Leg glucose and protein metabolism during an acute bout of resistance exercise in humans

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Leg glucose and protein metabolism during an acute bout of resistance exercise in humans. J Appl Physiol 97: 1379–1386, 2004. First published June 11, 2004; 10.1152/japplphysiol.00635.2003.—The present study investigated the responses of leg glucose and protein metabolism during an acute bout of resistance exercise. Seven subjects (5 men, 2 women) were studied at rest and during a strenuous lower body resistance exercise regimen consisting of ~8 sets of 10 repetitions of leg press at ~75% 1 repetition maximum and 8 sets of 8 repetitions of knee extensions at ~80% 1 repetition maximum. L-[^3-^14]C-phenylalanine was infused throughout the study for measurement of phenylalanine rates of appearance, disappearance, protein synthesis, and protein breakdown across the leg. Femoral arterial and venous blood samples were collected at rest and during exercise for determination of leg blood flow, concentrations of glucose, lactate, alanine, glutamine, glutamate, leucine, and phenylalanine, and phenylalanine enrichments. Muscle biopsies were obtained at rest and immediately after exercise. Leg blood flow was nearly three times (P < 0.009) higher and glucose uptake more than five times higher (P = 0.009) during exercise than at rest. Leg lactate release was 86 times higher than rest during the exercise bout. Although whole body phenylalanine rate of appearance, an indicator of whole body protein breakdown, was reduced during exercise; leg phenylalanine rate of appearance, rate of disappearance, protein synthesis, and protein breakdown did not change. Arterial and venous alanine concentrations and glutamate uptake were significantly higher during exercise than at rest. We conclude that lower body resistance exercise potently stimulates leg glucose uptake and lactate release. In addition, muscle protein synthesis is not elevated during a bout of resistance exercise.

Address for reprint requests and other correspondence: W. J. Durham, Pulmonary Medicine, One Baylor Plaza, Suite 520B, Baylor College of Medicine, Houston, TX 77030 (E-mail: wdurham@bcm.tmc.edu). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

EXERCISE IMPOSES EXTREME DEMANDS on skeletal muscle, as both a potent physiological stimulus for muscle adaptation and an acute test of the muscle’s capacity to repeatedly produce force. During the early (3–4 h) postexercise period, the metabolic response to exercise varies depending on the type of contractions performed. High-force contractions (resistance exercise) stimulate muscle protein synthesis (PS) and breakdown (PB) above preexercise rates (39), whereas the response to lower force contractions (aerobic exercise) appears to be intensity dependent, with PS and PB inhibited at higher intensities (cycling at 60% maximal oxygen uptake) (32, 33) but increased at lower intensities (walking at 40% maximal oxygen uptake) (14). Because the increase in PS is greater and persists longer than the increase in PB after resistance exercise (39), this type of exercise eventually leads to accretion of muscle protein (hypertrophy). Glucose metabolism in the early postexercise period is affected similarly by aerobic and resistance exercise, with both increasing insulin sensitivity (11, 23) and glycogen synthesis (37) above resting levels.

In contrast to the relatively well-studied postexercise responses, metabolic responses during a bout of exercise are not as well characterized. Although it is well known that glucose uptake is increased in contracting skeletal muscle during aerobic exercise (23), only one study has measured muscle PS during aerobic exercise (14). In that study, muscle PS during exercise was not different from control. Even less is known about the metabolic response to resistance exercise, as muscle PS and limb glucose uptake have not been reported. Thus one goal of the present study was to determine whether muscle PS and leg glucose uptake are stimulated by a bout of lower body resistance exercise. Furthermore, because the responses of glucose extraction and uptake to changes in blood flow may provide information about the balance between nutritive and nonnutritive blood flow (7, 12, 16, 19), we were interested in comparing the responses of glucose and phenylalanine transport to resistance exercise, because they would be predicted to respond similarly to alterations in nutritive blood flow (7).

Numerous factors present during resistance exercise have previously been shown to increase glucose uptake and muscle PS. Increased muscle tension and decreased cellular energy state, both of which occur during resistance exercise, have each been reported to promote glucose uptake (28, 36, 54). In addition, contraction-induced increases in skeletal muscle nitric oxide production and calcium release may stimulate muscle glucose uptake (3, 19, 43). Alterations in blood flow may also affect metabolism during resistance exercise. Postcontraction hyperemia increases glucose delivery to skeletal muscle, which may in turn increase muscle glucose uptake (19, 23–25). This laboratory has also reported a significant correlation between blood flow and muscle PS during hyperaminocademia (10). Thus we hypothesized that leg glucose uptake and muscle PS would be stimulated during a bout of resistance exercise.

