Age-related anabolic resistance after endurance-type exercise in healthy humans

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ABSTRACT Age-related skeletal muscle loss is thought to stem from suboptimal nutrition and resistance to anabolic stimuli. Impaired microcirculatory (nutritive) blood flow may contribute to anabolic resistance by reducing delivery of amino acids to skeletal muscle. In this study, we employed contrast-enhanced ultrasound, microdialysis sampling of skeletal muscle interstitium, and stable isotope methodology, to assess hemodynamic and metabolic responses of older individuals to endurance type (walking) exercise during controlled amino acid provision. We hypothesized that older individuals would exhibit reduced microcirculatory blood flow, interstitial amino acid concentrations, and amino acid transport when compared with younger controls. We report for the first time that aging induces anabolic resistance following endurance exercise, manifested as reduced (by ~40%) efficiency of muscle protein synthesis. Despite lower (by ~40–45%) microcirculatory flow in the older than in the younger participants, circulating and interstitial amino acid concentrations and phenylalanine transport into skeletal muscle were all equal or higher in older individuals than in the young, comprehensively refuting our hypothesis that amino acid availability limits postexercise anabolism in older individuals. Our data point to alternative mediators of age-related anabolic resistance and importantly suggest correction of these impairments may reduce requirements for, and increase the efficacy of, dietary protein in older individuals. Durham, W. J., Casperson, S. L., Dillon, E. L., Keske, M. A., Paddon-Jones, D., Sanford, A. P., Hickner, R. C., Grady, J. J., Sheffield-Moore, M. Age-related anabolic resistance after endurance-type exercise in healthy humans. FASEB J. 24, 4117–4127 (2010). www.fasebj.org

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Most individuals experience a gradual loss of muscle mass as they age, a process termed sarcopenia. When sarcopenic losses reduce skeletal muscle mass below a critical threshold, activities of daily living are compromised and disability ensues (1). Although multiple etiologic factors have been associated with sarcopenia (1–3), the nature of sarcopenic progression is unclear. In particular, it is uncertain whether there is an early stage of resistance to anabolic stimuli that precedes frank muscle loss. Although changes in basal muscle protein metabolism have been reported to occur in some (4–8) but not all (9–13) studies of otherwise healthy adults, aberrant responses to anabolic stimuli are thought to play an important role (14). Accordingly, evidence is accumulating that skeletal muscle of older individuals exhibits resistance to anabolic stimuli such as amino acids, insulin, and resistance exercise (15, 16).

Before circulating amino acids can be used for skeletal muscle protein synthesis, they must first leave skeletal muscle capillaries, traverse the interstitial fluid, and be transported into muscle fibers. Previous studies suggest that the transit of amino acids from the blood to the interstitial space occurs primarily via diffusion and is rate-limiting for the net uptake of circulating amino acids by muscle fibers (17–19). One mediator of this uptake by skeletal muscle may be the relative distribution of blood flow between routes of optimal nutrient transfer (termed “nutritive” flow routes) and suboptimal nutrient transfer (termed “non-nutritive” routes) (20, 21). Thus, reduced nutritive flow is one potential contributor to the blunted anabolic responsiveness of older individuals to anabolic stimuli. However, there is evidence that other factors are likely involved as well. Previous studies suggest that the capacity for translation by ribosomes and their associated factors is reduced by aging (22, 23). In addition, aging has been reported to decrease skeletal muscle capillarization and increase capillary basement membrane width (24), with unknown effects on transcapillary efflux of amino acids into the interstitial fluid.

To our knowledge, no previous studies have determined whether the generalized phenomenon of age-related amino acid resistance includes the period following endurance-type (“aerobic”) exercise. Although
such exercise stimulates skeletal muscle protein synthesis both acutely (25, 26) and chronically (27, 28) in the fasted state, it is not associated with hypertrophy (28), in contrast to resistance-type exercise. Divergent responses to resistance and endurance exercise may result from differences in energetic signaling responses (29–31), including intensity-dependent adaptive responses, such as mitochondrial biogenesis (32, 33). Such responses may interfere with stimulation of muscle protein synthesis by exogenous amino acids and/or increased muscle catabolism during postabsorptive conditions (27). Consistent with this notion, acute moderate-intensity (75% VO$_2$ peak) endurance-type cycling exercise (34) blunted the anabolic response to constant amino acid administration observed in healthy young subjects at rest (35, 36). In contrast, repetitive knee extension exercise (67% maximal work rate) for 1 hour did not interfere with the anabolic response to chronic amino acid administration (37), suggesting anabolic resistance induced by endurance exercise is specific to the type of exercise performed. However, whether lower-intensity endurance exercise, which provides less energetic stress and less stimulation of mitochondrial adaptive responses, blunts amino acid-induced anabolic responses is unknown. Further, whether such blunting occurs in older subjects in response to walking, the mode of endurance exercise most commonly engaged in by older individuals, has not been studied.

