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Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise

Syed S. I. Bukhari,1*, Bethan E. Phillips,2* Daniel J. Wilkinson,1 Marie C. Limb,1 Debbie Rankin,1 William K. Mitchell,1 Hisamine Kobayashi,2 Paul L. Greenhaff,1 Kenneth Smith,1 and Philip J. Atherton1

1Medical Research Council/Arthritis Research United Kingdom Centre of Excellence for Musculoskeletal Ageing Research, University of Nottingham, Derby, United Kingdom; and 2Ajinomoto Company Incorporated, Tokyo, Japan

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Bukhari SS, Phillips BE, Wilkinson DJ, Limb MC, Rankin D, Mitchell WK, Kobayashi H, Greenhaff PL, Smith K, Atherton PJ. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. Am J Physiol Endocrinol Metab 308: E1056–E1065, 2015. First published March 31, 2015; doi:10.1152/ajpendo.00481.2014.—Dysregulated anabolic responses to nutrition/exercise may contribute to sarcopenia; however, these characteristics are poorly defined in female populations. We determined the effects of two feeding regimes in older women (66 ± 5.0 yr; n = 8/group): bolus whey protein (WP-20 g) or novel low-dose leucine-enriched essential amino acids (EAA) [LEAA; 3 g (40% leucine)]. Using 13C6-phenylalanine infusions, we quantified muscle (MPS) and albumin (APS) protein synthesis at baseline and in response to both feeding (FED) and feeding plus exercise (FED-EX; 6 × 8 knee extensions at 75% 1-repetition maximum). We also quantified plasma insulin/AA concentrations, whole leg (LBF)/muscle microvascular blood flow (MBF), and muscle anabolic signaling by phosphoimmunoblotting. Plasma insulinemia and EAA/aemia were markedly greater after WP than LEAA (P < 0.001). Neither LEAA nor WP modified LBF in response to FED or FED-EX, whereas MBF increased to a similar extent in both groups only after FED-EX (P < 0.05). In response to FED, both WP and LEAA equally stimulated MPS 0–2 h (P < 0.05), abating thereafter (0–4 h, P > 0.05). In contrast, after FED-EX, MPS increased at 0–2 h and remained elevated at 0–4 h (P < 0.05) with both WP and LEAA. No anabolic signals quantifiably increased after FED, but p70 S6K1 Thr389 increased after FED-EX (2 h, P < 0.05). APS increased similarly after WP and LEAA. Older women remain subtly responsive to nutrition ± exercise. Intriguingly though, bolus WP offers no trophic advantage over LEAA.

skeletal muscle; blood flow; protein synthesis; aging; amino acids; exercise

ILL HEALTH ASSOCIATED WITH AGING represents a major socioeconomic burden, especially given shifting demographics toward a more aged, populous world. In particular, the loss of skeletal muscle mass associated with aging, or sarcopenia, is a major clinical issue. For instance, not only are there established links between low muscle mass and all-cause mortality per se (1), but also, lower skeletal muscle mass associated with sarcopenia leads to increased frailty, risk of falls, sedentarism, poor quality of life, and prevalence of metabolic comorbidities (17, 53).

The two major extrinsic influences over muscle mass are nutrition and physical activity. For example, oral intake of protein-based foods containing essential amino acids (EAA) leads to a transient (2–3 h; see Ref. 2) stimulation of muscle protein synthesis (MPS) in younger men. This brief increase in MPS above postabsorptive rates serves the purpose of replenishing protein stores lost during fasting, ensuring preservation of muscle protein mass. Similarly, physical activity is a prerequisite for maintenance of a healthy muscle mass. For example, inactivity (6, 20) causes muscle atrophy by inducing “anabolic resistance” to nutrition. Conversely, physical activity/exercise potentiates the trophic effects of nutrition; i.e., anabolic responses are greater when exercise and nutrient intake are combined to facilitate adaptation and repair. Thus, investigations into the effects of nutrition and the combination of nutrition and exercise are key to determining the regulation and dysregulation of muscle protein metabolism in older age.

