A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults

Kyle L Timmerman, Shaheen Dhanani, Erin L Glynn, Christopher S Fry, Micah J Drummond, Kristofer Jennings, Blake B Rasmussen, and Elena Volpi

ABSTRACT

Background: Nutrient stimulation of muscle protein anabolism is blunted with aging and may contribute to the development and progression of sarcopenia in older adults. This is likely due to insulin resistance of protein metabolism and/or endothelial dysfunction with a reduction in nutritive flow, both of which can be improved by aerobic exercise.

Objective: Our objective was to determine whether increasing physical activity can enhance the muscle protein anabolic effect of essential amino acid (EAA) + sucrose intake in older subjects by improving nutritive flow and/or insulin signaling.

Design: Using a randomized crossover design, we measured in older subjects (n = 6, 70 ± 3 y of age, BMI (in kg/m^2) of 25 ± 1) the acute effects of increasing physical activity with aerobic exercise, as compared with normal sedentary lifestyle, on the response of blood flow, microvascular perfusion, insulin signaling, and muscle protein kinetics to EAA+sucrose intake.

Results: No differences between treatment groups were found in the basal state. The change from the basal state in blood flow, muscle perfusion, phenylalanine delivery, net balance, and muscle protein synthesis during the consumption of EAA+sucrose was significantly higher after the exercise than after the control treatment (P < 0.05). Insulin signaling increased during EAA+sucrose ingestion in both groups (P < 0.05).

Conclusions: Our data indicate that a prior bout of aerobic exercise increases the anabolic effect of nutrient intake in older adults. This effect appears to be mediated by an exercise-induced improvement in nutrient-stimulated vasodilation and nutrient delivery to muscle rather than to improved insulin signaling. This trial was registered at clinicaltrials.gov as NCT00690534.


INTRODUCTION

The age-related loss of skeletal muscle mass and function, or sarcopenia, is a major contributor to physical frailty in older adults (1–5). The rate of muscle loss or gain is a function of the net balance between muscle protein synthesis and breakdown. Whereas healthy, active, older adults have a postabsorptive net protein balance comparable with that of healthy younger individuals (6), they are less responsive to the combined anabolic effect of elevated amino acid and insulin concentrations, mainly because of blunted protein synthesis (7, 8).

A reduced response to amino acids at low doses may contribute to the blunted protein anabolism observed in older adults after mixed nutrient intake (9). However, this phenomenon seems to be dependent on the leucine content of the amino acid mixture (10) and is not detectable when amino acids or intact protein are administered after exercise or at the standard doses of a main meal (11–15).

Growing evidence indicates that the inability to mount an anabolic response to a meal may be primarily attributable to a reduced responsiveness of skeletal muscle to the vasodilatory and protein anabolic effects of insulin (16–18). Insulin has been shown to elicit skeletal muscle anabolism in young healthy adults (19–23) but not in healthy nondiabetic older adults (7, 16, 18). This age disparity of the muscle protein anabolic response to hyperinsulinemia is a true insulin resistance, because it can be overcome by elevating insulin to supraphysiologic concentrations (24). In recent studies, we have found that insulin’s anabolic effects on skeletal muscle proteins are mediated by its ability to stimulate endothelial-dependent increases in blood flow, muscle perfusion, and amino acid delivery to the muscle, which are impaired in older adults (16, 18, 22). We have also shown that aerobic exercise before an insulin challenge can normalize vasodilation and restore muscle protein anabolism in response to isolated hyperinsulinemia in healthy older adults (25). However, whereas it is quite useful to define mechanisms, isolated hyperinsulinemia does not occur physiologically because insulin is normally secreted in conjunction with macronutrient intake. There is currently no direct evidence that increased aerobic activity may also enhance the muscle protein anabolic effects of essential amino acids (EAA) and carbohydrate intake in older adults.

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2 Supported by NIH R01 AG018311, P30 AG024832, S10 RR16650, T32 HD07539, and UL1 RR029876. Definity was provided by Lantheus Medical Imaging, North Billerica, MA, under Definity Research grant no. 26020.
3 Address correspondence to E Volpi, 301 University Boulevard, Galveston, TX 77555-0460. E-mail: evolpi@utmb.edu.
4 Abbreviations used: Akt, protein kinase B; EAA, essential amino acids; GC-MS, gas chromatography–mass spectrometry; ICG, indocyanine green; ITS-CRC, Institute for Translational Sciences–Clinical Research Center; mTOR, mammalian target of rapamycin; 4E-BP1, 4E-binding protein 1.

