Mixed muscle and hepatic derived plasma protein metabolism is differentially regulated in older and younger men following resistance exercise

M. Sheffield-Moore, D. Paddon-Jones, A. P. Sanford, J. I. Rosenblatt, A. G. Matlock, M. G. Cree, and R. R. Wolfe. Mixed muscle and hepatic derived plasma protein metabolism is differentially regulated in older and younger men following resistance exercise. Am J Physiol Endocrinol Metab 288: E922–E929, 2005. First published January 11, 2005; doi:10.1152/ajpendo.00358.2004.—We sought to determine whether exercise-induced muscle protein turnover alters the subsequent production of hepatically derived acute-phase plasma proteins, and whether age affects how these proteins are regulated. We measured arteriovenous (a-v) balance and the synthesis of mixed muscle protein, albumin (A) and fibrinogen (F) before exercise (REST) and from the beginning of exercise to 10, 60, and 180 min following a single bout of moderate-intensity leg extension exercise (POST-EX) in postabsorptive untrained older (n = 6) and younger (n = 6) men using 1-[ring-13H]phenylalanine (Phe). Subjects performed 6 sets of 8 repetitions of leg extension at 80% of their 1-RM (one-repetition maximum). All data are presented as the difference from REST (∆ from REST at 10, 60, and 180 min POST-EX). Mixed muscle fractional synthesis rate (FSR-M) increased significantly from the beginning of exercise until 10 min POST-EX in the older men (∆FSR-M: 0.044%/h), whereas FSR-M in the younger men was not elevated until 180 min POST-EX (∆FSR-M: 0.030%/h). FSR-A and FSR-F increased at all POST-EX periods in the older men (∆FSR-A: 10 min: 1.90%/day; 60 min: 2.72%/day; 180 min: 2.78%/day; ∆FSR-F: 10 min: 1.00%/day; 60 min: 3.01%/day; 180 min: 3.73%/day). No change occurred in FSR-A in the younger men, but FSR-F was elevated from the beginning of exercise until 10 and 180 min POST-EX (10 min: 3.07%/day and 180 min: 3.96%/day). Net balance of Phe was positive in the older men in the immediate POST-EX period. Our data indicate that mixed muscle and hepatic derived protein synthesis is differentially regulated in younger and older men in response to a single bout of moderate-intensity leg extension exercise. Moreover, our data suggest that with age may come a greater need to salvage or make available amino acids from exercise-induced muscle protein breakdown to mount an acute-phase response.

agging; leg extension; protein turnover; plasma proteins

IN RECENT YEARS, CONSIDERABLE ATTENTION has been directed toward studying exercise modes capable of stimulating skeletal muscle protein synthesis, with a highly directed effort focused on stemming the well-documented losses of muscular strength and mass in older individuals. The ability of resistance exercise to positively affect the quantity and quality of skeletal muscle tissue has considerable health benefits for all ages. Several studies have successfully demonstrated the anabolic potential of chronic resistance exercise training on muscle proteins in both young and older individuals (1, 17, 39, 40). However, although regular resistance exercise training in conjunction with nutrient intake can be a potent stimulator of muscle protein synthesis, net muscle protein balance generally remains negative in the recovery period of a single bout of resistance exercise in the absence of nutrient intake (4, 23–25). Although the reasons for this are not well understood, there exists an ongoing debate over the specific factors that influence the protein metabolic response of skeletal muscle to a single bout of resistance exercise in young healthy humans (36). The most often discussed factors include the effects of training status (27, 36), exercise intensity (27, 36), and type and timing of nutrient intake (5, 8, 9, 15, 21, 28, 33, 35). However, often overlooked is whether resistance exercise alters the economy of amino acids derived from exercise-induced muscle protein turnover and whether these amino acids are salvaged or partitioned by the liver to support the synthesis of both negative (i.e., albumin) and positive (i.e., fibrinogen) hepatic proteins, or are simply irrevocably lost.