The anabolic effects of protein and EAA have long been defined. However, a significant body of recent work has shown that the EAA leucine (LEU) serves not only as substrate for synthesis of other exogenous EAA (61). On this basis, the “dose response” of MPS to EAA/protein is not apparently driven by AA quantity per se but LEU content instead. This leaves open the possibility that lower doses of EAA enriched with LEU may provide a robust and less satiating (or caloric) anabolic alternative, e.g., to large boluses of high-quality protein in older age.

The rationale for the present study was threefold. First, older women are grossly underrepresented in terms of studies on the regulation of muscle protein metabolism with aging. Second, since there exists sexual dimorphism in muscle protein turnover in older age (50, 51), with markedly different feeding responses observed between older men and women (50), one cannot assume that prior research findings in older men are germane to older women. Therefore, an improved understanding of the regulation of muscle protein metabolism in older women, i.e., by nutrition and exercise combinations, is needed. Third, in the context of older women, who show an apparent...
increased level of anabolic resistance (vs. men; see Ref. 51), an investigation into novel nutritional interventions, which maximize anabolic responses to nutrition, is sorely needed. Although increasing protein content of meals and providing large boluses of protein are known to maximally stimulate MPS (62), few older individuals may be able or willing to consume such a satiating dose of protein without impacting habitual nutrient intake, hence the need for lower caloric supplements. Finally, albumin is an abundant protein used as a marker of general nutritional status, and low levels are associated with low protein intake (36), functional decline (49), reduced muscle mass (4), reduced strength (48), and numerous age-related diseases. Moreover, albumin synthesis is also affected by sex difference (females have lower synthesis rates than males) (56). Therefore, the albumin-synthetic response to anabolic stimuli, such as nutrition, provides another window of insight into sex differences in protein metabolism and is linked to skeletal muscle outcomes in ageing. Therefore, in this study we quantified the effects of two distinct nutritional interventions: 1) low-dose leucine-enriched amino acids [LEAA; 3 g of EAA (40% LEU)] nutrition or 2) large whey protein (WP) bolus (20 g) under rested conditions and in tandem with an acute bout of resistance exercise (RE; which is well known to potentiate the anabolic response; see Ref. 16) on MPS and plasma albumin synthesis in older women. It was hypothesized that LEAA would provide equivalent stimulation of MPS and albumin protein synthesis (APS) due principally to its leucine content and to WP in older women, and the addition of RE would potentiate this response.

MATERIALS AND METHODS

Subject characteristics and ethics. Ethical approval was obtained from the University of Nottingham Medical School Ethics Committee (United Kingdom), with all studies conducted in accordance with the Declaration of Helsinki and preregistered at www.clinicaltrials.gov (registration no. NCT02053441). Sixteen older postmenopausal women (n = 8 in each group) matched for age and BMI (age 66 ± 3 yr, BMI 29 ± 1 [means ± SE]; see Table 1 for subject characteristics) were recruited locally via advertisement through the mail. Exclusion criteria included impaired mobility, history of diabetes, cardiovascular, pulmonary, liver, or kidney disorders, those on contraindicated medications (nonsteroidal anti-inflammatory drugs, acetaminophen, hormone replacement therapy) and those currently undergoing active cancer therapies. All volunteers were then screened by a physician (≥1 wk prior to the study day) by means of a medical questionnaire, physical examination, and resting electrocardiogram, with exclusions for metabolic, respiratory, cardiovascular/vascular, or claudication-related (either symptomatic or on treatment) disorders or other symptoms of ill health. Subjects had normal blood chemistry and were normotensive (blood pressure < 140/90), and all subjects performed activities of daily living and recreation but did not routinely participate in any formal strenuous exercise regimes and were not on a weight loss diet. During the screening visit, knee extension one-repetition maximum (1-RM) was assessed, using the participants’ dominant/preferred leg, on a standard weighted gym knee extension machine (Technogym, Gambettola, Italy). In addition, lean body mass was assessed via dual X-ray absorptiometry and used to measure appendicular muscle mass and calculate skeletal muscle index (SMI) according to the following equation (38): SMI = (total body skeletal muscle mass/total body mass) × 100. All subjects gave their written, informed consent to participate after all procedures and risks were explained. Following screening subjects were randomly assigned, on the day of the acute study, to one of two groups receiving either WP (n = 8) or 2 LEAA (n = 8) (Table 2). Subjects were requested not to start any new diet or exercise program between screening and the study day and to refrain from vigorous exercise for 48 h prior to the acute study visit and arrive at 0800 on the morning of the study fasted overnight from 2000 the night before.