On the other hand, other factors may oppose the predicted increases in glucose uptake and PS. For example, in the fasting state, the concentration gradient between muscle and blood favors net efflux of amino acids. Thus, possible perfusion-induced increases in intracellular amino acid appearance from inward transport (and possibly PB) may be counteracted by...
increased outward transport of amino acids. Support for this scenario comes from the high correlation between inward and outward amino acid transport (8, 9, 11). Because transport of some amino acids is coupled to transport of other amino acids or ions, such as Na\(^+\), any perfusion-induced increase in amino acid transport would be dependent on stimulation of these complementary systems (e.g., the Na\(^+\)-K\(^+\)-ATPase) to maintain conditions favorable for transport (27). In addition, blood flow during resistance exercise is highly oscillatory due to the high intramuscular pressures that are generated during contractions (4, 5). Such high intramuscular pressures impede muscle blood flow, with the result that blood flow approaches zero during contractions but is greatly elevated after contractions (4, 5). This characteristic makes it difficult to predict what role, if any, blood flow plays in resistance exercise metabolism. Finally, muscle damage occurring during exercise may reduce muscle glucose uptake (17).

Recruitment of fast-twitch muscle fibers, along with the aforementioned reductions in cellular energy state and blood flow during high-intensity contractions, may result in significant production of ammonia from AMP and branched chain amino acid deamination (41, 45). One route for removal of exercise-induced ammonia is through production and release of glutamine and alanine by skeletal muscle (20). Accordingly, another hypothesis tested in the present study was that net glutamine and alanine release would be increased during a bout of resistance exercise. Furthermore, because one molecule of glutamate is needed for each molecule of glutamine formed, we hypothesized that leg glutamate uptake would also be stimulated.

METHODS

Subjects

Seven subjects (5 men, 2 women) consented to participate in this study after receiving a detailed written and verbal description of the study. The mean age, height, and weight (means ± SE) were 27 ± 3 yr, 1.75 ± 0.04 m, and 81 ± 7 kg, respectively. Leg volume was 10.8 ± 0.9 liters and was estimated from measurements of leg circumference and height at several points along the length of the leg. The one repetition maximum (1 RM; the maximum amount of weight that could be lifted one time) was 123 ± 13 kg for the two-legged leg press and 59 ± 6 kg for the one-leg extension. The study was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston and conformed with the standards of the Declaration of Helsinki regarding human subjects.

Isotopes

L-\([\text{ring-}^2\text{H}]\)phenylalanine was purchased from Cambridge Isotopes (Andover, MA) and dissolved in 0.9% saline before infusion. The infusate was filtered through a 0.2-μm filter as it was infused, using a calibrated Harvard syringe pump (Natick, MA). The tracer was infused for ≥2 h before collection of resting samples. The infusion protocol was intended to produce an isotopic steady state in the blood and intracellular pools. Our laboratory previously showed such a protocol to be effective for this purpose (Ref. 49; see also Methodological Considerations below).

Experimental Design

Each subject was studied during the hour before exercise and during a bout of lower body resistance exercise lasting ~45 min. Muscle biopsies and femoral arterial and venous samples were obtained at rest and during the bout of resistance exercise. Leg blood flow was also measured both at rest and during the resistance exercise bout.

Procedure

Subjects reported to the General Clinical Research Center (GCRC) at the University of Texas Medical Branch in Galveston the evening before the study and fasted from 10:00 PM until the completion of the study the next day. Preentry diet was not controlled, but subjects were asked to refrain from strenuous physical activity for 2 days before testing. Subjects had free access to water throughout the study. At ~7:00 AM, a 20-gauge polyethylene catheter was inserted into an antecubital vein, and, after a background sample was drawn, a primed (2 μmol/kg), constant (0.05 μmol·kg\(^{-1}·\)min\(^{-1}\)) infusion of L-\([\text{ring-}^2\text{H}]\)phenylalanine was started. At ~7:30 AM, the subject was transported to the Department of Radiology, where sheaths (6 Fr) were placed in the femoral artery and vein of one leg under local anesthesia (2% lidocaine, ~10 ml). Catheters (5 Fr) were inserted through the sheaths and guided, with the aid of fluoroscopy, to the femoral artery and vein of the other leg. This arrangement allowed for simultaneous femoral arterial and venous blood sampling from both legs. Once the catheters had been placed, the subject was transported to the Exercise Laboratory of the Shriners Burns Institute, affiliated with the GCRC of the University of Texas Medical Branch at Galveston, where the subject rested for at least 1 h. During this period, resting blood flow was measured, and arterial and venous blood samples and a muscle biopsy were taken. After this hour, the subject performed ~45 min of lower body resistance exercise. This exercise consisted of ~8 sets of 10 repetitions of leg press at ~75% of 1 RM followed by 8 sets of 8 repetitions of knee extension at ~80% of 1 RM. The fatiguing nature of the exercise bout is evidenced by the fact that, even with verbal encouragement, subjects required assistance (“spotting”) to complete the workout. At the conclusion of the workout, another muscle biopsy was taken.