There exists a complex relationship between albumin, fibrinogen, and muscle protein turnover. Numerous physiological and pathological conditions, including the process of aging, can alter the response of plasma proteins, with some studies demonstrating a coordinated increase in the fractional synthesis rates (FSR) of albumin and fibrinogen (14, 22, 26), whereas in others the responses have been shown to be directionally opposite (16, 32). For example, total liver protein synthesis is elevated in conditions such as sepsis, trauma, and inflammation, whereas muscle protein synthesis is blunted (26). In healthy humans, albumin and fibrinogen account for ~50 and 10% of total liver protein synthesis, respectively (20). Moreover, turnover of proteins via the energetic processes of protein synthesis and protein breakdown is estimated to account for ~20% of resting energy expenditure, with ~1–2% of all skeletal muscle turnover occurring daily (27). This, in turn, necessitates the availability of amino acids to support the continuous turnover of hepatic and muscle proteins.

Physical stress induced by exercise results in the mobilization of free amino acids, which may be used for protein synthesis, muscular energetics, and substrates for gluconeogenesis (29). We (31) recently demonstrated that an acute bout of moderate-intensity aerobic exercise induced a significant increase in muscle protein turnover and albumin and fibrinogen synthesis in postabsorptive older and younger men. Additionally, a few studies find that aging induces a proinflammatory state (22, 41) that may ultimately affect skeletal muscle protein metabolism by shifting a disproportionate amount of these free amino acids to hepatic protein synthesis.

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amino acids toward the splanchnic bed (7) due to the increased demand for synthesis of plasma proteins (22). Quantification of mixed muscle and hepatically synthesized plasma protein synthetic rates after resistance exercise could provide insight as to how protein metabolism is partitioned between tissues and organs in older persons. Therefore, we sought to test the hypothesis that a single bout of moderate-intensity resistance exercise would increase postexercise muscle protein turnover and that the increased release of amino acids from exercise-induced protein breakdown would translate into increased hepatic protein synthesis in younger and older men with normal physical capabilities.

METHODS

Subjects. Twelve healthy untrained men, six older [69 ± 1 (SE) yr] and six younger [29 ± 2 yr] were studied before (REST) and after (10, 60, and 180 min POST-EX) a single bout of moderate-intensity leg extension exercise. Younger subjects were recruited from within the community by use of institutionally approved advertisements, whereas older subjects were obtained from the volunteer database kept by the Sealy Center on Aging at the University of Texas Medical Branch (UTMB). Detailed explanation of the study procedures and risks was provided prior to obtaining informed consent, and all procedures conformed with both national and local ethics committee guidelines. Written informed consent was obtained on all subjects prior to conducting any study-related procedures, and were performed by using a consent form approved by UTMB’s Institutional Review Board. Volunteers were considered to be eligible if they were found to be healthy on the basis of the following: clinical history, physical exam, electrocardiogram, ankle brachial index, exercise stress test, blood pressure, complete blood count, blood chemistries including blood glucose and liver and kidney function tests, hepatitis panel, HIV test, and urinalysis. Exclusion criteria included the following: cardiac, liver, kidney, pulmonary, autoimmune, or vascular disease, coagulation disorders, hypertension, diabetes, obesity, cancer, anemia, drug or alcohol abuse, strength or being aerobically trained, impairment of activities of daily living (ADLs), anabolic or corticosteroid use, or inability to discontinue anti-inflammatory or prophylactic aspirin therapy (e.g., 14 days for aspirin). Volunteers were queried regarding their ADLs, including their history of recent and past physical activity and were asked to perform their regular activities and maintain their usual diet during the week preceding the study.

Prestudy testing. Subjects meeting the study inclusion and exclusion criteria were admitted as outpatients to the General Clinical Research Center (GCRC) at UTMB ~2 wk before the experimental protocol. Total body fat, leg lean mass, and leg fat mass were determined using dual-energy X-ray absorptiometry (DEXA). All subjects reported to the GCRC exercise lab for the determination of their leg extension one-repetition maximum (1-RM). After the tests, subjects were monitored, fed, and discharged.

Experimental protocol. The evening before the study, subjects were admitted to the GCRC and began an overnight fast from 2200 until completion of the study protocol. At 0600, polyethylene catheters were inserted into a forearm vein for infusion of labeled phenylalanine, into a vein of the opposite hand for arterialized blood sampling, and into the femoral artery and vein of one leg for blood sampling. The femoral arterial catheter was also used for the infusion of indocyanine green (ICG, IC-Green; Akorn, Buffalo Grove, IL). Anthropometric measurements of the study leg were obtained for purposes of calculating leg volume (19).