Study procedures. On the morning of the study (0800), subjects had an 18-g cannula inserted into the antecubital vein of one arm for a priming (0.4 mg/kg), constant infusion (0.6 mg·kg⁻¹·h⁻¹) of L-[ring-¹³C]phenylalanine (Isotec; Sigma Aldrich) tracer and a retrograde 14-g cannula inserted to sample arterialized blood from the dorsal capillary bed of the hand (using the “hot hand” method). Biopsies were taken 1 and 3 h after the commencement of tracer infusion to permit assessment of basal (postabsorptive) MPS. Subjects then performed a bout of unilateral knee extension RE previously shown (34, 35) to maximally stimulate MPS (6 × 8 repetitions at 75% of their predetermined 1-RM using the subjects’ dominant/preferred leg with 3 min rest) and also elevate leg blood flow (LBF) in elderly men (44) before their supplement was consumed. Each bout lasted 40–60 s. If the subject failed to complete eight repetitions, then an interest break was allowed before moving on to the next set. This happened regularly with sets 5 and 6. Immediately after the exercise, subjects consumed either 20 g of WP or 3 g of LEAA (“Amino L40”; Ajinomoto) prepared in water (250 ml). The AA composition of each supplement is given in Table 2. This unilateral study design meant that the nonexercised leg was exposed to the effect of feeding alone (“FED”), whereas the exercised leg was exposed to the combination of feeding and exercise (“FED-EX”). Subsequent biopsies were then taken 2 and 4 h after feeding to permit assessment of MPS over and within the intervening periods. Blood samples and blood flow/vascular measurements were collected as outlined in Fig. 1. Muscle biopsies were collected from m. vastus lateralis using the conchotome technique (14) after induction of local anesthesia via infiltration of 5 ml of 1% lignocaine. Muscle was washed in ice-cold phosphate-buffered saline, and visible fat and connective tissue removed were before being snap-frozen in liquid N2 and stored at −80°C until analysis. After completion of the study, cannulae were removed and subjects fed and monitored for a further 30 min before being provided transportation home.

Measurement of plasma insulin and AA concentrations. Arterialized venous plasma insulin concentration was measured using a

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**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>LEAA (3 g)</th>
<th>WP (20 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>66 ± 1</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29 ± 1</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>41 ± 2</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>EAA dose, g/kg LBM</td>
<td>0.075 ± 0.003</td>
<td>0.25 ± 0.01*</td>
</tr>
<tr>
<td>Appendicular lean mass, kg</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>SMI</td>
<td>25 ± 1</td>
<td>26 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. LEAA, leucine-enriched amino acids; WP, whey protein; BMI, body mass index; LBM, lean body mass; EAA, essential amino acids; SMI, skeletal muscle index. *P < 0.001 vs. LEAA.