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The purpose of the current study was to determine whether an acute and moderate increase in physical activity with aerobic exercise improves the response of muscle protein anabolism to subsequent intake of EAA+sucrose in older adults. We hypothesized that a moderately intense bout of aerobic exercise performed the night before ingestion of EAA+sucrose would enhance muscle vasodilation and/or insulin signaling and, consequently, improve muscle protein synthesis and net protein balance in sedentary healthy older adults.

SUBJECTS AND METHODS

Subjects

Six healthy independent but sedentary subjects were recruited through the Claude D. Pepper Older Americans Independence Center Volunteer Registry. A clinical history, physical examination, and laboratory tests—including a standard 75-g oral-glucose-tolerance test—were performed on each volunteer to determine eligibility. Volunteers with a stable body weight for ≥3 mo and screening results within the normal range were included in the study (Table 1). After approval by the Institutional Review Board of the University of Texas Medical Branch (Galveston, TX) and the US Food and Drug Administration (IND 73870), all subjects read and signed a written informed consent form before enrollment.

Study design

We used a randomized crossover design. Each subject participated in 2 EAA+sucrose ingestion experiments, separated by a 4–6-wk washout period during which subjects maintained their usual diet and activity level. The 2 experiments were identical, with the exception of the level of physical activity performed on the night before ingestion of EAA+sucrose: on one occasion subjects completed a bout of aerobic exercise (60–70% of heart rate reserve for 45 min on a treadmill), and on the other occasion they did not exercise (Figure 1).

Before both experiments, subjects were instructed to refrain from strenuous physical activity before admission to our Institute for Translational Sciences–Clinical Research Center (ITS-CRC). To monitor the subjects’ prestudy activity and ensure that our exercise intervention resulted in increased physical activity over the day preceding the exercise infusion experiment, we used StepWatch step activity monitors (Orthocare Innovations LLC), which subjects wore for the 3 d preceding admission to the ITS-CRC. The average number of daily steps over the 3 d preceding the control treatment were as follows: day 1: 3613 ± 514; day 2: 4984 ± 602; and day 3: 4217 ± 430. The average number of steps preceding the aerobic exercise treatment were as follows: day 1: 3999 ± 222; day 2: 4383 ± 543, and day 3: 6239 ± 277. Steps for days 1 and 2 were not significantly different between treatments, whereas steps on day 3 were significantly higher (P < 0.05) in the exercise treatment than in the control period. To control for macronutrient intake, subjects consumed a standardized research dinner (10 kcal/kg body weight; 60% carbohydrate, 20% fat, and 20% protein) and received a standard snack at ~2300. After the snack, subjects were allowed only water ad libitum until consuming the experimental meal on the following day.

On the next morning at ~0600, a polyethylene catheter was inserted into an antecubital vein for the infusion of stable-isotope tracers (Cambridge Isotopes Laboratories Inc). A retrograde catheter was placed in the contralateral hand and warmed to ~60°C to obtain arterialized blood for the measurement of systemic insulin, glucose, and indocyanine green (ICG) concentrations. With the use of local anesthesia and aseptic technique, 3-French (21 gauge) retrograde catheters were placed into the common femoral artery and vein of one leg for blood sampling. The arterial catheter was also used for the infusion of ICG. After background samples were obtained, a primed continuous infusion of L-[ring-13C6]phenylalanine (priming dose: 2 μmol/kg, infusion rate: 0.05 μmol·kg⁻¹·min⁻¹) and L-[6,6-2H2]glucose (priming dose: 19 μmol/kg, infusion rate: 0.05 μmol·kg⁻¹·min⁻¹) were started to measure phenylalanine and glucose kinetics.