Accordingly, in the current study we examined the response of muscle protein metabolism to endurance exercise in younger and older subjects during provision of amino acids. We also measured amino acid concentrations in the blood and interstitial fluid, determined phenylalanine transport rates in skeletal muscle, and utilized both contrast-enhanced ultrasound and the microdialysis ethanol technique, two independent methods for assessing skeletal muscle nutritive flow (38–46). We studied younger and older subjects who were free of disease and not taking medications in an attempt to study the effects of aging per se and reduce the potential independent effects of disease and pharmacological interventions on metabolic responses, as well as to complement investigations of older individuals who were less healthy or already exhibited markedly reduced muscle mass. We hypothesized that older subjects, as compared with the young, would be resistant to the anabolic effects of amino acids and that this resistance would be associated with reductions in postexercise nutritive blood flow, interstitial amino acid concentrations, and amino acid transport from the blood into skeletal muscle. Although we did find evidence for an age-related deficit in nutritive blood flow, our data comprehensively refute our hypothesis that impaired nutritive flow limits amino acid availability in healthy older individuals, as circulating and interstitial amino acid concentrations and phenylalanine transport into skeletal muscle were all equal or higher in older individuals than in the young. Instead, our data direct attention to metabolic and oxidative stress, altered skeletal muscle membrane integrity, and hemodynamic mechanisms apart from amino acid availability as potential mediators of age-related anabolic resistance following an acute bout of endurance-type exercise.

**MATERIALS AND METHODS**

**Subjects**

Seventeen healthy untrained men, 8 older [O; 67±1.6 (mean±s.e.) yr] and 9 younger (Y; 29.8±1.7 yr), were studied before and after chronic amino acid administration (walking) during continuous infusion of amino acids. Informed written consent, which was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB), was obtained from all volunteers prior to any study-related procedures. Volunteers were screened at the UTMB General Clinical Research Center (GCRC) to determine study eligibility. Exclusion criteria included the following: cardiac, liver, kidney, pulmonary, autoimmune or vascular disease; hypocoagulation or hypercoagulation disorders, diabetes, cancer, obesity, anemia, infectious diseases, or an allergy to iodides. Subjects taking antihypertensive or lipid-lowering medications, anabolic steroids, or corticosteroids in the past 6 mo were excluded, as were subjects unable to continue anti-inflammatory or prophylactic aspirin therapy or nutritional supplement use (for 14 d prior to their study date) or those engaged in regular aerobic or resistance exercise training. Older subjects had their ankle-brachial index (ABI) determined to screen for peripheral arterial disease of the legs. Subjects were instructed to continue all regular activities of daily living and maintain their usual diet during the week preceding the study.

**Prestudy testing**

Subjects were admitted as outpatients to the GCRC 2 wk prior to conducting the metabolism study. Total body fat, leg lean mass, and leg fat mass were determined by dual-energy X-ray absorptiometry (DEXA; Hologic, Inc., Natick, MA, USA). Following the DEXA, subjects were escorted to the UTMB heart station for determination of VO$_2$ peak. VO$_2$ peak was measured on a treadmill using expired gas analysis (SensorMedics, Yorba Linda, CA, USA) during a medically supervised progressive walk/run exercise test, as described previously (26).

**Experimental protocol**

The experimental protocol is outlined in Fig. 1. Subjects reported to the GCRC at noon the day before the study. Subjects were fed a standardized, meat-containing mixed meal the evening before the study and fasted from 10 PM until amino acids were initiated in the immediate preexercise (rest) period the following day. The morning of the study, polyethylene catheters were inserted into the antecubital vein of both arms for infusion of stable isotopes, amino acids. Informed written consent, which was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB), was obtained from all volunteers prior to any study-related procedures. Volunteers were screened at the UTMB General Clinical Research Center (GCRC) to determine study eligibility. Exclusion criteria included the following: cardiac, liver, kidney, pulmonary, autoimmune or vascular disease; hypocoagulation or hypercoagulation disorders, diabetes, cancer, obesity, anemia, infectious diseases, or an allergy to iodides. Subjects taking antihypertensive or lipid-lowering medications, anabolic steroids, or corticosteroids in the past 6 mo were excluded, as were subjects unable to continue anti-inflammatory or prophylactic aspirin therapy or nutritional supplement use (for 14 d prior to their study date) or those engaged in regular aerobic or resistance exercise training. Older subjects had their ankle-brachial index (ABI) determined to screen for peripheral arterial disease of the legs. Subjects were instructed to continue all regular activities of daily living and maintain their usual diet during the week preceding the study.

