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

Gianni Biolo, Bradley D. Williams, R.Y. Declan Fleming, and Robert R. Wolfe

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 substantially 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, 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

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 ±
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.

**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 5-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, MD). 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-[1-13C]phenylalanine was started, followed by 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 infusions rates (IR) and priming doses (PD) were used: l-[1-13C]phenylalanine, 0.65 µmol · kg⁻¹ · min⁻¹; l-[2-15N]lysine, 0.65 µmol · kg⁻¹ · min⁻¹; l-[1-13C]alanine, 0.65 µmol · kg⁻¹ · min⁻¹; and the first muscle biopsy was performed. Then, intravenous infusions of l-[1-13C]leucine, l-[2-15N]lysine, and l-[1-13C]alanine and the intramuscular insulin infusion were started as described. Blood glucose was measured at basal and 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.

**Postexercise recovery: basal postabsorptive state.** At 6:00 a.m., after an overnight fast, the subject was studied in the basal postabsorptive state during hyperinsulinemia. In the two postexercise studies, the subject was studied in the postabsorptive state or during hyperinsulinemia.

**Isotopes.** l-[1-13C]Leucine (98% enriched), l-[ring-13C]phenylalanine (99% enriched), and l-[2-15N]lysine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA); l-[1-13C]leucine (98% enriched), l-[2-15N]lysine (98% enriched), l-[1-12C,6-15N]histidine (98% enriched), and l-[ring-13C]phenylalanine (98% enriched) were purchased from Tracer Technologies (Somerville, MA). l-[1-13C]alanine (99% enriched) was purchased from Isotec (Miamisburg, OH).

**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.** The isotopic enrichment of free amino acids in blood and muscle samples was measured by gas-chromatography mass spectrometry (GCMS) (model 5985B; Hewlett-Packard, Palo Alto, CA) by chemical ionization and selected ion monitoring (10–12, 18, 22). The 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. 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. The isotopic enrichment of free amino acids in blood and muscle samples was measured by gas-chromatography mass spectrometry (GCMS) (model 5985B; Hewlett-Packard, Palo Alto, CA) by chemical ionization and selected ion monitoring (10–12, 18, 22). The 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. The isotopic enrichment of free amino acids in blood and muscle samples was measured by gas-chromatography mass spectrometry (GCMS) (model 5985B; Hewlett-Packard, Palo Alto, CA) by chemical ionization and selected ion monitoring (10–12, 18, 22). The 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.
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 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 non-protein utilization. Each kinetic parameter is defined as follows (see Biolo et al. [18] for the derivation of the equations):

\[
NB = (C_A - C_V) \times BF \tag{1}
\]

Inward transport \[\frac{[E_M - E_V]}{[E_A - E_M]} \times C_V + C_A] \times BF \tag{2}\]

Intracellular utilization \[\frac{[C_A \times E_A - C_V \times E_V]}{E_M} \times BF \tag{3}\]

Intracellular appearance \[\frac{[C_A \times E_A - C_V \times E_V]}{E_M} \times BF - NB \tag{4}\]

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:

\[
\text{Nonprotein alanine utilization} = \frac{\text{intracellular alanine utilization} - \text{intracellular phenylalanine appearance} \times 2.80}{(\text{intracellular phenylalanine utilization} \times 2.80)} \tag{5}
\]

\[
\text{De novo alanine synthesis} = \frac{\text{intracellular alanine appearance} - \text{intracellular phenylalanine appearance} \times 2.80}{(\text{intracellular phenylalanine utilization} \times 2.80)} \tag{6}
\]

where 2.80 is the molar ratio alanine/phenylalanine in human muscle protein (18).

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

\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.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
 & Femoral artery (nmol/ml whole blood) & Femoral vein (nmol/ml whole blood) & Muscle free pool (nmol/ml intracellular water) \\
\hline
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* & 2,166 ± 260 \\
Postexercise & & & \\
Basal & 253 ± 24 & 274 ± 27 & 3,742 ± 789‡ \\
Insulin & 331 ± 34 & 318 ± 31 & 3,061 ± 629* \\
\hline
\end{tabular}
\caption{Free amino acid concentration in femoral artery and vein and skeletal muscle}
\end{table}

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.
Insulin effects on muscle protein after exercise

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). 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. Atrial values of leucine concentrations significantly decreased during insulin infusion both at rest and after exercise.

\[
FSR = \frac{\Delta E}{[(E_{M(1)} + E_{M(2)})/2] \times T} \times 60 \times 100
\]

where \(E_{M(1)}\) and \(E_{M(2)}\) are the \(\nu\)-ring\(^{13}C\)_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.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Free amino acid enrichments in femoral artery and vein and skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>Femoral artery</td>
</tr>
<tr>
<td>Rest</td>
<td>0.0800 ± 0.0031</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0905 ± 0.0026*</td>
</tr>
<tr>
<td>Postexercise</td>
<td>0.0764 ± 0.0024†</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0845 ± 0.0023*</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.0761 ± 0.0024</td>
</tr>
<tr>
<td>Rest</td>
<td>0.0886 ± 0.0025*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0700 ± 0.0047</td>
</tr>
<tr>
<td>Postexercise</td>
<td>0.0813 ± 0.0045*</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.0795 ± 0.0040</td>
</tr>
<tr>
<td>Rest</td>
<td>0.0935 ± 0.0050*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0756 ± 0.0045</td>
</tr>
<tr>
<td>Postexercise</td>
<td>0.0781 ± 0.0046*</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.0605 ± 0.0028</td>
</tr>
<tr>
<td>Rest</td>
<td>0.0642 ± 0.0043</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0595 ± 0.0083</td>
</tr>
<tr>
<td>Postexercise</td>
<td>0.0557 ± 0.0064</td>
</tr>
</tbody>
</table>

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 $\sim 70\%$ from the basal resting value. Insulin infusion increased leg blood flow by $\sim 40\%$ 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 ($1.17 \pm 0.46$ ml · min$^{-1}$ · 100 ml$^{-1}$ leg volume) than after exercise ($0.50 \pm 0.36$ ml · min$^{-1}$ · ml$^{-1}$ leg volume; $P < 0.01$).

### TABLE 3

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

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; $^\dagger P < 0.05$, postexercise basal vs. rest basal; $^\ddagger P < 0.05$, postexercise insulin vs. rest basal; $^\ddagger P < 0.05$ 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 of muscle amino acids 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 reflect 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 of muscle protein from 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.
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.

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 (30–41). 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-

TABLE 4
Intracellular alanine metabolism in skeletal muscle

<table>
<thead>
<tr>
<th></th>
<th>Nonprotein utilization</th>
<th>De novo synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest Basal</td>
<td>1,226 ± 138</td>
<td>1,301 ± 145</td>
</tr>
<tr>
<td>Rest Insulin</td>
<td>1,576 ± 178</td>
<td>1,665 ± 182</td>
</tr>
<tr>
<td>Postexercise Basal</td>
<td>1,481 ± 166</td>
<td>1,572 ± 164</td>
</tr>
<tr>
<td>Postexercise Insulin</td>
<td>3,380 ± 648*†‡</td>
<td>3,347 ± 626*†‡</td>
</tr>
</tbody>
</table>

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.
The increase in alanine turnover in response to the combination of insulin and exercise (48–50). Furthermore, a positive interaction involving a direct activation of the protein synthetic process has been shown to be regulated by both insulin (45–47). 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 stimulation of alanine transport was observed during insulin infusion in the postexercise recovery period.

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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