After a 4-h basal period, subjects began consuming a solution of EAA+sucrose containing 20 g crystalline EAA (Sigma Aldrich; 20% histidine, 10% isoleucine, 18% leucine, 16% lysine, 3% methionine, 16% phenylalanine, 14% threonine, and 12% valine) and 35 g carbohydrate (sucrose), which were dissolved in 540 mL flavored water. To maintain the isotopic steady state in the arterial blood, based on our previous experience in older adults given phenylalanine and glucose tracers at the same rate (18), we enriched the meal with 9% L-[ring-13C6]phenylalanine and 2% D-[6,6-2H2]glucose. The enriched drink was given in small boluses (30 mL) every 10 min for 3 h to minimize fluctuations in blood phenylalanine and glucose concentrations, thus maintaining a metabolic steady state. Throughout the infusion experiment, frequent Doppler ultrasound (HD3-5000 ultrasound system; Philips ATL Ultrasound) measurements of the superficial femoral artery were taken to determine leg blood flow. Leg blood flow was also measured between 2.5 and 3 h (basal state) and between 5.5 and 6 h (EAA+sucrose) via infusion of ICG (0.5 mg/mL) into the common femoral artery and subsequent blood sampling from the femoral vein and hand vein. After ICG infusion, Perflutren Lipid Microspheres (Definity; Lantheus Medical Imaging) were infused into the antecubital vein to measure blood perfusion into the vastus lateralis muscle by contrast-enhanced ultrasound imaging. After perfusion was measured between 3.5 and 4 h (basal state) and 6.5 and 7 h (EAA+sucrose), 4 blood samples were taken from the femoral artery and vein to measure the concentrations of amino acids, glucose, insulin, and free phenylalanine and glucose enrichments. Muscle biopsy samples were taken from the lateral aspect of the vastus lateralis at 2, 4, 5.5, and 7 h of tracer infusion with the use of aseptic technique, local anesthesia, and a 5-mm Bergström biopsy needle. The first 2 biopsy samples (basal period) were

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### Table 1

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Subjects (n = 3 M, 3 F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>70 ± 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.70 ± 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Leg volume (mL)</td>
<td>9489 ± 801</td>
</tr>
</tbody>
</table>

*All values are means ± SEs.*
the sampling sites were taken from the same incision at different angles to ensure that the sampling sites were ≥5 cm apart. Biopsy samples 3 and 4 (EAA+sucrose period) were collected from a new incision ~10 cm proximal to the first incision. On collection, biopsied muscle tissue was rinsed in ice-cold saline, blotted, and frozen in liquid nitrogen. Samples were then stored at −80°C until analyzed. After the last biopsy sample was collected, the tracer infusion was stopped, the catheters were removed, and the subjects were fed and discharged from the ITS-CRC after 2 h of observation.

Analytic methods

Plasma glucose concentrations were measured with the use of an automated glucose analyzer (Yellow Springs Instrument Co). Insulin concentrations were measured by enzyme-linked immunosorbent assay (Linco) by using a microplate reader (Bio-Rad). Serum ICG concentrations in the femoral vein and hand vein were measured spectrophotometrically (Beckman Coulter) at λ = 805, which allowed for the calculation of blood flow as previously described (26, 27).

Muscle microvascular perfusion was measured in the basal period and during EAA+sucrose consumption by using contrast-enhanced ultrasound as previously described (17, 18, 28–31). Ultrasound imaging of the vastus lateralis muscle was performed in a transaxial plane ~15–20 cm above the patella over the midportion of the vastus lateralis muscle by using a P4-2 phased array transducer. A suspension of perflutren lipid microspheres (Definity; Lantheus Medical Imaging) was infused intravenously (3.5 mL/min for 8 min) by using a mechanical index of 1.3 and a compression of 80%. Once infused intravenously, the systemic microsphere concentrations reached steady state over the midportion of the vastus lateralis muscle by using a P4-2 phased array transducer. A suspension of perflutren lipid microspheres (Definity; Lantheus Medical Imaging) was infused intravenously (3.5 mL/min for 8 min) by using a mechanical index of 1.3 and a compression of 80%. Once infused intravenously, the systemic microsphere concentrations reached steady state (28).

Muscle tissue samples were used to measure phosphorylated and total protein expression of protein kinase B (Akt), mammalian target of rapamycin (mTOR), and 4E-binding protein 1 (4E-BP1). The primary phospho-antibodies (Cell Signaling) used were phospho-Akt (serine 473, 1:1000), phospho-mTOR (serine 2448, 1:1000 in 5% nonfat dairy milk), and phospho-4E-BP1 (threonine 37/46, 1:1000). A dilution of 1:1000 was used for total expression of each protein. Fifty micrograms of total protein homogenate was loaded in duplicate into each lane, and the samples were separated on a 7.5% or 15% polyacrylamide gel (150 V, 1 h) (Criterion; Bio-Rad), depending on the size of the target protein. Specific details of our immunoblotting technique have been reported elsewhere (32). All samples were normalized to a rodent internal loading control, and final data were reported as phosphorylated protein relative to total protein.