Baseline blood samples were drawn ~150 min prior to exercise (time = −195 min in Fig. 1) for the analysis of background isotopic enrichment and ICG concentration. Thereafter, blood samples were taken at t = −120, −105, −90, and −75 min for the analysis of isotopic enrichment, ICG concentration, insulin concentration, and glucose concentration. In addition, the t = −75-min sample was also analyzed for amino acid concentrations (see below). Following exercise, blood samples were obtained at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min for measurements...
of isotopic enrichment, ICG concentration, insulin concentration, and glucose concentration. In addition, the 60, 120, and 180 min postexercise samples were analyzed for amino acid concentrations, as described below.

A primed (2 μmol·kg⁻¹) continuous infusion of L-[ring-¹³C₆] phenylalanine (Phe) (0.08 μmol·kg⁻¹·min⁻¹) was started (t = −300 min) and continued until the end of the study (t = +180 min). Blood samples (at −300, −195, −150, −120, −45, 0, 60, 120, and 180 min postexercise) were analyzed for amino acid concentrations, as described below.

Leg blood flow

Leg plasma flow was determined utilizing the ICG dye dilution technique and converted to leg blood flow using hematocrit as described previously (47, 48).

Contrast-enhanced ultrasound

Imaging of the vastus lateralis muscle was performed in a transaxial plane ~15–20 cm above the patella over the midportion of the muscle using a P4–2 phased array transducer interfaced with the HDI-5000 ultrasound system (Philips ATL Ultrasound, Andover, MA, USA). Power Doppler imaging was performed as described by others (51–53). In summary, an intravenous infusion (3.5 ml·min⁻¹ for 8 min) of a suspension of perflutren microbubbles (Definity) was performed at −t = −85 min at rest and again at −50 min postexercise. Contrast-enhanced ultrasound was only performed 2 times because the FDA limits the amount of Definity that can be infused per day. A mechanical index of 1.3 was used and a compression of 80%. Once the systemic microbubble concentrations reached steady-state (~2 min), background images were obtained at a frame rate of 1 s⁻¹. Intermittent imaging was then performed using an internal timer at pulsing intervals (PI) ranging from 1 to 25 s, thus allowing progressively greater replenishment of the ultrasound beam elevation between destructive pulses. Depth, focus, and gain were optimized at the beginning of each study and held constant throughout. Data were recorded on an SVHS tape and digitized for analysis using an offline system. A minimum of 3 images were acquired at each PI. The background-subtracted video intensity (VI) at each PI was measured from a region of interest (ROI) within the vastus lateralis muscle. PI vs. VI data were curve fitted to the function:

\[ y = A \left(1 - e^{-\beta t}\right) \]

where y is the video intensity at a PI of t, A is the plateau video intensity [an index of microvascular blood volume (MBV)], and β is the rate of microvascular refilling [an indicator of microvascular flow velocity (MFV)] (52). The product MBV × MFV is a measure of microvascular blood flow (MBF).

Microdialysis

Three CMA 60 microdialysis probes (30 mm, 20-kDa cutoff; CMA Microdialysis, Solna, Sweden) were inserted percutaneously into the vastus lateralis muscle of one leg with an 18-gauge needle following 1% lidocaine administration ~20 cm above the patella. Microdialysis probes were perfused at a rate of 5.0 μl·min⁻¹ using a CMA 102 microinfusion pump (CMA Microdialysis) with a solution consisting of Na⁺ (147 mM), K⁺ (4 mM), Ca²⁺ (2.5 mM), CI⁻ (156 mM), and 40 g·L⁻¹ Dextran 70. EtOH (5 mM) was included in the microdialysis perfusion medium to monitor skeletal muscle nutritive blood flow in the area of the microdialysis probe. Microdialysis probe recovery for phenylalanine and leucine was determined by the internal reference technique (54) by adding 0.108 μCi·ml⁻¹ of Na⁻[³H]phenylalanine and 0.108 μCi·ml⁻¹ of D⁻[¹³C]leucine (Amersham Pharmacia Biotech, Piscataway, NJ, USA) to the perfusate. Probe recovery was determined by measuring disintegrations per minute (DPM) of ¹³C Leu and ³H Phe in the perfusate and dialysate, placing 10 μl in 15 ml of scintillation fluid and counting for 10 min on a LS 6500 multipurpose scintillation counter (Beckman Coulter, Fullerton, CA, USA). In vivo recovery was calculated using the following formula:

\[ \text{dialysate dpm} = \frac{\text{perfusate dpm} - \text{dialysate dpm}}{\text{perfusate dpm}} \]

As described previously (55), recoveries for phenylalanine and leucine were used for lysine and alanine, respectively. For all other interstitial amino acids, the average of the phenylalanine and leucine recoveries was applied to estimate interstitial concentrations and allow relative comparisons between young and old. Once inserted, probes were perfused for 45 min to reach equilibrium, after which dialysate samples were collected in 30-min aliquots during the preexercise and postexercise time.
periods in sealed microvials (Milian, Geneva, Switzerland) that were weighed before and after dialysate collection to determine dialysate volume. The microvials for each 30-min collection were immediately stored at 4°C until an aliquot was removed later in the day for ethanol analysis, with the remaining volume stored at −80°C.