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**Table 2. Essential amino acid composition of both types of feed**

<table>
<thead>
<tr>
<th>AA</th>
<th>LEAA (3 g), g</th>
<th>WP (20 g), g</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>0.32</td>
<td>1.4</td>
</tr>
<tr>
<td>L-Valine</td>
<td>0.33</td>
<td>1.2</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.28</td>
<td>1.4</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.02</td>
<td>0.4</td>
</tr>
</tbody>
</table>

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00481.2014 • www.ajpendo.org
high-sensitivity human insulin enzyme-linked immunosorbent assay (ELISA; DRG Instruments, Marburg, Germany). For AA analyses, equal volumes of arterialized plasma and 10% sulfosalicylic acid were mixed and cooled to 4°C for 30 min. Samples were centrifuged at 8,000 g to pellet the precipitated protein, and the supernatant fluid was passed through a 0.22-μm filter before analysis with a dedicated AA analyzer (Biochrom 30; Biochrom, Cambridge, UK) using lithium buffers. All 20 AA concentrations were measured by comparison to a standard AA mix with norleucine as an internal standard.

**Measurement of LBF and muscle microvascular blood flow.** LBF was measured using Doppler ultrasound, with a 9-3 mHz probe positioned over the origin of the common femoral artery. Flow was estimated as the product of vessel cross-sectional area and mean velocity over six cardiac cycles. Contrast-enhanced ultrasound (CEUS) was used to measure MBF, as described previously (45). Briefly, Sonovue microbubbles were infused via an antecebular fossa vein (Bracco, Milan, Italy). An iU22 ultrasound scanner (Phillips Healthcare, Reigate, UK) with 40-mm linear 9-3 mHz probes (Phillips L9-3) firmly secured on both anterior thighs (and measured individually for the FED and FED-EX legs) was used to register microbubble appearance within the quadriceps muscle at rest and in response to exercise (and thus skeletal muscle tissue MBF (45). In total, two flash/replenishment recordings were made. Offline region-of-interest analysis using Q-Lab software (Phillips Healthcare, Surrey, UK) was used to measure the plateau (“A” value), the product of which is proportional to volume blood flow rate constant (“β” value), and also the rate constant (“β” value), and the product of which is proportional to volume blood flow. Micron bleeding was computed over six cardiac cycles. Contrast-enhanced ultrasound (CEUS) was used to measure MBF, as described previously (45). Offline region-of-interest analysis using Q-Lab software (Phillips Healthcare, Surrey, UK) was used to measure the plateau (“A” value), the product of which is proportional to volume blood flow rate constant (“β” value), and also the rate constant (“β” value), and the product of which is proportional to volume blood flow. Micron bleeding was computed over six cardiac cycles.

**Measurement of MPS.** Myofibrillar proteins were isolated, hydrolyzed, and derivatized using our standard techniques (61). Briefly, ~25 mg of muscle biopsy tissue was homogenized in ice-cold homogenization buffer [50 mm Tris-HCl (pH 7.4), 50 mm NaF, 10 mm β-glycerophosphate disodium salt, 1 mm EDTA, 1 mm EGTA, and 1 mm activated Na3VO4 (all Sigma-Aldrich, Poole, UK)] and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK) at 10 μl/μg of tissue. Homogenates were rotated for 10 min, and the supernatant was collected by centrifugation at 13,000 g for 5 min at 4°C. The resulting pellet was washed three times with homogenization buffer, and 0.3 M NaOH was added to facilitate the separation of the soluble myofibrillar fraction from the insoluble collagen fraction by subsequent centrifugation. The myofibrillar fraction was then removed and precipitated using 1 M perchloric acid. Isolated albumin-bound AA were released by acid hydrolysis with 0.1 M hydrochloric acid and Dowex H+ ion exchange resin (50W-X8-200; Sigma Aldrich, Poole, UK) heated overnight at 110°C before purification by ion exchange chromatography; AA were derivatized as their N-acetyl-N-propyl esters, and incorporation of 1-[ring-13C6]phenylalanine into albumin was assayed by gas chromatography-combustion-isotope ratio mass spectrometry (Delta Plus XP; ThermoFisher Scientific, Hemel Hempstead, UK). Fractional synthesis rates of albumin were determined using a standard precursor-product model:

\[
\text{APS} \, (\% \cdot h^{-1}) = \frac{\Delta E_a}{E_p \cdot t} \times 100
\]