Free phenylalanine enrichment and 13C6 concentrations in blood and tissue fluid were measured by gas chromatography–mass spectrometry (GC-MS; 6890 Plus GC, 5973N MSD, 7683 autosampler, Agilent Technologies) after addition of an internal standard ([L-15N]phenylalanine), extraction, purification, and tert-butyldimethylsilyl derivatization (33). [13C6]Glucose enrichment was measured by GC-MS after extraction by ion-exchange chromatography and penta-acetate derivatization (33). Muscle tissue samples were mechanically homogenized, and intracellular free amino acids and muscle proteins were extracted as described previously (34). The incorporation of labeled phenylalanine into the mixed muscle proteins was measured by GC-MS, after protein hydrolysis and amino acid extraction, by using the external standard curve approach (35).

Calculations

The kinetics of intracellular free phenylalanine in response to the intake of EAA+sucrose preceded by either an acute bout of aerobic exercise or rest were calculated by using 2-pool, 3-pool,
and precursor-product models. With the 2-pool model, phenylalanine enrichments and concentrations in the femoral artery and vein were measured to estimate muscle protein synthesis, breakdown, and net balance without any consideration of the intracellular recycling of amino acids from breakdown to synthesis (33). The 3-pool model also included intracellular amino acid kinetics.

The 2-pool and 3-pool model parameters were calculated as follows:

\[
\text{Delivery to the leg} = C_A \times BF
\]  
\[
\text{Output from the leg} = C_V \times BF
\]  
\[
\text{Net Balance} = NB = (C_A - C_V) \times BF
\]  
\[
\text{Leg Rate of Appearance} = \text{Leg Ra} = BF \times C_A \times [(E_A/E_V) - 1]
\]  
\[
\text{Leg Rate of Disappearance} = \text{Leg Rd} = \text{Leg Ra} + NB = BF \times [(C_A \times E_A/E_V) - C_V]
\]  
\[
\text{Transport Into the Muscle} = F_{M,A} = \left(\frac{[C_V \times (E_M - E_V)/(E_A - E_M)] + C_A}{BF}\right)
\]  
\[
\text{Transport Out of the Muscle} = F_{V,M} = \left(\frac{[C_V \times (E_M - E_V)/(E_A - E_M)] + C_V}{BF}\right)
\]  
\[
\text{Release from Proteolysis} = F_{M,0} = F_{M,A} \times (E_A/E_M - 1)
\]  
\[
\text{Intracellular Availability} = F_{M,0} + F_{M,A}
\]  
\[
\text{Utilization for Protein Synthesis} = F_{0,M} = F_{M,0} + NB
\]  
\[
\text{Recycling} = F_{M,0} - \text{Leg Ra}
\]  
\[
\text{Protein Synthesis Efficiency} = F_{0,M}/(F_{M,A} + F_{M,0})
\]

where \(C_A\) and \(C_V\) are the blood amino acid concentrations in the femoral artery and vein, respectively; \(E_A, E_V\), and \(E_M\) are the amino acid enrichments, expressed as tracer/tracee ratios, in the femoral artery, vein, and muscle, respectively; and \(BF\) is leg blood flow as calculated from the steady state ICG concentration values in the common femoral and wrist veins (26, 27). Data were expressed per 100 mL leg volume.

With the precursor-product model, we calculated the fractional synthetic rate (FSR) of mixed muscle proteins as follows:

\[
\text{FSR} = \left(\frac{\Delta E_p}{t}\right)/\left(\frac{E_M'(1) + E_M'(2)}{2}\right) \times 60 \times 100
\]  

where \(\Delta E_p\) is the increment in protein-bound phenylalanine enrichment between 2 biopsy samples, \(t\) is the time between the 2 biopsy samples, and \(E_M'(1)\) and \(E_M'(2)\) are the phenylalanine enrichments in the free intracellular pool in the 2 biopsy samples. Data are expressed as percentages per hour (33).

Leg glucose utilization was calculated as the net glucose uptake across the leg:

\[
\text{Glucose Uptake} = (G_A - G_V) \times BF
\]

where \(G_A\) and \(G_V\) are blood glucose arterial and venous concentrations, respectively. Data are reported per 100 mL leg volume.

Basal endogenous glucose production was calculated, by using the single-pool model, as the ratio between the tracer infusion rate and the arterial glucose enrichment (\(E_A\)) (33):

\[
\text{Endogenous Glucose Production} = \text{Whole Body Glucose Utilization} = i/E_A
\]

This equation was also used during nutrient intake because the nutritive solution was enriched with labeled glucose to match the basal arterial glucose enrichment (see above).