Ethanol concentration in each perfusate and dialysate sample was measured according to the method described by Hickner et al. (56, 57). Briefly, 150 μl of reagent mixture consisting of glycine-hydradine buffer at pH 8.9 (74 μM Na₄P₂O₇·2 2H₂O, 22 μM glycine, and 60 μM hydradine) and 0.54 μM NAD⁺ was added to a 96-well plate. Then, 2 μl of sample was added, followed by 20 μl of enzyme (1.7 mg alcohol dehydrogenase in 1 ml ddH₂O). Ethanol concentrations were measured in the perfusate and dialysate solutions by fluorometric-enzymatic assay (Fluoraskan II; MTX Labs Systems, Inc., Vienna, VA, USA), with the results expressed as the ethanol outflow/inflow concentration ratio:

\[
\frac{C_{\text{out}}}{C_{\text{in}}} = \frac{[\text{ethanol}]_{\text{collected dialysate}}}{[\text{ethanol}]_{\text{inflused perfusion medium}}}
\]

where \( C_{\text{out}} \) is the concentration of the dialysate and \( C_{\text{in}} \) is the concentration of the perfusate. Sample determinations from each time period (rest, 0–60 min postexercise, 61–120 min postexercise, 121–180 min postexercise) were averaged. The ethanol outflow/inflow (O/I) ratio is inversely related to local nutritive blood flow (57).

**Analytical methods**

Phenylalanine enrichments and concentrations in arterial and venous blood samples were determined after the addition of an internal standard, deproteinization with sulfosalicylic acid, extraction using cation exchange chromatography, and tert-butylidimethylsilyl (t-BDMS) derivatization using gas-chromatography mass-spectrometry (GCMS) in electron impact mode (GC HP 5890, MSD HP 5898; Hewlett Packard, Palo Alto, CA, USA).

Muscle samples were weighed, and the proteins were precipitated with 800 μl of 10% perchloric acid. Tissue homogenization and centrifugation were performed twice, and the supernatant was collected. The remaining pellet was then washed 3 times and centrifugation were performed twice, and the supernatant was then washed 3 times with 800 μl of buffer. Ethanol concentrations were measured in the perfusate and dialysate solutions by fluorometric-enzymatic assay (Fluoraskan II; MTX Labs Systems, Inc., Vienna, VA, USA), with the results expressed as the ethanol outflow/inflow concentration ratio:

\[
\frac{C_{\text{out}}}{C_{\text{in}}} = \frac{[\text{ethanol}]_{\text{collected dialysate}}}{[\text{ethanol}]_{\text{inflused perfusion medium}}}
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where \( C_{\text{out}} \) is the concentration of the dialysate and \( C_{\text{in}} \) is the concentration of the perfusate. Sample determinations from each time period (rest, 0–60 min postexercise, 61–120 min postexercise, 121–180 min postexercise) were averaged. The ethanol outflow/inflow (O/I) ratio is inversely related to local nutritive blood flow (57).

**Calculations**

Skeletal muscle phenylalanine transmembrane transport and intracellular kinetics were studied using established techniques, as described previously (47, 58).

Outward transport efficiency % = 100 × \( \frac{F_{\text{V,M}}}{F_{\text{M,A}} + F_{\text{M,O}}} \)

Synthetic efficiency (%) = 100 × \( \frac{F_{\text{R,M}}}{F_{\text{M,A}} + F_{\text{M,O}}} \)

Synthetic clearance (ml·min⁻¹) = \( \frac{F_{\text{R,M}}}{C_{\text{A}}} \)

Anabolic clearance (ml·min⁻¹) = \( \frac{NB}{C_{\text{A}}} \)

Mixed muscle fractional synthesis rate (FSR; %/h) was calculated from the incorporation of Phe into protein, using the precursor-product model:
where \( E_{\text{in}} \) and \( E_{\text{out}} \) are the enrichments of bound Phe in the first and second muscle biopsies of a time period, \( t \) is the time interval between biopsies (min), and \( E_{\text{M}} \) is the mean Phe enrichment in the muscle intracellular pool (59).