The details of the experimental protocol are outlined in Fig. 1. A primed (2 μmol/kg) continuous infusion of L-[ring-2H5]phenylalanine (0.05 μmol·kg⁻¹·min⁻¹) was started (time 0) and maintained for the duration of the experiment (~405 min) after a blood sample was obtained for the measurement of background phenylalanine enrichment and concentration and ICG concentration. To prevent shifts in plasma volume and alterations in plasma proteins, subjects remained supine when not performing the exercise bout. At the end of the REST period, subjects were transferred from the bed to the leg extension machine. Resistance exercise consisted of six sets of eight repetitions at 80% of their predetermined 1-RM, with two warm-up sets of eight repetitions each performed at 50 and 100 lb, respectively. Table 1 outlines the subject characteristics and exercise parameters for the younger and older men. Immediately after exercise, subjects were transferred back to bed and remained supine for the entire POST-EX period. Muscle biopsies (~80–100 mg of tissue) were taken at the time points indicated in Fig. 1. The first POST-EX biopsy sample was taken at 10 min to allow for transfer of the subject to the bed from the leg extension machine. Biopsy samples were taken from the lateral portion of the vastus lateralis muscle of the leg, ~20 cm above the knee, using a 5-mm Bergström needle. Two separate biopsy sites were prepared for the collection of the four biopsies. Each biopsy site was used to obtain a biopsy both distal and proximal (i.e., superior) to the incision site. The tissue was rinsed, blotted, and immediately frozen in liquid nitrogen and stored at −80°C until analysis. Leg blood flow

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Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 3</td>
<td>67 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181 ± 3</td>
<td>178 ± 2</td>
<td>0.39</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78 ± 3</td>
<td>86 ± 2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>20 ± 1</td>
<td>26 ± 2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total leg volume, liters</td>
<td>10 ± 0.5</td>
<td>11 ± 0.2</td>
<td>0.361</td>
</tr>
<tr>
<td>Studied leg lean mass, g</td>
<td>9,708 ± 524</td>
<td>9,933 ± 321</td>
<td>0.632</td>
</tr>
<tr>
<td>Studied leg fat mass, g</td>
<td>2,816 ± 255</td>
<td>3,657 ± 445</td>
<td>0.131</td>
</tr>
<tr>
<td>Mean 1-RM, kg</td>
<td>227 ± 18</td>
<td>168 ± 12</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. 1-RM, one-repetition maximum.

was measured during REST (130–150 min), EX (15–25 min), and twice during the POST-EX (50–60 and 130–150 min) period using the ICG dye dilution method (16). Femoral arterial and venous blood samples were taken throughout the REST and POST-EX periods to measure phenylalanine, albumin, and fibrinogen concentrations and phenylalanine enrichment. The tracer infusion was stopped, and all catheters were removed upon completion of the study. A meal was provided and subjects were monitored for 2 h before discharge.

Analytic methods. Phenylalanine enrichments and concentrations in arterial and venous blood samples were determined after the addition of an internal standard, deproteinization with sarsosulfosalicylic acid, extraction with cation exchange chromatography, and tert-butyldimethylsilyl (t-BDMS) derivatization using gas chromatography-mass spectrometry (GC-MS) in electron impact mode (GC HP 5890, MSD HP 5989; Hewlett Packard, Palo Alto, CA).

Muscle samples were weighed and the proteins precipitated with 450 μl of 10% sarsosulfosalicylic acid. An internal standard containing phenylalanine (m + 10) was added to measure intracellular concentration. Tissue homogenization and centrifugation were performed on three separate occasions, and the supernatant was collected. The enrichment and concentration of free tissue phenylalanine were determined on its t-BDMS derivative using GC-MS (GC 8000 series, MD 800; Fisons Instruments, Manchester, UK) (38). Calculation of phenylalanine intracellular concentration was then possible by accounting for the tissue value in addition to the ratio of intracellular to extracellular water (0.16) (3). The remaining pellet containing mixed muscle proteins was repeatedly washed and then dried at 110°C for 24 h in 6 N HCl. Amino acids in the hydrolysate were extracted and derivatized, as described above for the plasma samples, before the measurement of phenylalanine enrichment using GC-MS monitoring the ions 237 and 239 and using the standard curve approach as described by Calder et al. (10).