### Statistical analyses

Statistical analyses were performed by using the statistical software SAS version 9.2 (SAS Institute Inc). The primary endpoints were measures of blood flow, muscle perfusion, muscle protein synthesis, and net muscle phenylalanine balance. Secondary endpoints were all remaining measures of muscle amino acid turnover and glucose kinetics. Paired \(t\) tests were used to make 3 comparisons: 1) a comparison of basal values between treatments (control and exercise conditions) to determine whether basal values were significantly different between the 2 treatment conditions, 2) a comparison of basal with EAA+sucrose values within a treatment (control or exercise condition) to determine whether there were significant within-treatment effects, and 3) a comparison of delta scores (EAA+sucrose values – basal values) between treatments (control and exercise conditions) to determine whether the magnitude of the effect was significantly different between treatments. Paired \(t\) tests were additionally used to make comparisons of step counts on the 3 d preceding each treatment. For data that did not pass the normality test, the values were transformed by using the natural ln or the reciprocal of the value. Blood flow, phenylalanine delivery to the leg, phenylalanine output from the leg, and phenylalanine rate of disappearance were transformed by using ln. Phenylalanine transport into the muscle was transformed by using the reciprocal of the value. The medians and interquartile ranges for these variables are reported elsewhere (see Supplemental Table 1 under “Supplemental data” in the online issue). Statistical analyses were performed on the transformed data,
RESULTS

Blood flow and microvascular perfusion

No basal differences for blood flow or microvascular perfusion were found between the treatment conditions (control and exercise). During the ingestion of EAA+sucrose, the change in blood flow from the basal state was significantly greater after the exercise than after the control treatment \( P < 0.05 \). This difference was due to a significant increase \( P < 0.05 \) in blood flow from the basal state with EAA+sucrose in the exercise condition, which was not observed in the control condition \( P = \text{NS} \) (Figure 2A; \( P < 0.05 \)). Microvascular perfusion increased significantly within the exercise treatment during EAA+sucrose ingestion, and there was a trend for the change in perfusion to be higher after the exercise than after the control treatment (Figure 2B; \( P = 0.07 \)). Representative contrast ultrasound images are shown for one subject during each of the 4 conditions (control: basal; control: EAA+sucrose; exercise: basal; and exercise: EAA+sucrose) (Figure 3).

Insulin and glucose kinetics

Data for insulin and glucose kinetics are presented in Table 2. No treatment differences for either insulin or glucose were found in the basal period. Systemic and femoral vein concentrations of insulin increased significantly during nutrient consumption under both treatment conditions \( P < 0.05 \). Arterial and venous glucose increased during nutrient consumption compared with basal concentrations during both treatments \( P < 0.05 \). Leg glucose uptake increased \( P < 0.05 \) and endogenous glucose production decreased \( P < 0.05 \) with nutrient consumption under both treatment conditions, with no differences in delta scores between treatments.

Phenylalanine concentrations and enrichments

The concentrations of phenylalanine in the artery, vein, and muscle (Table 3) were not different between treatments in the basal period and increased significantly during consumption of EAA+sucrose under both treatment conditions. No differences were found in the delta scores for these concentrations between treatments. Consumption of EAA+sucrose resulted in a slight, yet significant \( P < 0.05 \), increase in phenylalanine enrichment in femoral vein and muscle under both treatment conditions, and no differences in the delta scores between treatments were observed. A slight yet significant decrease in phenylalanine enrichment in the artery was found during consumption of EAA+sucrose under both treatment conditions \( P < 0.05 \).

Phenylalanine kinetics

No differences in phenylalanine kinetics in the basal state were found between treatment conditions (Table 3). Phenylalanine increased significantly during nutrient consumption under both treatments \( P < 0.05 \). Arterial and venous glucose increased during nutrient consumption compared with basal concentrations during both treatments \( P < 0.05 \). Leg glucose uptake increased \( P < 0.05 \) and endogenous glucose production decreased \( P < 0.05 \) with nutrient consumption under both treatment conditions, with no differences in delta scores between treatments.
to positive during EAA+sucrose consumption (37 ± 10 nmol · min⁻¹ · 100 mL leg volume⁻¹ = net muscle protein accretion). In contrast, net balance remained negative (−2 ± 2 nmol · min⁻¹ · 100 mL leg volume⁻¹) during EAA+sucrose consumption after the control treatment (Figure 2C; P < 0.05). The change in net balance was significantly greater after aerobic exercise than after the control treatment. The changes in efficiency of protein synthesis and intracellular recycling of protein were not different between treatments because of an outlier. However, when the raw values for these 2 variables were compared by using a paired t test between treatments, they were significantly higher during EAA+sucrose consumption after the exercise treatment than after the control treatment.

