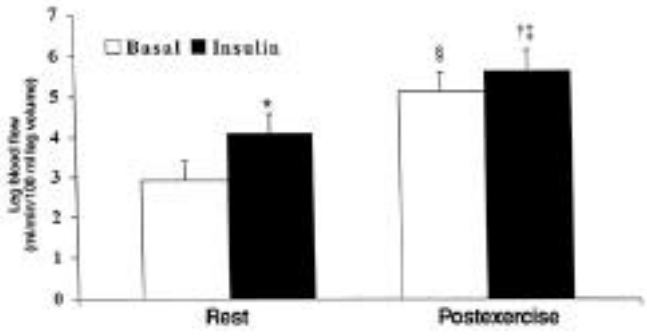

FIG. 2. Insulin effects on leg blood flow at rest and in the postexercise recovery. Values are means ± SE. *$P < 0.05$ insulin vs. basal; §$P < 0.05$ postexercise basal vs. rest basal; †$P < 0.05$ postexercise insulin vs. rest basal; ‡$P < 0.05$ postexercise insulin vs. rest insulin.

TABLE 3
Leg muscle amino acid kinetics

	Net balance	Intracellular utilization	Intracellular appearance from protein breakdown	Inward transport
Phenylalanine				
Rest				
Basal	–16 ± 2	30 ± 7	46 ± 8	72 ± 11
Insulin	3 ± 3*	51 ± 4*	48 ± 3	79 ± 12
Postexercise				
Basal	–8 ± 3	65 ± 10†	74 ± 10†	50 ± 5
Insulin	12 ± 5*‡	64 ± 9‡	52 ± 9*	65 ± 29
Leucine				
Rest				
Basal	–20 ± 9	160 ± 12	180 ± 15	142 ± 18
Insulin	23 ± 3*	200 ± 13	178 ± 12	195 ± 37
Postexercise				
Basal	14 ± 7†	276 ± 64	263 ± 64	170 ± 12
Insulin	34 ± 5‡	249 ± 53	178 ± 12	151 ± 25
Lysine				
Rest				
Basal	–20 ± 4	231 ± 11	250 ± 12	135 ± 10
Insulin	9 ± 10*	266 ± 19	257 ± 15	228 ± 40
Postexercise				
Basal	–11 ± 11	343 ± 42†	354 ± 40†	276 ± 30†
Insulin	33 ± 9‡	336 ± 32‡	303 ± 33	291 ± 25‡
Alanine				
Rest				
Basal	–111 ± 21	1,297 ± 140	1,408 ± 153	419 ± 52
Insulin	-82 ± 26	1,695 ± 185	1,777 ± 187	590 ± 93
Postexercise				
Basal	–110 ± 23	1,632 ± 164	1,743 ± 162	579 ± 71
Insulin	62 ± 23*‡§	3,529 ± 637*‡§	3,467 ± 619*‡§	1,114 ± 110*‡§

Data are means ± SE and are expressed as nmol · min^{-1} · 100 ml^{-1} leg volume. Intracellular utilization is protein synthesis for phenylalanine and lysine, protein synthesis plus other fates for leucine; intracellular appearance is from proteolysis for phenylalanine, leucine, and lysine and from proteolysis plus de novo synthesis for alanine. *$P < 0.05$, insulin vs. basal; †$P < 0.05$, postexercise basal vs. rest basal; ‡$P < 0.05$, postexercise insulin vs. rest basal; §$P < 0.05$ postexercise insulin vs. rest insulin.

Table 3 shows the insulin effects on leg muscle amino acid kinetics at rest and after exercise. During insulin infusion at rest and after exercise, the net balance of phenylalanine and lysine, which reflects balance between muscle protein synthesis and breakdown, significantly increased, that is, shifted from a net output (negative values) in the postabsorptive state to a net uptake (positive value) during insulin infusion. Phenylalanine and lysine balance tended to be greater in the postexercise recovery than at rest both in basal postabsorptive state (i.e., less negative) and during hyperinsulinemia (i.e., more positive). However, the insulin-mediated changes from basal of phenylalanine and lysine balance were not significantly different at rest and after exercise (for phenylalanine, 19 ± 4 and 21 ± 6 nmol · min^{-1} · 100 ml^{-1} leg volume, respectively; for lysine, 28 ± 10 and 44 ± 14 nmol · min^{-1} · 100 ml^{-1} leg volume, respectively). Leucine balance, which reflects simultaneous changes of protein balance and leucine catabolism, was negative in the basal state at rest and became positive after exercise and during insulin infusions.