### Statistical analysis

The outcome variables were all continuous and approximately normally distributed. A repeated-measures general linear mixed model (GLMM) was used to analyze the treatment effects over time. Initial assessments for time \( \times \) group interactions suggested no evidence of interaction in the model, and this term was dropped for all subsequent models. The models were fit in the MIXED procedure in SAS (60). To obtain meaningful (positive) values for 3-pool model fluxes calculated using both muscle and blood enrichments (e.g., \( F_{\text{MA}} \)), the muscle intracellular enrichment must be lower than that of the blood. In one younger and one older subject, this condition was not met during the postexercise period, and, as a result, only \( F_{\text{in}}, F_{\text{out}}, \) and NB were included in analyses for these subjects. In addition, in another older subject, the NB 60-min postexercise was negative (and >7 s.d. away from the mean) despite receiving an amino acid infusion; model fluxes from this subject at this time point were excluded from statistical analysis. We used a 2-sided a level of significance of 0.05 to assess statistical significance. Correlation coefficients are presented as Supplemental Data.

### RESULTS

#### Subject characteristics

Subject characteristics are presented in Table 1. Except for age and aerobic capacity (\( V_{\text{O}_{2}\text{peak}} \)), subjects were well matched, with similar heights, weights, BMI values, leg volumes, leg lean and fat mass, and body composition.

#### Leg blood flow, microvascular blood flow, and interstitial ethanol exchange

Total leg blood flow did not differ between young and older groups at any time (Fig. 2A). MBF (MBV \( \times \) MFV) was significantly elevated relative to rest period at 60 min postexercise in both young and old (Fig. 2B; \( P=0.006 \) for time main effect). In addition, MBF was significantly higher in the young than in the older group \( (P=0.03) \). At 60 min postexercise, skeletal muscle microvascular blood volume was higher than at rest in both young and old (Fig. 2C; \( P=0.0006 \) for time main effect). MFV did not differ between young and old (Fig. 2D), and there was no change from baseline at 60 min postexercise. There was a marginal \( (P=0.06) \) effect of age on interstitial fluid ethanol exchange, whereas the effect of time on this variable was highly \( (P=0.0006) \) significant (Fig. 2E), with means of 0.47 \( \pm \) 0.02, 0.40 \( \pm \) 0.02, 0.42 \( \pm \) 0.02, and 0.42 \( \pm \) 0.02 for rest and 0–60, 61–120, and 121–180 min postexercise, respectively, in the repeated mixed model.

### TABLE 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Younger men ( (n=9) )</th>
<th>Older men ( (n=8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>30 ( \pm ) 2</td>
<td>67 ( \pm ) 2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 ( \pm ) 1</td>
<td>176 ( \pm ) 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ( \pm ) 2</td>
<td>84 ( \pm ) 4</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>27 ( \pm ) 1</td>
<td>27 ( \pm ) 1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>22 ( \pm ) 1</td>
<td>24 ( \pm ) 1</td>
</tr>
<tr>
<td>Total fat-free mass (kg)</td>
<td>55 ( \pm ) 2</td>
<td>59 ( \pm ) 2</td>
</tr>
<tr>
<td>Right leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leg lean mass (kg)</td>
<td>9.6 ( \pm ) 0.3</td>
<td>9.7 ( \pm ) 0.5</td>
</tr>
<tr>
<td>Total leg fat mass (kg)</td>
<td>3.0 ( \pm ) 0.3</td>
<td>3.2 ( \pm ) 0.2</td>
</tr>
<tr>
<td>Total leg volume (L)</td>
<td>8.8 ( \pm ) 4</td>
<td>8.5 ( \pm ) 3</td>
</tr>
<tr>
<td>Left leg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leg lean mass (kg)</td>
<td>9.6 ( \pm ) 0.3</td>
<td>9.7 ( \pm ) 0.5</td>
</tr>
<tr>
<td>Total leg fat mass (kg)</td>
<td>3.1 ( \pm ) 0.3</td>
<td>3.2 ( \pm ) 0.2</td>
</tr>
<tr>
<td>Total leg volume (L)</td>
<td>8.6 ( \pm ) 4</td>
<td>8.4 ( \pm ) 3</td>
</tr>
<tr>
<td>( V_{O_{2}\text{peak}} ) (ml kg(^{-1}) min(^{-1}))</td>
<td>52 ( \pm ) 4(^{a} )</td>
<td>41 ( \pm ) 2(^{a} )</td>
</tr>
</tbody>
</table>

Values are expressed as means \( \pm \) s.e. \(^{a}P \leq 0.05\); significant difference between groups.