Albumin concentration was measured spectrophotometrically at λ = 628 nm (Sigma Diagnostics, St. Louis, MO), and fibrinogen concentration was determined at the UTMB Clinical Laboratory by the previously described Clauss method (11) using the MDA Fibriquik assay (Biomerieux, Durham, NC). The fractional synthesis rates (FSRs) of albumin and fibrinogen were determined by GC-MC using protein-bound phenylalanine. First, plasma albumin and fibrinogen were purified from 1 ml of plasma, as previously described (13). Subsequently, plasma proteins were hydrolyzed in 6 N HCl at 110°C, and the amino acids derived from the hydrolyzed proteins were processed, extracted, and derivatized, as described above for the plasma and muscle samples, before the measurement of phenylalanine enrichment using GC-MS.

ICG concentration in infusate and serum samples was measured spectrophotometrically at λ = 805 nm.

Calculations. Parameters of phenylalanine kinetics were calculated using both the arteriovenous (a-v) balance method (38) and the three-pool model (3) for comparison. Phenylalanine was used because it is an essential amino acid and is not oxidized in the muscle tissue. Therefore, phenylalanine utilization in the muscle is a direct index of muscle protein synthesis and its release from the muscle a measure of muscle proteolysis.

The a-v balance method provides an estimate of muscle protein synthesis, breakdown, and net balance and is dependent on the measurement of phenylalanine enrichments and concentrations in the femoral artery and vein. These parameters are based on the extraction of labeled phenylalanine from the femoral artery, the appearance of unlabeled phenylalanine from the muscle in the femoral vein, and the net a-v difference in phenylalanine concentrations, respectively (38). The three-pool model is an expansion of the a-v balance method and relies not only on the measurement of phenylalanine enrichments and concentrations in the femoral artery and vein but also on the direct measurement of phenylalanine enrichment in the free tissue water. Thus the direct measurement of phenylalanine intracellular utilization for protein synthesis and release from protein breakdown is possible. In addition, it is possible to calculate the rate of phenylalanine transport from the artery into the muscle tissue and from the muscle tissue into the venous blood. The a-v balance method and the three-pool model are well established and have been presented elsewhere (38). Both kinetic methods are presented to demonstrate the agreement between the two methods. Data are presented per 100 ml of leg volume.

Leg plasma flow was calculated using the dye dilution technique, as previously described (18). Leg blood flow was calculated by correcting the plasma flow by the hematocrit.

The FSR of mixed muscle proteins (FSR-M) was calculated from the incorporation rate of L-[ring-3H]phenylalanine into the proteins and the free-tissue phenylalanine enrichment using the precursor-product model previously described (38):

\[
FSR - M = \left( \frac{\Delta E_{PB}}{t} \right) \left( \frac{E_{PB1}}{E_{PB2}} \right) \cdot 60 \cdot 100
\]

where \( \Delta E_{PB} \) is the increment of protein-bound phenylalanine enrichment between two sequential biopsies, \( t \) is the time interval between the two sequential biopsies, and \( E_{PB1} \) and \( E_{PB2} \) are the phenylalanine enrichments (tracer-to-tracer ratio) in the free muscle pool in two subsequent biopsies. The results are presented as percent per hour.

Albumin (FSR-A) and fibrinogen FSR (FSR-F) are calculated in a similar manner as described above:

\[
FSR = \left( \frac{\Delta E_{PB}}{t} \right) \left( \frac{E_{PB1}}{E_{PB2}} \right) \cdot 60 \cdot 100 \cdot 24
\]

\( \Delta E_{PB} \) is the increment of protein-bound phenylalanine enrichment between two sequential blood draws, \( t \) is the time interval between the two sequential blood draws, and \( E_{PB1} \) and \( E_{PB2} \) are the phenylalanine enrichments (tracer-to-tracer ratio) in the blood pool from two subsequent draws. The results are presented as percent per day.