Blood samples were taken from the femoral artery and the femoral vein of each leg approximately every 15 min at rest and after sets 3–6 of knee extension during the resistance exercise regimen for determinations of glucose, lactate, phenylalanine, alanine, glutamine, leucine, and glutamate concentrations, phenylalanine enrichment, and leg blood flow. In preliminary experiments (n = 2), we determined that arterial and venous concentrations and enrichments obtained during a set of contractions were not significantly different from those obtained 1 min after the set of contractions. Thus, in the remaining subjects, samples obtained 1 min into the rest period were considered representative of the concentrations and enrichments throughout the workout. Blood flow was calculated using intra-arterial indocyanine green (ICG) dye infusion and femoral venous blood sampling.

Leg Blood Flow

Blood flow was determined as previously described (49), with minor modification. Briefly, ICG dye (0.5 mg/ml) was infused into one femoral artery at a rate of 1 ml/min. Blood samples were taken from the femoral vein of the leg into which the dye was infused to measure dye dilution and from the contralateral femoral vein to measure recirculation of dye (29). Because both legs were catheterized, it was not necessary to momentarily interrupt the arterial ICG infusion to obtain arterial samples, as was necessary in some previous studies (48).

Plasma Volume Changes

Plasma volume changes induced by resistance exercise were measured in five subjects by the method of Dill and Costill (18).

Blood and Muscle Processing

Blood. Blood samples were obtained from the femoral artery and vein. Blood glucose and lactate concentrations were determined en-
zymatically (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH). Samples for phenylalanine concentration and enrichment measurements were immediately precipitated in preweighed tubes containing 15% sulfosalicylic acid (1 ml/ml blood) and a preweighed amount of the internal standard l-[ring-13C6]phenylalanine (50 μM, ~100 μl/ml blood) (8, 39). Blood samples were processed as previously described (39) for the synthesis and measurement of the tertiary-butyl dimethylsilyl derivative of phenylalanine by gas chromatography-mass spectrometer (Hewlett Packard 5890, series II), using electron impact ionization and selective ion monitoring of m/e 234 (m + 0), 235 (m + 1), 239 (m + 5), and 240 (m + 6). Correction of overlapping spectra was made as described previously (55). Femoral arterial and venous alanine, glutamine, leucine, and glutamate concentrations were determined by high-performance liquid chromatography. These samples were not collected in the first subject.

Muscle. Intracellular enrichment in muscle was determined in muscle biopsies as previously described (8, 39). As for the blood, the tertiary-butyl dimethylsilyl derivative of the intracellular fraction was formed and analyzed by gas chromatography-mass spectrometer. For measurement of intracellular concentrations, an internal standard containing 3 μM l-[ring-13C6]phenylalanine was added (2 μl/mg wet wt) to the intracellular samples. Selective ion monitoring of m/e 234 (m + 0), 235 (m + 1), 239 (m + 5), and 240 (m + 6) was carried out for the intracellular samples. Due to technical difficulties and small sample size in two subjects, intracellular concentrations are for five subjects.

Calculations and Statistics

The net balance (NB), rate of appearance (Ra), and rate of disappearance (Rd) across the leg were calculated using the equations

\[
R_a = R_d - NB
\]

\[
R_d = (E_a[A] - E_v[V]) \cdot BF/E_m
\]

\[
NB = ([A] - [V]) \cdot BF
\]

where \(E_a\), \(E_v\), \([A]\), and \([V]\) are the phenylalanine enrichments and concentrations in the femoral artery and vein, respectively, and BF is leg blood flow. Because phenylalanine is neither oxidized nor produced in muscle, \(R_a\) and \(R_d\) reflect the appearance of amino acids in plasma from muscle PB and the incorporation of amino acids from plasma into muscle protein, respectively. \(R_a\) and \(R_d\) do not include amino acids that are released in the process of breakdown that are directly reincorporated into protein. Total PS and PB (including intracellular cycling of amino acids from breakdown directly back into protein) were calculated using the equations

\[
PB = PS - NB
\]

\[
PS = (E_a[A] - E_v[V]) \cdot BF/E_m
\]

where \(E_m\) refers to the enrichment of phenylalanine in the muscle intracellular free amino acid pool.