**Muscle-protein fractional synthetic rate**

The fractional synthetic rate of mixed skeletal muscle protein was not different in the basal state between treatment conditions (control: 0.059 ± 0.005%/h; exercise: 0.054 ± 0.008%/h), but there was a trend for the delta score to be higher after the exercise than after the control treatment (Figure 2D; P = 0.06).

**Cell signaling**

Phosphorylation of skeletal muscle Akt, mTOR, and 4E-BP1 did not differ in the basal state between treatment conditions (Figure 4). Akt phosphorylation increased with EAA+sucrose consumption 1.5 h after nutrient intake began under both treatment conditions (P < 0.05), and no difference in delta scores was found between treatments at 1.5 or 3.0 h of EAA+sucrose consumption. mTOR phosphorylation increased from the basal state at 1.5 and 3.0 h of nutrient consumption intake under both treatment conditions (P < 0.05), and no difference in delta scores was found between treatment conditions. No significant differences in 4E-BP1 were observed.

**DISCUSSION**

The novel finding of this study was that an acute increase in physical activity with a moderate bout of aerobic exercise enhanced the subsequent anabolic response to intake of amino acids and carbohydrate in healthy older adults, which suggests that aerobic exercise may be an important tool for the prevention and

**TABLE 2**

Insulin and glucose concentrations and kinetics in healthy older subjects in the basal state and during EAA+sucrose ingestion and the change (delta score) from the basal state during EAA+sucrose ingestion after a prior bout of aerobic exercise (n = 6) or a control period (n = 6)†

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Aerobic exercise</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>EAA+ sucrose</td>
<td>Delta score</td>
<td>Basal</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic concentration (pmol/L)</td>
<td>28 ± 4</td>
<td>62 ± 11*</td>
<td>34 ± 8</td>
<td>31 ± 9</td>
</tr>
<tr>
<td>Femoral vein concentration (pmol/L)</td>
<td>24 ± 4</td>
<td>58 ± 10*</td>
<td>34 ± 8</td>
<td>23 ± 6</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial concentration (mmol/L)</td>
<td>5.1 ± 0.1</td>
<td>5.6 ± 0.2*</td>
<td>0.4 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Venous concentration (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.4 ± 0.2*</td>
<td>0.4 ± 0.2</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Leg uptake (µmol · min⁻¹· 100 mL leg volume⁻¹)</td>
<td>0.10 ± 0.03</td>
<td>0.36 ± 0.18*</td>
<td>0.26 ± 0.13</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>Endogenous production (µmol · kg⁻¹· min⁻¹)</td>
<td>9.3 ± 0.4</td>
<td>5.9 ± 0.2*</td>
<td>−3.4 ± 0.3</td>
<td>9.4 ± 0.3</td>
</tr>
</tbody>
</table>

†All values are means ± SEs. A randomized crossover design was used. *Significantly different from basal, P < 0.05. EAA, essential amino acids.
phenylalanine kinetics (nmol/L) and concentration (μmol/L) 

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>EAA+ sucrose</th>
<th>Delta score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral artery</td>
<td>63 ± 5</td>
<td>187 ± 11*</td>
<td>124 ± 12</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>68 ± 5</td>
<td>187 ± 10*</td>
<td>119 ± 11</td>
</tr>
<tr>
<td>Muscle</td>
<td>63 ± 6</td>
<td>136 ± 16*</td>
<td>73 ± 13</td>
</tr>
</tbody>
</table>

Phenylalanine enrichment (tracer/tracer, %) 

<table>
<thead>
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<th></th>
<th>Basal</th>
<th>EAA+ sucrose</th>
<th>Delta score</th>
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</thead>
<tbody>
<tr>
<td>Femoral artery</td>
<td>9.7 ± 0.4</td>
<td>9.2 ± 0.3*</td>
<td>-0.46 ± 0.25</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>7.9 ± 0.4</td>
<td>8.5 ± 0.3*</td>
<td>0.63 ± 0.24</td>
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<tr>
<td>Muscle</td>
<td>6.1 ± 0.5</td>
<td>7.5 ± 0.3*</td>
<td>1.3 ± 0.30</td>
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</table>