In the basal postabsorptive state, the rates of intracellular phenylalanine and lysine utilization for protein synthesis were significantly greater 3 h after exercise than at rest. Insulin infusion at rest significantly increased the rate of phenylalanine utilization for protein synthesis and tended to increase the rate of lysine utilization for protein synthesis. Insulin infusion after exercise did not change the rates of phenylalanine and lysine utilization for protein synthesis from the control postexercise values. Intracellular leucine utilization, which reflects simultaneous changes of protein synthesis and leucine catabolism, did not change significantly after exercise or during insulin infusions.

In the control basal postabsorptive state, the FSR of muscle protein was significantly greater during the first 3 h after exercise (0.0946 ± 0.0167% per hour) than at rest (0.0475 ± 0.0054% per hour; $P < 0.05$). Insulin infusion tended to increase muscle FSR from the basal postabsorptive values both at rest (0.0745 ± 0.0097% per hour) and after exercise (0.1215 ± 0.0159% per hour). Whereas muscle FSR was significantly greater during hyperinsulinemia after exercise than during hyperinsulinemia at rest ($P < 0.05$), the FSR during insulin infusion after exercise was not significantly greater than the basal value after exercise (Fig. 3).

In the basal postabsorptive state, the rates of intracellular phenylalanine and lysine appearance from protein breakdown were significantly greater after exercise than at rest. Also, intracellular leucine appearance tended to be greater after exercise than at rest. Insulin infusion at rest did not significantly change intracellular phenylalanine, lysine, and leucine appearance from protein breakdown. In contrast, insulin infusion after exercise significantly decreased phenylalanine appearance from protein breakdown and tended to decrease leucine and lysine appearance from protein breakdown. Figure 4 shows the insulin-mediated changes from the basal postabsorptive state of phenylalanine, leucine, and lysine release from muscle protein breakdown at rest and after exercise. During the postexercise recovery, the suppressive effect of insulin on amino acid release from protein breakdown was significantly greater than the insulin-mediated changes at rest.

Alanine net balance was negative in the basal postabsorptive state at rest and after exercise, reflecting alanine release from net protein breakdown and net alanine de novo synthesis from pyruvate. The rate of alanine release from leg muscle was not significantly changed by insulin infusion at rest. In contrast, insulin infusion during the postexercise recovery shifted alanine balance from negative to positive values, that is, from net release to net uptake. The rate of intracellular utilization of alanine (Table 3) is the sum of utilization for protein synthesis and catabolism of the amino acid, whereas the rate of intracellular alanine appearance (Table 3) is the sum of release from protein breakdown and de novo synthesis. The rates of alanine de novo synthesis and catabolism are shown in Table 4. The rates of alanine catabolism and de novo synthesis tended to be greater after exercise than at rest in the basal postabsorptive state. Insulin infusion at rest slightly but not significantly increased both alanine catabolism and de novo synthesis. In contrast, insulin infusion after exercise doubled both the rate of alanine catabolism and the rate of alanine synthesis. In the basal postabsorptive state at rest and after exercise as well as during insulin infusion at rest, the rate of alanine synthesis was greater than that of catabolism, thereby accounting for net alanine release in these experimental conditions. In contrast, during insulin infusion after exercise, alanine catabolism tended to be greater than alanine synthesis, thus explaining the shift of net alanine balance from net release to net uptake observed in this experimental condition.

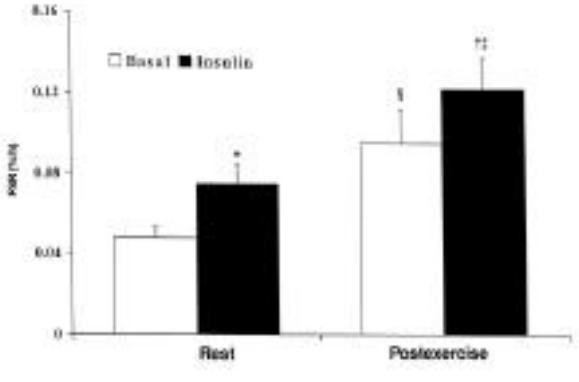

FIG. 3. Fractional synthetic rate (FSR) of muscle protein at rest and after exercise in the basal state and during insulin infusion. *$P < 0.05$ insulin vs. basal; §$P < 0.05$ postexercise basal vs. rest basal; ‡$P < 0.05$ postexercise insulin vs. rest insulin.