Statistics. Statistical analyses were performed with Dunnett’s one-tailed simultaneous 95% confidence limits (CI), using 90% upper and lower limits on each of the following primary and secondary parameters. Primary parameters include FSR-M, FSR-A, and FSR-F. Secondary parameters include phenylalanine net balance, three-pool model-derived protein synthesis (\( F_{\text{syn}} \)) and breakdown (\( F_{\text{bnd}} \)), and two-pool model-derived protein synthesis [rate of disappearance (\( R_{\text{d}} \))] and protein breakdown [rate of appearance (\( R_{\text{a}} \))]. Statistical analysis was carried out using NCSS (2004). All primary and secondary data are presented as the difference from REST (\( \Delta \) from REST at 10, 60, and 180 min POST-EX). Tertiary parameters and descriptive data are presented as means ± SE.

RESULTS

Subjects’ characteristics. Height, total leg volume, leg lean mass, and leg fat mass were similar in the younger and older men. However, body weight and percent body fat were significantly higher in the older men. Moreover, the younger men exercised at a higher workload (80% of 1-RM) than the older
Table 2. Basal and POST-EX values of FSR-M, FSR-A, FSR-F, and leg blood flow in healthy younger and older men at rest and from beginning of exercise to 10, 60, and 180 min POST-EX

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>10 min POST-EX</th>
<th>60 min POST-EX</th>
<th>180 min POST-EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger men (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSR-M (%/h)</td>
<td>0.072 ± 0.003</td>
<td>0.072 ± 0.005</td>
<td>0.091 ± 0.007</td>
<td>0.102 ± 0.013</td>
</tr>
<tr>
<td>FSR-A, %/day</td>
<td>6.0 ± 1.0</td>
<td>6.0 ± 0.6</td>
<td>6.6 ± 0.5</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>FSR-F, %/day</td>
<td>14.2 ± 1.7</td>
<td>16.3 ± 1.7</td>
<td>16.9 ± 1.5</td>
<td>18.2 ± 2.0</td>
</tr>
<tr>
<td>Leg blood flow, ml/min⁻¹·100 ml leg vol⁻¹</td>
<td>2.7 ± 0.3</td>
<td>8.4 ± 12</td>
<td>3.9 ± 1.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Older men (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSR-M (%/h)</td>
<td>0.076 ± 0.004</td>
<td>0.120 ± 0.018</td>
<td>0.089 ± 0.013</td>
<td>0.079 ± 0.014</td>
</tr>
<tr>
<td>FSR-A, %/day</td>
<td>4.0 ± 0.3</td>
<td>5.9 ± 0.4</td>
<td>6.7 ± 0.2</td>
<td>6.7 ± 0.4</td>
</tr>
<tr>
<td>FSR-F, %/day</td>
<td>6.8 ± 0.6</td>
<td>7.9 ± 0.5</td>
<td>9.8 ± 0.6</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>Leg blood flow, ml/min⁻¹·100 ml leg vol⁻¹</td>
<td>3.3 ± 0.2</td>
<td>11.9 ± 1.1</td>
<td>4.1 ± 0.7</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. POST-EX, postexercise; FSR-M, FSR-A, and FSR-F, fractional synthesis rates of mixed muscle, albumin, and fibrinogen, respectively.

Phenylalanine concentrations and enrichments. Phenylalanine concentrations and enrichments in the femoral artery, vein, and muscle of the younger and older men were unchanged in both groups of men in the POST-EX periods. However, arterial enrichments in the older men remained relatively constant throughout 60 min POST-EX but increased slightly by 180 min POST-EX. Furthermore, phenylalanine enrichments in the vein and muscle in the older men slowly increased throughout the POST-EX period.

Leg phenylalanine kinetics. Phenylalanine rate of disappearance (Rd) and rate of appearance (Ra), indexes of muscle protein synthesis and breakdown, respectively, were significantly increased above REST from the beginning of exercise to 10 min POST-EX in the older men only (Rd: 10 min = 146.528, 90% CI = 84.662 to 208.39; Ra: 10 min = 94.293, 90% CI = 31.094 to 157.492). Consequently, only the older men displayed a positive net balance of phenylalanine from REST to 10 min POST-EX (10 min = 52.235, 90% CI = 31.937 to 72.534; Fig. 6).