Leg amino acid kinetics were calculated using a previously described three-compartment model (8), which employs the following equations

\[
\text{Inflow to leg (F_in) = } [A] \cdot BF
\]

\[
\text{Outflow from leg (F_out) = } [V] \cdot BF
\]

\[
\text{NB = } ([A] - [V]) \cdot BF
\]

\[
\text{Inward transport (F_{in,A}) = } \left( \frac{[E_a - E_v]}{[E_a - E_m]} \right) \cdot [V] + [A] \cdot BF
\]

\[
\text{Outward transport (F_{out,A}) = } \left( \frac{[E_m - E_v]}{[E_a - E_m]} \right) \cdot [V] + [V] \cdot BF
\]

\[
\text{Arteriovenous shunting } = F_m - F_{M,A}
\]

Phenylalanine fractional utilization (sometimes also referred to as synthetic efficiency) was calculated by dividing PS by the intracellular

\[
R_d \text{ of phenylalanine (inward transport + PB). Whole body phenylalanine } R_w \text{ was also calculated by dividing the infusion rate (F) of labeled phenylalanine (in } \mu\text{mol } \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \text{) by the arterial phenylalanine enrichment.}
\]

\[
R_w = F/E_m
\]

Glucose uptake and lactate uptake/release were calculated by multiplying the arteriovenous difference for each by the leg blood flow.

Data Analysis

Data were statistically evaluated using a paired, two-tailed r-test (Microsoft Excel 97). A P value of <0.05 was considered statistically significant. Data are presented as means ± SE.

RESULTS

Blood flow was nearly three times higher during exercise than at rest (P < 0.009; Fig. 1). In response to resistance exercise, plasma volume was reduced by 8 ± 1%. Arterial and venous glucose and lactate concentrations were significantly elevated during exercise (Table 1). Resistance exercise caused a 26-fold increase in the lactate arteriovenous difference (Table 1). Glucose extraction tended to be higher during exercise, increasing by 113% (P = 0.06) relative to the resting value (Table 1). Lactate release and glucose uptake were both higher during exercise than at rest, increasing to 86 and 5 times the resting levels, respectively (Fig. 2). Arterial and venous phenylalanine enrichments (P < 0.01) were significantly greater during exercise than at rest (Table 2). Whole body phenylalanine \(R_a\) was slightly but significantly (P = 0.0066) lower during resistance exercise than at rest (35.75 ± 1.25 and 38.37 ± 1.42 μmol·kg⁻¹·h⁻¹, respectively). Leg phenylalanine concentration and enrichment at rest and during resistance exercise

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial [glucose], mM</td>
<td>4.58±0.09</td>
<td>5.97±0.32</td>
<td>0.002</td>
</tr>
<tr>
<td>Venous [glucose], mM</td>
<td>4.46±0.08</td>
<td>5.71±0.32</td>
<td>0.004</td>
</tr>
<tr>
<td>a-v Glucose, mM</td>
<td>0.12±0.04</td>
<td>0.26±0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Arterial [lactate], mM</td>
<td>0.65±0.08</td>
<td>5.66±0.85</td>
<td>0.0008</td>
</tr>
<tr>
<td>Venous [lactate], mM</td>
<td>0.68±0.07</td>
<td>6.40±0.84</td>
<td>0.0004</td>
</tr>
<tr>
<td>a-v Lactate, mM</td>
<td>−0.03±0.02</td>
<td>−0.75±0.18</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE. Brackets denote concentration, a-v, arteriovenous.

Fig. 1. Leg blood flow at rest and during resistance exercise. *P < 0.009 vs. rest per leg; P < 0.002 per 100 ml leg.
nine R_a, R_d, PS, PB, and NB during resistance exercise were not significantly different from rest (Fig. 3). Both leg inflow and outflow of phenylalanine more than doubled during resistance exercise (Table 3). Arteriovenous shunting of phenylalanine during exercise was nearly 400% of the preexercise value (Table 3). This was not simply due to the elevation in blood flow, as the proportion of the arterial delivery of phenylalanine that was shunted to the vein (shunting/inflow) was significantly higher during exercise as well (Table 3). Inward and outward transport both tended to be higher but did not reach the level of significance (Table 3). Arterial and venous alanine concentrations were higher during exercise than at rest, whereas venous glutamate concentrations were significantly increased by exercise (Table 4). There were no significant concentration changes for any of the other amino acids analyzed (Table 4). The only amino acid exhibiting a significant change in uptake or release was glutamate, whose uptake significantly increased by ~50% during the resistance exercise bout (Table 4).