#### Amino acid concentrations

Because older individuals have elevated first-pass splanchnic extraction of oral amino acids (61), we provided amino acids via infusion in an attempt to normalize delivery of exogenous amino acids to the circulation. Arterial concentrations of several amino acids, including leucine, were marginally higher \( (P=0.04, 0.08, 0.08, \) for leucine, phenylalanine, and essential amino acids, respectively) in the older than in the younger group (Fig. 3, Supplemental Table 1). However, this was not due to an elevated overall \( (i.e.\), endogenous+exogenous) rate of appearance of amino acids in the older subjects, based on the fact that whole-body phenylalanine \( R_{a} \) was fairly well matched in the two groups (Fig. 3G), with values in the younger slightly (but significantly; \( P=0.007 \)) higher than in the older. As expected, total whole-body phenylalanine \( R_{a} \) increased in response to the amino acid infusion (Fig. 3G, \( P<0.0001 \) for time main effect). Endogenous phenylalanine \( R_{a} \) was reduced in both groups in response to the amino acid infusion and exercise and was significantly lower in the older group (Fig. 3H; \( P=0.007 \) and \( P=0.02 \) for age and time effects, respectively).

Interstitial concentrations of many amino acids were significantly correlated with plasma concentrations (Supplemental Table 3). In particular, plasma-interstitial fluid correlations for the branched chain amino acids and the sum of the essential amino acids were significant \( (P<0.0001) \) and moderately high \( (R^{2}=0.65–0.76) \). As in the plasma, several amino acids were present at higher concentrations in the interstitium of the older individuals than in the younger (Fig. 3D and Supplemental Table 2). Relative interstitial ammonia and urea levels were also significantly higher in the older group (Supplemental Table 2). We were not able to determine interstitial levels of all species measured in plasma. In particular, the summed interstitial summed essential amino acids do not include histidine, as we were...
Inward transport of subjects, although there were marginal group effects for variables were found to differ significantly between the young and older sub-
did not differ between young and older individuals. \( P = 0.0006 \). D) Microvascular flow velocity did not differ between age groups or times. E) Interstitial ethanol exchange was marginally different between age groups \(( P = 0.06 )\), with a significant effect of time. \( P = 0.0006 \) for time effect.

not able to measure interstitial histidine levels in all subjects.

**Phenylalanine kinetics**

Phenylalanine concentration and enrichment data are presented in Supplemental Table 4 and demonstrate that subjects were in isotopic steady state during the postexercise period. Consistent with previous studies \((9, 10)\), basal muscle protein metabolism was similar in subjects at rest and 0.098 \( \mu M \) at rest and at 60 and 180 min postexercise, respectively, in the young; 3.3 \( \pm 0.4 \), 7.3 \( \pm 1.2 \), 5.2 \( \pm 0.8 \) \( \mu M \) at rest and at 60 and 180 min postexercise, respectively, in the older subjects).

**Sensitivity of muscle protein synthesis to amino acids**

Because recent evidence suggests that older individuals exhibit resistance to anabolic stimuli, in particular, amino acids, we performed a number of calculations assessing amino acid handling and anabolic sensitivity in the young and older groups. Fractional outward transport and synthetic efficiency, two reciprocally related factors, increased and decreased, respectively, with time after exercise and were significantly different between young and old, with older subjects exhibiting greater fractional outward transport and lower synthetic efficiency (Fig. 4). We also calculated synthetic efficiency by dividing the FSR by the mean postexercise rate of phenylalanine appearance and obtained results similar to those obtained using the 3-pool model (Supplemental Fig. 1).

**Relationships between amino acid concentrations and muscle anabolism**

Correlations between circulating and interstitial amino acid concentrations and protein synthesis, protein breakdown, and the net balance between synthesis and breakdown, using all time points \((i.e., \text{rest and } 60 \text{ and } 180 \text{ min postexercise})\) are presented in Supplemental Tables 6 and 7. For the infused amino acids, the correlations between the interstitial or plasma concen-

**FSR**

Mixed muscle protein FSR was significantly higher postexercise than during rest \(( P < 0.001 \) for time main effect), without age-related differences \((0.061 \pm 0.005 \% h^{-1})\) in the young subjects at rest and \(0.070 \pm 0.006 \% h^{-1}\) in the older subjects at rest; \(0.098 \pm 0.009 \% h^{-1}\) in the young subjects postexercise and \(0.111 \pm 0.011 \% h^{-1}\) in the older subjects postexercise).

**Serum insulin concentrations**

Insulin concentrations responded differently to the combined intervention of amino acid infusion and exercise in the younger and older subjects \((\text{group} \times \text{time interaction } P = 0.01)\), with older subjects exhibiting higher insulin concentrations at 60 min postexercise than the younger subjects \((3.0 \pm 0.8, 3.2 \pm 1.1, \text{and } 3.6 \pm 1.1 \mu M \text{ at rest and at } 60 \text{ and } 180 \text{ min postexercise}, \text{respectively, in the young}; 3.3 \pm 0.4, 7.3 \pm 1.2, 5.2 \pm 0.8 \mu M \text{ at rest and at } 60 \text{ and } 180 \text{ min postexercise, respectively, in the older subjects})\).
trations and protein synthesis generally suggested a weak to moderate relationship, whereas the correlations with protein breakdown suggested at most a weak relationship (Supplemental Fig. 2 and Supplemental Tables 6 and 7). In contrast, correlations between net balance and the interstitial or plasma concentrations of the infused amino acids suggested a moderate to strong association (Supplemental Fig. 2 and Supplemental Tables 6 and 7).