The differences from REST in protein synthesis (Fsyn) and protein breakdown (Fb) derived from the three-pool model are displayed in Figs. 7 and 8, respectively. Only the older men displayed increases in protein synthesis (10 min = 208.092, 90% CI = 106.027 to 310.157) and protein breakdown (10 min = 155.857, 90% CI = 53.358 to 258.356) from the beginning of exercise to 10 min POST-EX.
Arterial phenylalanine transport appears to be increased by 10 min POST-EX in the older men, with transport from the muscle and a-v shunting demonstrating elevations from REST to 10 min POST-EX in both groups.

Plasma albumin and fibrinogen concentrations. Plasma albumin and fibrinogen concentrations in the artery and vein remained unchanged in both younger and older men during all POST-EX periods (Table 4).

**DISCUSSION**

The purpose of this study was to examine the postexercise metabolism of mixed muscle and hepatically synthesized acute-phase plasma proteins (i.e., albumin and fibrinogen) in younger and older men after a single bout of moderate-intensity leg extension exercise by measuring the incorporation of labeled phenylalanine (FSR). Our results demonstrate that both younger and older men are capable of stimulating mixed muscle and hepatic derived plasma protein FSR above the resting values, albeit with some slight differences in their postexercise response. In particular, the increase in FSR of mixed muscle from the older men occurred very rapidly and was short-lived, whereas the induction of mixed muscle FSR in the younger men took until 180 min postexercise, with the magnitude of change from rest being less than that of the older men. Furthermore, fibrinogen FSR increased in both the younger and older men throughout the entire postexercise period, with albumin stimulation occurring only in the older men. More importantly, the leg extension exercise stimulated muscle protein breakdown only in the older men, perhaps making available more amino acids to mount an acute-phase response. Regardless, the results clearly demonstrate a differential hepatic and mixed muscle response between young and old men after resistance exercise.

Short-term (~2-wk program) resistance exercise has been shown to acutely increase myosin heavy chain (MHC) and mixed muscle protein synthesis rates similarly in younger and older individuals (17). Furthermore, several studies have also shown that a single bout of resistance exercise containing multiple exercises increases mixed muscle proteolysis for up to 24 h postexercise (4, 6, 23, 24) in young individuals, indicating that muscle protein breakdown after exercise is an important component of the postexercise metabolic response in young.
However, there remains an ongoing debate over the specific factors that influence the net protein metabolic response of skeletal muscle to a single bout of resistance exercise. Factors such as age, exercise intensity, and prior training status have been discussed as possible explanations for the differing results of many resistance exercise studies (36). Often overlooked in this debate is the complex relationship and likely interdependence between the metabolism of the hepatic synthesized proteins such as albumin and fibrinogen and skeletal muscle proteins.

We (31) recently showed that a single bout of moderate-intensity aerobic exercise induced short-term increases in muscle and plasma protein synthesis in both younger and older men. Taken together, the results from the present study and from our recently published aerobic study (31) demonstrate that moderate-intensity exercise is sufficient to stimulate muscle and plasma protein synthesis in both older and younger men. Thus these results suggest that, regardless of the type of exercise performed (i.e., aerobic or resistance), a moderate bout of exercise is capable of conferring significant protein metabolic changes in both older and younger men (31).

It should be pointed out that, although both the younger and older men demonstrated an increase in protein synthesis in response to resistance exercise, their protein metabolic responses are by no means identical. For example, the younger men exhibited somewhat of a delayed response in mixed muscle protein synthesis and did not respond at all with respect to the negative acute-phase protein albumin. Conversely, the older men had a quick but short-lived response in the muscle and a very robust albumin and fibrinogen response. Moreover, the younger men demonstrated no increases in protein breakdown in response to leg extension exercise, whereas the older men matched their immediate postexercise increase in protein synthesis with a concurrent increase in protein breakdown. Therefore, the net increase in protein synthesis was great enough to result in a short-lived positive net balance of phenylalanine during exercise until 10 min after exercise. Perhaps the differential response between young and old is simply due to an insufficient exercise stimulus caused by a more moderate-intensity exercise session. For example, even though both groups exercised at 80% of their predetermined 1-RM, it is possible that untrained younger men require a higher-intensity bout of leg extension exercise, or perhaps the involvement of more leg muscle groups such as from additional leg exercises is necessary. It is also worth noting that younger and older men tend to exhibit differences in body composition. Although there were no statistical differences in leg lean or fat mass between our two groups of men, the older men had a tendency to have more leg fat. It is therefore possible that some or part of the response seen in the present study could simply be due to the slight differences in body composition between younger and older men.