DISCUSSION

Glucose Uptake During Resistance Exercise

Resistance exercise training has been reported to increase glucose tolerance and insulin sensitivity (1) and is recommended for individuals with Type 2 diabetes because it preserves muscle mass during weight loss and may reduce abdominal obesity. However, to our knowledge, this is the first report of the effects of an acute bout of resistance exercise on leg glucose uptake. Here, we show that, during an acute bout of resistance exercise, glucose uptake is elevated to greater than five times the resting level. This result is consistent with a previous report by Tesch et al. (46) showing increased glucose and glucose-6-phosphate concentrations in skeletal muscle during an acute bout of resistance exercise, although glucose uptake was not measured in the study.

To place our results in context with respect to other types of exercise, we have compared the elevation in glucose uptake in response to resistance exercise with that observed during other types of exercise (Table 5). It is difficult to compare resistance exercise, which is performed intermittently, with dynamic exercise, which is performed continuously and typically elicits much greater total energy expenditure. To facilitate comparison, we have also presented leg glucose uptake for the various types of exercise normalized to leg blood flow because blood flow typically increases to match the metabolic needs of the muscle and thus increases with increasing exercise intensity.

Table 2. Phenylalanine concentration and enrichment at rest and during exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest (μM)</th>
<th>Exercise (μM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial [phenylalanine], μM</td>
<td>54±2</td>
<td>58±3</td>
<td>0.04</td>
</tr>
<tr>
<td>Venous [phenylalanine], μM</td>
<td>59±3</td>
<td>59±3</td>
<td>0.7</td>
</tr>
<tr>
<td>a-v Phenylalanine, μM</td>
<td>-4.4±0.9</td>
<td>-1.6±0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Intracellular [phenylalanine], μM(n=5)</td>
<td>55±8</td>
<td>61±10</td>
<td>0.36</td>
</tr>
<tr>
<td>E_a</td>
<td>0.0788±0.0027</td>
<td>0.0845±0.0027</td>
<td>0.007</td>
</tr>
<tr>
<td>E_v</td>
<td>0.0664±0.0040</td>
<td>0.0788±0.0028</td>
<td>0.001</td>
</tr>
<tr>
<td>E_a - E_v</td>
<td>0.0124±0.0022</td>
<td>0.0056±0.0009</td>
<td>0.006</td>
</tr>
<tr>
<td>E_m (n=6)</td>
<td>0.0492±0.0040</td>
<td>0.0549±0.0050</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 3. Phenylalanine kinetics at rest and during resistance exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest (μM·min⁻¹·100 µL·1⁻¹)</th>
<th>Exercise (μM·min⁻¹·100 µL·1⁻¹)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflow</td>
<td>279±55</td>
<td>795±117</td>
<td>0.006</td>
</tr>
<tr>
<td>Outflow</td>
<td>304±59</td>
<td>819±120</td>
<td>0.008</td>
</tr>
<tr>
<td>Inward transport</td>
<td>125±32</td>
<td>187±53</td>
<td>0.059</td>
</tr>
<tr>
<td>Outward transport</td>
<td>150±32</td>
<td>211±54</td>
<td>0.096</td>
</tr>
<tr>
<td>Shunting</td>
<td>155±51</td>
<td>608±120</td>
<td>0.008</td>
</tr>
<tr>
<td>Fractional utilization</td>
<td>0.20±0.05</td>
<td>0.21±0.04</td>
<td>0.93</td>
</tr>
<tr>
<td>Inward transport/inflow</td>
<td>0.47±0.10</td>
<td>0.24±0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>Shunting/inflow</td>
<td>0.53±0.10</td>
<td>0.76±0.07</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are nmol phenylalanine-min⁻¹·100 µL·1⁻¹.
of selected amino acids