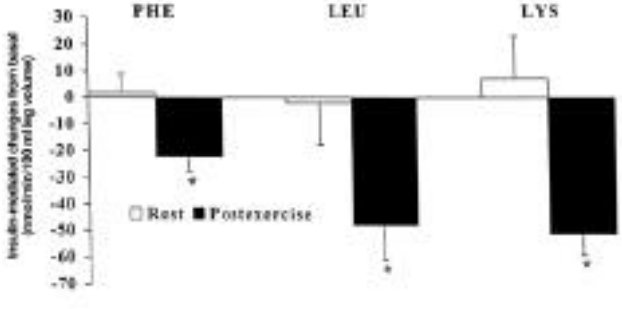

FIG. 4. Insulin-mediated changes of phenylalanine (PHE), leucine (LEU), and lysine (LYS) intracellular appearance from protein breakdown in skeletal muscle at rest and in the postexercise recovery. *$P < 0.05$ postexercise vs. rest.

TABLE 4
Intracellular alanine metabolism in skeletal muscle

	Nonprotein utilization	De novo synthesis
Rest		
Basal	1,226 ± 138	1,301 ± 145
Insulin	1,576 ± 178	1,665 ± 182
Postexercise		
Basal	1,481 ± 166	1,572 ± 164
Insulin	3,380 ± 648*†‡	3,347 ± 626*†‡

Data are means ± SE and are expressed as nmol · min^{-1} · 100 ml^{-1} leg volume. *P < 0.05, insulin vs. basal; †P < 0.05, postexercise insulin vs. rest basal; ‡P < 0.05 postexercise insulin vs. rest insulin.

After exercise in the basal postabsorptive state, the rates of leucine, lysine, and alanine transport were greater than at rest, but statistical significance was achieved only for lysine. During insulin infusion at rest, the rates of leucine, lysine, and alanine transport slightly but not significantly increased from basal (Table 3 and Fig. 5). Insulin infusion after exercise did not significantly change phenylalanine, leucine, or lysine transport. In contrast, insulin infusion after exercise greatly accelerated the rate of inward alanine transport (Table 3, Fig. 5).

DISCUSSION

In this study, we have evaluated the individual and combined effects of physical exercise and physiologic hyperinsulinemia on protein synthesis and breakdown, amino acid transport, glucose uptake, and alanine metabolism in leg skeletal muscle. The effect of exercise was determined at the end of 3-h recovery after a heavy leg resistance workout. Insulin was directly infused into the femoral artery to minimize systemic effects. We found that *1*) during the postexercise recovery in the postabsorptive state, the rates of both protein synthesis and breakdown were greater than at rest; *2*) hyperinsulinemia at rest increased muscle protein synthesis without affecting protein breakdown; *3*) hyperinsulinemia after exercise did not further increase muscle protein synthesis but blunted the exercise-mediated acceleration of protein breakdown; *4*) the insulin stimulatory effects on inward alanine transport and glucose uptake were three times greater during postexercise recovery than at rest (also, insulin after exercise doubled the rate of nonprotein alanine utilization—i.e., to pyruvate and further fates—and shifted muscle alanine balance from net release to net uptake); and *5*) the insulin effects on phenylalanine, leucine, and lysine transport were similar at rest and after exercise.