Phenylalanine kinetics (nmol · min⁻¹ · 100 mL leg volume⁻¹) 

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>EAA+ sucrose</th>
<th>Delta score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery to the leg</td>
<td>143 ± 22</td>
<td>473 ± 88*</td>
<td>330 ± 76</td>
</tr>
<tr>
<td>Output from the leg</td>
<td>154 ± 23</td>
<td>475 ± 89*</td>
<td>320 ± 74</td>
</tr>
<tr>
<td>Leg rate of appearance</td>
<td>36 ± 8</td>
<td>37 ± 8</td>
<td>1.0 ± 2.5</td>
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<tr>
<td>Leg rate of disappearance</td>
<td>24 ± 7</td>
<td>35 ± 7</td>
<td>10.6 ± 5</td>
</tr>
<tr>
<td>Transport into the muscle</td>
<td>72 ± 17</td>
<td>217 ± 54*</td>
<td>143 ± 52</td>
</tr>
<tr>
<td>Transport out of the muscle</td>
<td>83 ± 17</td>
<td>217 ± 55*</td>
<td>134 ± 51</td>
</tr>
<tr>
<td>Release from proteolysis</td>
<td>42 ± 10</td>
<td>42 ± 9</td>
<td>0.15 ± 3.20</td>
</tr>
<tr>
<td>Utilization for protein synthesis</td>
<td>30 ± 8</td>
<td>40 ± 8</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>Intracellular availability</td>
<td>114 ± 25</td>
<td>257 ± 56*</td>
<td>143 ± 51</td>
</tr>
<tr>
<td>Efficiency of synthesis</td>
<td>24 ± 3</td>
<td>19 ± 4</td>
<td>-5 ± 4</td>
</tr>
<tr>
<td>Recycling (%)</td>
<td>14 ± 1</td>
<td>12 ± 3</td>
<td>-0.02 ± 0.03</td>
</tr>
</tbody>
</table>

1All values are means ± SEs. *Significantly different from basal, P < 0.05. 1Exercise: EAA+ sucrose delta score > control: EAA+ sucrose delta score (P < 0.05). EAA, essential amino acids.

treatment of sarcopenia. This positive effect of acute aerobic exercise on skeletal muscle protein anabolism appears to be linked to an increase in microvascular perfusion and amino acid delivery, rather than to an improvement in skeletal muscle insulin signaling.

Exercising the night before nutrient intake significantly elevated total leg blood flow, microvascular perfusion and amino acid delivery to the leg, muscle protein synthesis, and net balance in these older adults to a greater extent than did the control treatment. The resulting increase in delivery of amino acids likely contributed to the enhanced anabolic response to nutrients. Conversely, when the same older subjects consumed the same nutrients after a sedentary period, blood flow and microvascular perfusion did not increase, and amino acid delivery to the leg increased less than after aerobic exercise, and no significant changes in either muscle protein synthesis or net balance was found.

Hyperaminoacidemia with hyperinsulinemia increases muscle protein synthesis (7, 8) and stimulates blood flow (8) and microvascular perfusion (30) in young subjects, whereas older subjects have been reported to be resistant to the anabolic effects of these anabolic stimuli (7, 8), which is consistent with our current findings in the control, resting condition. Of note, this was the first study to show that nutrient consumption fails to stimulate muscle microvascular flow and perfusion in older adults. These data support the notion that there is a link between endothelial function and muscle protein anabolism that is not only limited to isolated anabolic stimuli (eg, isolated hyperinsulinemia) as previously reported (16–18, 22, 24), but is also critical in complex physiologic conditions such as the response to mixed nutrients (ie, EAA+ sucrose) in older adults. The nutrient-induced vasodilation was likely due to a direct effect of endogenous hyperinsulinemia on endothelial-dependent nitric oxide synthesis—a process that is impaired in sedentary older adults (8, 16, 36, 37) but can be restored by aerobic physical activity (25, 38).

Whereas insulin and glucose kinetics were equivalent in response to nutrient ingestion, regardless of treatment (control or aerobic exercise), significant treatment differences in phenylalanine kinetics were observed. Phenylalanine delivery to the leg was elevated to a greater extent when consumption of nutrients was preceded by aerobic exercise than when preceded by a sedentary behavior. Thus, it was not surprising to find that phenylalanine utilization for protein synthesis was also markedly elevated during EAA+ sucrose intake after aerobic exercise only.