Table 4. Femoral arterial and venous concentrations of selected amino acids

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial [alanine], µM</td>
<td>296±34</td>
<td>535±51</td>
<td>0.00006</td>
</tr>
<tr>
<td>Venous [alanine], µM</td>
<td>354±38</td>
<td>554±51</td>
<td>0.0005</td>
</tr>
<tr>
<td>a-v Alanine, µM</td>
<td>−58±13</td>
<td>−19±9</td>
<td>0.019</td>
</tr>
<tr>
<td>a-v × Plasma flow, nmol/min⁻¹·100ml leg⁻¹</td>
<td>−100±20</td>
<td>−118±67</td>
<td>0.8</td>
</tr>
<tr>
<td>Arterial [glutamine], µM</td>
<td>605±30</td>
<td>639±38</td>
<td>0.6</td>
</tr>
<tr>
<td>Venous [glutamine], µM</td>
<td>639±38</td>
<td>633±37</td>
<td>0.9</td>
</tr>
<tr>
<td>a-v Glutamine, µM</td>
<td>−34±11</td>
<td>−12±9</td>
<td>0.12</td>
</tr>
<tr>
<td>a-v × Plasma flow, nmol/min⁻¹·100ml leg⁻¹</td>
<td>−59±18</td>
<td>−77±68</td>
<td>0.8</td>
</tr>
<tr>
<td>Arterial [leucine], µM</td>
<td>115±9</td>
<td>116±10</td>
<td>0.9</td>
</tr>
<tr>
<td>Venous [leucine], µM</td>
<td>117±9</td>
<td>114±11</td>
<td>0.7</td>
</tr>
<tr>
<td>a-v Leucine, µM</td>
<td>−1±2</td>
<td>2±2</td>
<td>0.3</td>
</tr>
<tr>
<td>a-v × Plasma flow, nmol/min⁻¹·100ml leg⁻¹</td>
<td>−1±3</td>
<td>7±11</td>
<td>0.5</td>
</tr>
<tr>
<td>Arterial [glutamate], µM</td>
<td>23±5</td>
<td>23±4</td>
<td>0.9</td>
</tr>
<tr>
<td>Venous [glutamate], µM</td>
<td>7±1</td>
<td>14±3</td>
<td>0.01</td>
</tr>
<tr>
<td>a-v Glutamate, µM</td>
<td>16±4</td>
<td>8±2</td>
<td>0.03</td>
</tr>
<tr>
<td>a-v × Plasma flow, nmol/min⁻¹·100ml leg⁻¹</td>
<td>29±8</td>
<td>46±9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. Brackets denote concentration.

When expressed in this manner, resistance exercise was similar to walking or supine cycling at 60% maximal oxygen uptake (Table 5). It should be noted, however, that resistance exercise protocols vary with respect to the intensity (% of 1 RM) and volume (sets × repetitions), as well as the type of exercises and contractions (shortening or concentric vs. lengthening or eccentric) performed. Thus it is possible that different protocols might elicit different responses than those observed in the present study.

Several nonmutually exclusive mechanisms may explain our results. First, it has been proposed that blood flow is an independent modulator of insulin- (6) and exercise-induced (23) glucose uptake. Because blood flow was greatly elevated during the resistance exercise bout, it is possible that increased glucose delivery may have augmented glucose uptake. However, it is noteworthy that glucose uptake would have more than doubled during exercise, even if the increase in blood flow had not occurred because glucose extraction was 113% higher (P = 0.06) during the exercise period. Other potential mechanisms for the present results are high muscle tension (28, 36), hypoxia caused by the reduction in blood flow occurring during each set of contractions, and activation of AMP-activated protein kinase (46, 54), because each of these are associated with increased muscle glucose uptake.

Protein Metabolism During Resistance Exercise

The anabolic response to a bout of resistance exercise is known to persist for 24–48 h after the cessation of exercise (15, 39). However, the responses of skeletal muscle PS and the breakdown during a bout of resistance exercise have not been previously reported. In the present study, we have calculated PS and PB across the leg by using the intracellular enrichment. When calculated in this manner, the calculated values account for intracellular amino acid recycling and thus reflect the true rates of muscle PB and PS, respectively (see Methodological Considerations below). Here, we show that muscle PS and PB do not change during an acute bout of resistance exercise lasting ~45 min. Thus stimulation of PS and PB in response to resistance exercise either occurs exclusively during the postexercise period or requires >45 min to be realized. In addition, contraction-induced hyperemia does not appear to influence muscle PS or breakdown during a resistance exercise bout. However, whole body phenylalanine Rₐ was slightly reduced during exercise, suggesting reduced PB in other tissues (non-exercised muscle, gut, liver, and/or skin).

Why is muscle PS refractory to such a large increase in amino acid delivery during resistance exercise? One factor may be a reduced sensitivity of inward transport to increased delivery of amino acids during exercise. When inward transport during exercise was normalized for arterial delivery, the resulting value was only half of what it was at rest (Table 3). As a result, although arterial delivery increased by 180% during exercise, inward transport only increased by ~50% (P = 0.059). This suggests that either much of the additional blood flow is through nonnutritive routes or that amino acid transport is less sensitive to increases in amino acid delivery during resistance exercise. Because the glucose responses were consistent with an increase rather than a decrease in nutritive blood flow, it appears that inward transport becomes less sensitive to increased amino acid delivery during resistance exercise. Because transport of many amino acids relies on transport of other amino acids or ions (27), it is possible that these systems do not keep pace with the stimulation of blood flow/amino acid delivery, thus limiting the response to exercise. Nevertheless, in the absence of other changes, a 50% increase in inward transport would be predicted to stimulate muscle PS. However, outward transport also tended to be higher during exercise and