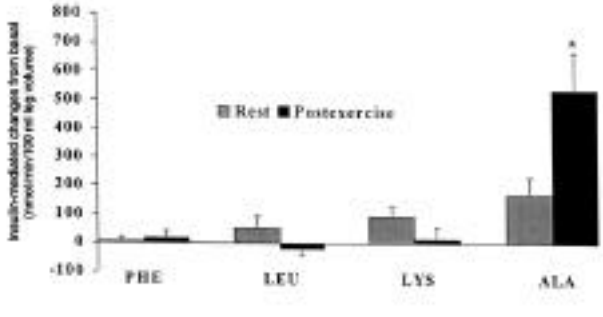

FIG. 5. Insulin-mediated changes of phenylalanine (PHE), leucine (LEU), lysine (LYS), and alanine (ALA) inward transport in skeletal muscle at rest and in the postexercise recovery. *P < 0.05 postexercise vs. rest.

The differential insulin effects on muscle proteolysis at rest and after exercise we observed in this study are consistent with expectations from in vitro findings. In normal physiologic conditions there are three systems responsible for protein breakdown: lysosomal, ubiquitin-proteasome, and Ca^{++}-dependent (14). The most important degradative system for myofibrillar proteins in testing skeletal muscle is the ubiquitin-proteasome system, which is located in the cytosol (25,26). The lysosomal proteases (14), such as the cathepsins, are predominantly involved in the breakdown of nonmyofibrillar proteins (27). Insulin apparently plays a major role in the regulation of lysosomal degradation of nonmyofibrillar proteins (28–30). Insulin failed to reduce hydrolysis of myofibrillar proteins both in vitro (27,31,32) and in vivo (33), although an insulin-mediated decrease of the ubiquitin-proteasome pathway has been recently demonstrated in insulinopenic rats (34,35). In normal conditions, the lysosomal system does not contribute significantly to overall proteolysis in skeletal muscle (27,36). However, the contribution of lysosomes to muscle proteolysis may increase in particular physiologic and pathologic conditions such as insulin deficiency (14), starvation (27), severe trauma (37), infections (38), and physical exercise (39–41). Kasperek et al. (41) observed that, in rat skeletal muscle, the rate of total protein breakdown was increased by exercise and involved increased activation of the lysosomal pathways. In contrast, the breakdown of myofibrillar protein was unchanged. These in vitro findings may explain the enhanced sensitivity to the suppressive effect of insulin on muscle protein breakdown we have observed after exercise. This interpretation is consistent with our earlier observation that whereas amino acid infusion did not suppress resting muscle protein breakdown, it also eliminated the postexercise stimulation in breakdown. The lysosomal system is also known to be responsive to changes in amino acid concentration (42,43).

The rate of muscle protein synthesis was determined by two independent methods. The arteriovenous balance technique allows simultaneous determinations of muscle protein synthesis and breakdown at the end of the study period, whereas the direct incorporation technique allows the assessment of the rate of protein synthesis over the entire study period (i.e, the tracer incorporation period). Both methods indicated that insulin and prior exercise each stimulated muscle protein synthesis independently. However, whereas the arteriovenous balance technique indicated that insulin infusion after exercise did not further increase protein synthesis, the direct incorporation technique suggested that insulin infusion may stimulate protein synthesis after exercise as well as when given at rest. The apparent discrepancy between the results of the two methods was not statistically significant, but it is possible that if we had run more studies, the FSR during insulin infusion would have become statistically greater than the control value. Even so, the results of the two methods would not be contradictory, because the arteriovenous method determines the rate of protein synthesis at the end of each study period (rest or postexercise recovery with or without insulin infusion), whereas the direct incor-

poration technique provides an average rate of protein synthesis over each 3 h tracer incorporation period. Thus, the possible difference between the results of the two methods may suggest a stimulatory insulin effect on muscle protein synthesis during the early postexercise recovery which may decrease by 3 h after exercise, when the arteriovenous model data was collected.

The mechanism of the insulin action on protein synthesis involves a direct activation of the protein synthetic process at the nuclear and cytoplasmic levels (44). However, the adequate availability of amino acids as precursors for synthesis is required for a stimulatory effect of insulin to be reflected in an increased production of protein. After exercise, the rates of intracellular amino acid appearance from protein breakdown and inward transmembrane transport are accelerated (10). Such increased amino acid availability may have contributed to the postexercise stimulation of protein synthesis. Insulin infusion after exercise suppressed muscle protein breakdown. In contrast to the situation at rest, after exercise insulin failed to stimulate muscle blood flow. The absence of a stimulatory effect of insulin on muscle blood flow after exercise minimized any effect of insulin on the increased transport of amino acids from blood. Therefore, availability of intracellular amino acids likely becomes limiting for protein synthesis, as reflected by the decrease in intracellular amino acid concentrations. It may be that this problem could be overcome by the ingestion of extra amino acids during the infusion of insulin.