Energetic and anabolic signaling response (AMPK and mTOR activation)

To address the possibility that the exercise bout represented a greater metabolic stress in the skeletal muscle of the older subjects and thereby interfered with anabolic signaling responses, we assessed skeletal muscle AMPKα phosphorylation (Thr172) and mTOR (Ser2448) phosphorylation (Supplemental Fig. 3). AMPKα phosphorylation was on average higher in the older subjects than in the young at each time point, but this difference did not reach statistical significance ($P=0.12$). Likewise, there were no group differences in mTOR phosphorylation; however, there was a significant effect of time ($P=0.01$).

Leg glucose uptake

Leg glucose uptake in the younger group was $70.9 \pm 7.3$, $97.9 \pm 29.2$, and $73.1 \pm 9.5 \text{ mol} \cdot \text{min}^{-1} \cdot \text{leg}^{-1}$ at rest and at 60 and 180 min postexercise, respectively. In the older group, the leg glucose uptake was $60.4 \pm 2.7$, $88.2 \pm 14.2$, and $68.1 \pm 5.6 \text{ mol} \cdot \text{min}^{-1} \cdot \text{leg}^{-1}$ at rest and at 60 and 180 min postexercise, respectively. Leg glucose uptake did not differ significantly in young and older subjects and did not exhibit a significant effect of time.

DISCUSSION

Loss of skeletal muscle mass is a common consequence of aging. Although age-related comorbidities can con-
or amino acid administration occurs in the reduction of muscle protein synthesis in response to protein intake, evident in a reduced sensitivity of muscle protein synthesis and breakdown. Rather, it was a subclinical phenomenon observed in this study did not manifest as marked changes in the overall balance between muscle protein synthesis and breakdown. Rather, it was a subclinical entity, evident in a reduced sensitivity of muscle protein synthesis to stimulation by amino acids.

Previous studies have determined that the stimulation of muscle protein synthesis in response to protein or amino acid administration occurs via an unidentified sensing mechanism responsive to elevated extracellular concentrations and/or increased intracellular rates of appearance of essential amino acids (15, 35, 47, 62–63). In the current study, we compared anabolic sensitivity in the younger and older groups by expressing anabolic responses relative to the intracellular rate of appearance of phenylalanine and the arterial phenylalanine concentration (Fig. 4 and Supplemental Figs. 1 and 4). These calculations uniformly demonstrated marked amino acid resistance in skeletal muscle of the older subjects. These findings are consistent with previous studies in which the response of skeletal muscle protein synthesis to amino acids was found to be blunted in the old under nonexercised conditions (14) and demonstrate that age-related anabolic resistance extends to the period following an acute bout of endurance-type exercise.

In this study, we hypothesized that reduced “nutritive” blood flow in older vs. younger subjects would be associated with reduced interstitial amino acid concentrations and inward phenylalanine transport, which would thereby limit muscle protein synthesis by limiting essential amino acid availability. In theory, impaired endothelial function could interfere with the response of skeletal muscle to elevated plasma amino acids by reducing capillary blood flow and thus limiting transcapillary amino acid exchange with the interstitial fluid. Our results comprehensively refute our hypothesis. Despite evidence of reduced nutritive blood flow by two independent measures, the older individuals had equal or greater amino acid availability than the young, based on circulating and interstitial amino acid concentrations and intracellular appearance rates of phenylalanine. Thus, age-related anabolic resistance to amino acids is apparently not mediated via reduced amino acid availability secondary to impaired hemodynamics. However, we cannot rule out a role for impaired microvascular flow in anabolic resistance, but by some mechanism other than amino acid availability [e.g., reduced hormone/nutrient delivery (66) and/or reduced removal of cellular “waste” products such as ammonia (Supplemental Table 2)], as both capillary recruitment and microvascular blood flow were positively correlated with net balance.