Two previous studies failed to show an acute response in muscle protein synthesis to resistance exercise (30, 34), and in these studies young trained subjects were used, also suggesting a lack of sufficient exercise stimulus (29). Given that the younger men did not display an increase in protein breakdown after exercise, this supports our belief that the exercise bout may not have been sufficiently intense to substantially alter protein turnover in the young or that protein breakdown is delayed beyond 3 h postexercise. Alternatively, it is possible that the young have fewer free amino acids available such as from splanchnic derived amino acids resulting from fasting-induced protein breakdown. Conversely, one could hypothe-

<table>
<thead>
<tr>
<th>Younger men</th>
<th>REST</th>
<th>10 min POST-EX</th>
<th>60 min POST-EX</th>
<th>180 min POST-EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>4.4±0.4</td>
<td>4.8±0.4</td>
<td>5.1±0.4</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>Vein</td>
<td>4.7±0.4</td>
<td>5.3±0.5</td>
<td>4.6±0.2</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>213±13</td>
<td>221±15</td>
<td>197±12</td>
<td>208±14</td>
</tr>
<tr>
<td>Vein</td>
<td>220±12</td>
<td>232±13</td>
<td>203±13</td>
<td>209±13</td>
</tr>
<tr>
<td>Older men</td>
<td></td>
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</tr>
<tr>
<td>Albumin</td>
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</tr>
<tr>
<td>Artery</td>
<td>3.7±0.2</td>
<td>4.6±0.3</td>
<td>4.2±0.3</td>
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<tr>
<td>Vein</td>
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<td>4.1±0.2</td>
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<td>Fibrinogen</td>
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<td></td>
</tr>
<tr>
<td>Artery</td>
<td>292±13</td>
<td>342±27</td>
<td>281±13</td>
<td>297±18</td>
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<tr>
<td>Vein</td>
<td>306±17</td>
<td>339±27</td>
<td>300±15</td>
<td>295±19</td>
</tr>
</tbody>
</table>

Values are means ± SE: albumin in g/dl, fibrinogen in mg/dl.
size that the reason the older men had a more vigorous acute-phase response is either due to a disproportionate shifting of free amino acids normally used for muscle protein synthesis to support their greater hepatic and/or splanchnic demand, or simply because the amino acids supplied from muscle protein breakdown in the older men resulted in a more pronounced hepatic derived plasma protein response. Moreover, it is also important to note that the exercise stimulus may have had differential effects on the individual muscle proteins just as it had on the hepatically derived proteins. Therefore, it is impossible from this study to determine whether individual muscle proteins would have been altered by the exercise stimulus. It has also been suggested that the catabolism of albumin in muscle makes available essential amino acids utilizable for local protein synthesis (12, 13), with a strong positive correlation having been shown between plasma albumin concentration and muscle mass in the elderly (2). Interestingly, a recent study by Trappe et al. (37), using a microdialysis approach, showed that proteolysis is not increased in the first 24 h after a single resistance exercise bout containing multiple exercises in the two main skeletal muscle proteins myosin and actin, respectively. This suggests that myosin and actin are not significant components of the protein breakdown seen in mixed muscle of young or older individuals after resistance exercise. Finally, given that both groups had similar exercise-induced hyperemia, it is unlikely that blood flow would explain the apparent differences in timing or magnitude of the postexercise protein metabolic response between the younger and older men.

In conclusion, our results demonstrate that both younger and older men are capable of stimulating mixed muscle and hepatic derived plasma protein synthesis in response to a single bout of moderate-intensity resistance exercise. However, because only the older men demonstrated an increase in the release of amino acids from muscle protein breakdown following exercise, it is possible that with age comes a greater need to salvage amino acids from muscle breakdown to mount an acute-phase response. Finally, although these results may also imply that younger men require a higher-intensity threshold of resistance exercise for the induction of synthesis and breakdown in mixed muscle, they also clearly demonstrate the existence of a differential hepatic and mixed muscle response between younger and older men following a single bout of resistance exercise.

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