Our previous studies (10,12) showed that the individual effects of insulin and prior exercise on skeletal muscle also involved the stimulation of transport of selected amino acids. The present study shows minimal effects of insulin on the transport of the essential amino acids phenylalanine, leucine, and lysine after exercise. In contrast, insulin and prior exercise in combination resulted in a positive interaction with regard to the stimulation of alanine transport. Such interaction was synergistic because the insulin-mediated stimulation of alanine transport was three times greater after exercise than at rest. Alanine is a major substrate for transport system A, a sodium-dependent system that maintains a steep transmembrane gradient of amino acid concentrations (45,46). This system has been shown to be regulated by both insulin (45–47) and exercise (48–50). Furthermore, a positive interaction between insulin and exercise with regard to the regulation of the A transport system was expected on the basis of previous in vitro studies that used nonmetabolizable alanine analogs (such as the aminoisobutyric acid) to assess transport kinetics (49,50). In contrast, insulin is not known to directly affect the transport of the essential amino acids tested (48). The modest increases in transport of these amino acids in the resting state when insulin was infused was likely due to the increase in blood flow, and the absence of any effect of insulin on the transport of these amino acids after exercise was probably due to the lack of an effect of insulin on blood flow.

The response of alanine transport to insulin infusion after exercise was paralleled by a similar acceleration of intracellular alanine turnover (alanine appearance and utilization). The increase in alanine turnover in response to the combination of insulin and exercise was accounted for by increases in the rates of nonprotein alanine utilization and de novo synthesis of the amino acid. Nonetheless, the rate of nonprotein utilization of alanine increased more than the rate of alanine de novo synthesis. Consequently, net alanine balance across muscle tissue shifted from net release to net uptake during insulin infusion after exercise.

Alanine constitutes a major carrier of nitrogen among body tissues. The rate of alanine de novo synthesis in skeletal muscle is a function of availability of pyruvate (51,52), which is primarily derived from glycolysis. In most circumstances, the rate of alanine synthesis exceeds that of utilization. Consequently, alanine is constantly released from skeletal muscle. Evidence indicates that alanine synthesis is increased by hyperinsulinemia and exercise (12,53). In our study, the rate of alanine utilization exceeded the rate of synthesis during insulin infusion after exercise. Nonprotein alanine utilization involves synthesis of pyruvate, which may have a number of different metabolic fates, including oxidation and synthesis of glycogen. Thus, we may hypothesize that the simultaneous accelerations of the rates of inward transport and of nonprotein utilization of alanine we have observed during insulin infusion in the postexercise recovery could contribute to replenish muscle glycogen stores depleted during exercise.

The synergistic action of prior exercise and insulin infusion on alanine transport closely paralleled the interaction between insulin and exercise on muscle glucose uptake. In fact, as for alanine transport, the insulin-mediated increase of glucose uptake was three times greater after exercise than at rest. Insulin-stimulated glucose metabolism in muscle has been widely studied after exercise (5). Such increased insulin effects include glucose transport and glycogen synthesis (5). It is noteworthy that the enhanced hypoglycemic action of insulin after exercise occurred in the absence of an effect on blood flow, as there is evidence that at least part of the stimulatory effect of insulin on muscle glucose uptake is mediated by an increase in blood flow (54). The fact that glucose uptake was stimulated after exercise by insulin, and that protein breakdown was suppressed by insulin after exercise, support the notion that there was no deficiency in either insulin binding to its receptor or pathways of insulin signaling within the cell. These observations support the notion stated above, that the lack of an effect of insulin on protein synthesis after exercise reflected a deficiency in amino acid availability rather than a defect in the direct stimulatory effect of insulin on protein synthesis.

In conclusion, this study shows that the abilities of insulin to stimulate glucose uptake and alanine transport and to suppress protein breakdown in skeletal muscle are increased after resistance exercise. Other insulin-sensitive processes, such as stimulation of muscle protein synthesis and of blood flow, are not augmented by prior exercise.

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