Interestingly, consumption of nutrients after aerobic exercise resulted in a modest, yet statistically significant, increase in phenylalanine rate of appearance. This response was somewhat unexpected, because elevated concentrations of insulin have been reported by some to blunt proteolysis (39, 40). However, in older subjects it has been suggested that insulin’s ability to decrease protein breakdown could have been due to a kinetic artifact induced by increased blood flow (washout effect). However, if the effects of exercise on proteolysis were solely due to a washout effect, we should have observed an equal increase in protein synthesis and proteolysis during nutrient intake, and no changes in net balance (which is the difference between synthesis and breakdown) would have been observed. This was not the
protein breakdown rate was simply reflective of increased intracellular protein turnover during nutrient intake after exercise. Specifically, whereas the delta scores of amino acid recycling from breakdown to synthesis and protein synthesis efficiency were not significantly different, when we compared the mean values during nutrient ingestion we found that the exercise treatment resulted in higher amino acid recycling from muscle protein breakdown to synthesis and higher protein synthesis efficiency than did the control treatment. Increased tissue exposure (muscle perfusion) to nutrients (amino acids) and hormones (insulin) probably increased amino acid turnover and were the driving forces behind the elevated protein synthesis, protein synthesis efficiency and net muscle protein anabolism observed during EAA+sucrose consumption after aerobic exercise in our group of older adults.

Because aerobic exercise can improve insulin signaling, we measured Akt/PKB and components of the mTOR signaling pathway. Akt/PKB phosphorylation increased 1.5 h after nutrient consumption was started under both treatment conditions. There was a trend for the change in Akt/PKB phosphorylation from the basal state (P = 0.12; 1.5 h during nutrient consumption) to be higher during EAA+sucrose consumption after the exercise than after the sedentary control condition. However, mTOR signaling increased to the same extent during nutrient consumption, regardless of treatment. It is possible that while nutrient intake activated the translational machinery to comparable levels under both treatment conditions, substrate (amino acids) availability and/or differences in the amount of muscle tissue exposed to the dietary stimuli were the rate-limiting steps for increased synthesis. In other words, the increased amino acid delivery and perfusion observed during nutrient consumption after the exercise treatment allowed for a significantly greater increase in muscle protein synthesis. However, others have also reported a disassociation between the effects of amino acid intake and mTOR signaling in skeletal muscle (42). We cannot exclude that under certain conditions, such as those of this and the other study (42), cell signaling events were missed because of the timing of the muscle biopsy sample collection.

Limitations of the current study include the following. First, we cannot draw final conclusions regarding the potential long-term benefits of aerobic exercise or increased physical activity level on muscle protein turnover after ingestion of a regular meal because the nutritional stimulus did not contain lipids and was given acutely, in a continuous manner. Second, we collected only 2 biopsy samples during the consumption of EAA+sucrose. Thus, it is possible that we missed treatment-related differences in mTOR signaling that may have occurred during nutrient consumption. Third, whereas we had enough power to detect changes in the primary outcomes (blood flow, protein synthesis, and net balance), the limited sample size did not allow for extensive extrapolation of the results of this study to the general population. Larger clinical trials are warranted. Fourth, the study was not powered to detect interactions for secondary variables, such as amino acid recycling and protein synthesis efficiency. Thus, these data should be interpreted as preliminary estimates of treatment differences for future experiments. Finally, the study was unblinded, because the treatment was exercise. However, because of the objective, the biological nature of the primary outcome measures and the randomized crossover design, we believe that the results of this study are unbiased.
In conclusion, the results of this study indicate that in healthy older adults a moderate acute increase in physical activity with aerobic exercise enhances the skeletal muscle protein synthetic and anabolic responses to subsequent amino acid and carbohydrate intake via improved nutrient delivery. It is important to underscore that, whereas the exercise bout was moderately intense (70% heart rate reserve), the overall level of physical activity remained well below the recommended 10,000 steps/day (43). These very encouraging acute data suggest that aerobic exercise and/or moderately increasing physical activity status may help prevent and treat muscle loss with aging.

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The authors' responsibilities were as follows—EV: designed the research; KLT, SD, ELG, CSF, MJD, and EV: conducted the research; KLT, KJ, BBR, and EV: analyzed the data; KLT, BBR, and EV: wrote the manuscript; and KTL and EV: had primary responsibility for the final content. The authors reported no conflicts of interest.

REFERENCES