<table>
<thead>
<tr>
<th>Activity Type</th>
<th>Blood Flow, l/min⁻¹·leg⁻¹</th>
<th>Glucose Uptake, µmol/leg⁻¹·min⁻¹</th>
<th>Uptake/Flow, µmol/ml</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.650</td>
<td>80</td>
<td>0.12</td>
<td>32</td>
</tr>
<tr>
<td>Roller skiing</td>
<td>0.515</td>
<td>65</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Knee extensions</td>
<td>6.75</td>
<td>65</td>
<td>0.18</td>
<td>51</td>
</tr>
<tr>
<td>Upright cycling</td>
<td>3.35</td>
<td>1.190</td>
<td>0.33</td>
<td>32</td>
</tr>
<tr>
<td>Supine cycling</td>
<td>6.65</td>
<td>2.400</td>
<td>0.41</td>
<td>52</td>
</tr>
<tr>
<td>Walking</td>
<td>6.1</td>
<td>1.490</td>
<td>0.24</td>
<td>35</td>
</tr>
<tr>
<td>Resistance exercise</td>
<td>~75–80%</td>
<td>1.412</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

VO₂max, maximal oxygen uptake; 1 RM, 1 repetition maximum.
apparently counteracted the increase in inward transport. Thus it appears that increased delivery of amino acids as a result of accelerated flow stimulated neither net uptake of essential amino acids nor muscle PS. This response is in contrast to the situation in which amino acid delivery is increased as a consequence of increased concentrations of essential amino acids (49). It could be that, for synthesis to be stimulated, the concentration gradient of amino acids must be reversed, with plasma concentrations being greater than intracellular concentrations. Alternatively, previous work in our laboratory (56) indicates that changes in inward transport occur primarily to maintain constant intracellular concentrations of essential amino acids, which in turn might be affected directly by changes in outward transport or by muscle PS and PB. Because neither synthesis nor breakdown was altered during exercise, the trend for inward transport to be increased during exercise may reflect the response to increased outward transport, which also tended to be higher during exercise.

Consistent with the report of Tipton et al. (49), we found that phenylalanine $R_a$ and $R_d$ were not significantly different from rest during an acute bout of resistance exercise. Although often used synonymously with PS and PB, phenylalanine $R_a$ and $R_d$ refer to the rates of release and uptake, respectively, of phenylalanine across the leg. As such, phenylalanine $R_d$ represents a minimal estimate of PS, accounting for the contribution of plasma amino acids to overall PS. As the amino acids used for PS arise from both inward amino acid transport and from PB, phenylalanine $R_a$ underestimates true PS to the extent that amino acids from PB are reutilized for PS without being released to the blood. It is interesting to note that $R_d$/PS (the fractional contribution of plasma amino acids to overall PS; mathematically equivalent to $E_d/E_a$) was $\sim 0.6$ both at rest and during resistance exercise. Thus, as at rest, the majority of amino acids used for synthesis during resistance exercise are of plasma origin. Phenylalanine $R_a$ refers to the $R_a$ of phenylalanine across the leg. Phenylalanine release by skeletal muscle may be affected by inward and outward amino acid transport and PS in addition to PB; thus phenylalanine $R_a$ may reflect changes in muscle PB.

**Leg Alanine and Glutamine Release**

Muscle ammonia production is greatly stimulated during intense contractions (2, 45). One route of removal of this ammonia is via synthesis of alanine and glutamine from pyruvate/lactate and glutamate, respectively, which are then transported out of the muscle (20). However, despite elevated rates of leg glutamate uptake and lactate production during exercise, we did not observe a significant increase in leg alanine or glutamine release, although there was considerable variation between subjects. One possibility for the lack of a significant response is that leg ammonia release is very high during resistance exercise, as has been shown previously during intense dynamic exercise (22). Also, it has been previously suggested that the ATP-dependent glutamine synthase reaction is inhibited when muscle energy demand is high (22). In addition, in the case of alanine, the high lactate release across the leg may have reduced the availability of pyruvate for formation of alanine.

**Methodological Considerations**

For measurement of PS and PB using tracer uptake, either the intracellular pool of the amino acid being studied must not change or account must be taken of any changes that do occur. This is necessary to avoid falsely attributing changes in amino acid uptake to changes in PS in situations in which the pool size of free amino acids change. In the present study, as in the study of Tipton et al. (49), intracellular phenylalanine concentrations did not change during resistance exercise. This finding, along with the fact that changes in muscle volume are likely to be small (see below), suggests that changes in phenylalanine pool size are unlikely to have affected our results.