The markedly higher interstitial ammonia levels (Supplemental Table 2) in skeletal muscle of the older subjects were unexpected. Skeletal muscle ammonia production can occur by several mechanisms involving deamination of AMP or amino acids (67–69), although the relative importance of these are still not certain, with the exception of very intense exercise, during which AMP deamination is generally agreed to be the most important pathway for skeletal muscle ammoniagenesis. Recent human and animal studies suggest that the susceptibility of older skeletal muscle to metabolic stress is increased, on the basis of activation of the energy sensor AMPK (10, 70). Such findings are consistent with the notion that mitochondrial ATP-generating capacity is reduced with aging (71). It is conceivable that this greater metabolic stress in older skeletal muscle results in significant ammonia production from AMP deamination. In the current study, skeletal muscle AMPK phosphorylation was, on average, higher in the older than in the younger subjects, but this difference did not reach statistical significance. The finding that arterial glutamine levels were significantly higher in the old than in the young, whereas interstitial concentrations were not different between the two groups, could also indicate that activity of glutaminase, which catalyzes conversion of glutamine to ammonia and glutamate, is higher in skeletal muscles of older vs. younger individuals. However, the facts that glutamate, aspartate, and alanine were given in the amino acid infusion and no tracers were infused for the purpose of studying glutamine trafficking. A) Intracellular rate of appearance was significantly different between young and older subjects, with a significant effect of time. *P = 0.01 vs. younger group; **P = 0.0005 for time effect. B) Outward transport efficiency was different between young and older subjects, with a significant effect of time. *P = 0.05 vs. younger group; **P = 0.0001 for time effect. C) Synthetic efficiency was higher in young than in older subjects, with a significant effect of time. *P = 0.05 vs. younger group; **P = 0.0001 for time effect.

Figure 4. Skeletal muscle phenylalanine trafficking. A) Intracellular rate of appearance was significantly different between young and older subjects, with a significant effect of time. *P = 0.01 vs. younger group; **P = 0.0005 for time effect. B) Outward transport efficiency was different between young and older subjects, with a significant effect of time. *P = 0.05 vs. younger group; **P = 0.0001 for time effect. C) Synthetic efficiency was higher in young than in older subjects, with a significant effect of time. *P = 0.05 vs. younger group; **P = 0.0001 for time effect.
amino acid deamination prevent any strong conclusions regarding which, if any, amino acid deamination pathways were differentially stimulated in older and younger skeletal muscle.

Cirulating levels of 3-methylhistidine, an accepted marker of myofibrillar protein breakdown (72), were significantly higher in the old than in the young (Supplemental Table 1). However, the fact that neither skeletal muscle protein breakdown nor whole body phenylalanine $\Delta R$ were greater in the old subjects is inconsistent with greater release of 3-methylhistidine from protein breakdown of skeletal muscle or other tissues. An alternative explanation is that skeletal muscle sarcolemmal integrity is compromised with aging, possibly due to chronic suboptimal nutrition (73, 74), with the result that exchange between muscle fibers and the interstitial fluid is increased, as suggested previously (75). The significantly greater fractional outward transport from skeletal muscle in the older subjects is consistent with such an interpretation, as our model of phenylalanine kinetics does not distinguish between transporter-mediated exchange and amino acid exchange occurring through a compromised sarcolemma. Notably, a metabolomic investigation found that 3-methylhistidine is a sensitive marker of oxidative stress (76), suggesting that age-related oxidative stress may have contributed to reduced sarcolemmal integrity in the older subjects. Likewise, cystine, the oxidized form of cysteine, was also higher in both interstitial fluid and plasma of the older subjects than in the young, confirming previous reports of higher circulating cystine levels in the elderly (77–80) and consistent with the development of redox stress with aging (81–83).

By design, we studied healthy older subjects who do not take prescription medications, are glucose tolerant, and do not participate in a regular exercise program. This allowed us to study the effects of aging per se in the absence of comorbidities or pharmacological effects. However, our results, therefore, may underestimate the extent of impairment in anabolic responsiveness in older individuals who are not as healthy. Likewise, by selecting glucose-tolerant elderly subjects, we have minimized the likelihood of observing impairments in leg glucose uptake. Future studies will be important to determine the degree of impairment in less healthy populations.

Regardless of how it occurs, the existence of age-related anabolic resistance to amino acids, as found in this study, as well as in several previous ones, suggests two general, nonexclusive approaches for treating or preventing sarcopenia: increase ingestion of amino acids and/or protein by older individuals; and increase the anabolic sensitivity of older skeletal muscle to amino acids. The first approach may be viable in principle, as rates of skeletal muscle protein synthesis in older subjects have equaled those of younger subjects in some (84, 85) (as well as the present study), but not all (14), studies when large enough amounts of protein or amino acids were given. However, the diminished anabolic responsiveness of older individuals to amino acids, as well as the uncertain effects of long-term high protein/amino acid diets on deleterious signaling in tissues other than skeletal muscle (86, 87), implies that increasing anabolic sensitivity is the preferable approach. The present study suggests that interventions to reduce metabolic and redox stress and maintain sarcolemmal integrity are worthy of investigation in this regard.

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