It should be pointed out that changes in glucose pool size likely occur during resistance exercise (46). This increase probably reflects increases in glucose transport and increases in glucose released from glycogen by debranching enzyme (35, 46). However, as we did not attempt to determine the intracellular fate of the glucose taken up by muscle, changes in pool size do not affect our findings regarding glucose uptake.

Accurate measurement of blood flow is also necessary if true rates of PS, PB, and glucose uptake are to be calculated by uptake from the blood. In the present study, we chose to use the blood flow measured 1 min after a set of repetitions as a representative blood flow for the entire exercise bout. This time point represents the midpoint of the rest period and the median flow after each set contractions. This value should be approximately equal to the average flow for the 2-min rest period between sets (4). The actual average blood flow for the entire resistance exercise bout (during and between each set of contractions) may have been slightly less because blood flow is impeded during a set of high-resistance contractions. However, this should not qualitatively affect our results because, even considering the extreme situation in which blood flow is zero throughout, each set of contractions (lasting $\sim 30$ s) only reduces the blood flow values used for calculation of balance data by 20%.

The use of ICG to measure blood flow in the present study is in theory problematic. Blood flow measurement by constant-indicator infusion requires that the leg ICG pool size is constant, i.e., that the rate ICG enters the leg equals the rate ICG exits the leg. The pool size of ICG in the leg can change two ways: 1) by a change in the plasma volume of the leg and/or 2) by a change in the ICG concentration in the leg. After a bout of resistance exercise, both plasma volume and leg ICG concentration change as the body returns to the resting state. In practice, however, the effects of these changes are small in the context of the present study. The equation for calculating leg blood flow using constant ICG infusion is

$$\text{Blood flow} = \frac{F_i(C_i - C_v) / [(C_i - C_v)(1 - \text{Hct})]}{C_v}$$

where $F_i$ is the infusion rate of ICG into the femoral artery, $C_i$ is the concentration of the infused ICG, $C_v$ is the concentration of ICG in the femoral vein of the leg into which ICG was infused, $C_v$ is the concentration of ICG in the femoral vein of the noninfused leg, and Hct is hematocrit (20). Because $C_i > C_v$, the numerator can be approximated by $F_iC_i$ and will not change appreciably regardless of the blood flow. Because plasma volume changes $\sim 0.5\%/\text{min}$ during the first 15 min of recovery from resistance exercise (40), the difference of $1 - \text{Hct}$ will also remain essentially constant over the course of
taking a blood sample. The difference $C_v - C_r$ will increase as the blood flow drops and $C_v$ increases. Because passage of ICG from the artery to the vein requires $\sim 7$ s (38), the measured value of $C_v - C_r$ will differ from the true value by the amount that this difference changes during the mean transit time (also $\sim 7$ s) of ICG from the femoral artery to the femoral vein. Assuming that the difference $C_v - C_r$ doubles over the course of 2 min of recovery (i.e., blood flow is reduced by approximately one-half), the error in $C_v - C_r$ will be

\[ 7 \text{ s/120 s } \times 100\% = 5.8\% \]

Because the differences reported in this study were $>50\%$, the relatively small error associated with a non-steady state is unlikely to affect our conclusions.

It is reasonable to question whether the intracellular phenylalanine enrichments were valid for calculating rates of muscle PS and PB during exercise. Several factors suggest that they were. First, because the exercise biopsy was taken immediately after the last set of contractions (i.e., during the rest period before the next set of contractions, had there been another set), we maximized the time available ($\sim 45$ min) for a new steady state to be achieved. In addition, the intracellular enrichment during exercise was not significantly different from the value measured at rest, suggesting that an exercise-induced effect, if present, was small. Importantly, the fact that intracellular enrichment changed so little during exercise strongly suggests that our measured rates of PS and PB are valid, because the intracellular pool of labeled and unlabeled phenylalanine was apparently relatively constant over the time that the arterial and venous blood samples were collected. Finally, it is worth noting that, given the direction of the slight change in enrichment (an increase), any further change in enrichment would have only reduced the differences between resting and exercise measurements.

A final consideration is the effect of changes in interstitial phenylalanine pool size occurring during the exercise bout. We did not measure interstitial phenylalanine concentrations and enrichments in the present study. However, previous studies suggest that resistance exercise-induced increases in muscle volume (the sum of changes in interstitial and intracellular volume) are $\approx 10\%$ (40) and thus unlikely to significantly affect our results.

In conclusion, in the present study, we have shown, for the first time in humans, that resistance exercise is a potent stimulator of glucose uptake, increasing rates of uptake to roughly six times the resting level. In addition, we have shown that muscle PS and PB are not significantly affected during resistance exercise.

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