where ΔEa is the change in labeling of albumin phenylalanine between two blood samples, Ep is the mean enrichment over time of the precursor (taken as arterialized plasma 1-[ring-13C6]phenylalanine labeling), and t is the time in hours between samples.
FSR (%/h) = \frac{\Delta E_m}{E_p \cdot t} \times 100

where $\Delta E_m$ is the change in enrichment of bound $\text{L}^{\text{ring}}\text{C}^{13}\text{C}_\text{phenylalanine}$ in two sequential biopsies, $t$ is the time interval between two biopsies in hours, and $E_p$ is the mean free $\text{L}^{\text{ring}}\text{C}^{13}\text{C}_\text{phenylalanine}$ enrichment in the intramuscular pool.

Immunoblotting for Akt/mTORC1 signaling pathway activity (i.e., phosphorylation). Immunoblotting was performed as described previously (3) using the sarcoplasmic fraction collected during MPS preparation described above. Sarcoplasmic protein concentrations were determined using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and adjusted to 2 µg/lane and separated electrophoretically at 200 V for 1 h. Proteins were wet transferred at 100 V for 45 min and subsequently blocked in 2.5% nonfat milk in 1× Laemmli buffer. Each sample was loaded onto precast 12% Bis-Tris Criterion XT gels (Bio-Rad, Hemel Hempstead, UK) at ambient temperature for 1 h, followed by 3× 5 min washes in TBST. Membranes were exposed to Chemiluminescent HRP Substrate (Millipore, Billerica, MA) for 5 min and bands quantified by Chemidoc XRS (Bio-Rad, Hertfordshire, UK). All signals were within the linear range of detection, and loading anomalies were corrected to coomassie (59). Blotting data were captured using peak density analysis.

Statistical analyses. Data are presented as means ± SE, except where indicated. Demographic, anthropometric, and CEUS comparisons were analyzed within or between groups by unpaired or paired t-tests (all 2-tailed) as appropriate. All other data sets were analyzed using two-way repeated measures ANOVA (feed type x time) with differences located via Holm Sidak posttests using GraphPad Prism version 5, with $P$ values calculated using GraphPad Quickcalcs (GraphPad Software, San Diego, CA). $P$ values of <0.05 were considered significant.

RESULTS

Plasma AA and insulin concentrations. Groups were matched for appendicular lean mass (LEAA 17 ± 1 vs. WP 17 ± 1 kg) and skeletal muscle index (LEAA 25 ± 1 vs. WP 26 ± 2) but received markedly different ($P < 0.01$) quantities of EAA (i.e., LEAA 0.075 ± 0.003 vs. WP 0.25 ± 0.01 g/kg lean body mass). Plasma AA concentrations increased rapidly in response to LEAA and WP and peaked at ~40–60 min ($P < 0.05$; Fig. 2, A–E). Plasma availability of AA, EAA, branched-chain AA, and LEU was significantly greater and
more sustained in response to WP than LEAA (i.e., returning to baseline concentrations 60–80 min after LEAA vs. 115–160 min after WP, \( P < 0.001 \)). Plasma insulin concentration increased over postabsorptive values (~5 mU/L, both groups) by 20 min in response to both LEAA and WP (to 14 ± 3 vs. 20 ± 3 mU/L, respectively, \( P < 0.05 \)). However, whereas plasma insulin returned to basal concentration 40 min after LEAA intake, (Fig. 2F), WP led to insulin concentrations that remained elevated up to (and beyond) 60 min (18 ± 3 mU/L).

**LBF and MBF.** LBF was not different between groups at baseline (LEAA 0.27 ± 0.03 vs. WP 0.34 ± 0.04 l/min) and was unaltered in response to FED and FED-EX 60–80 min postfeeding, when AA concentrations peaked (\( P > 0.05 \); Fig. 3, A and C). Similarly MBF, measured 60–80 min postingestion of supplement, was not increased in the rested leg, i.e., FED only, in either group, and although MBF was numerically greater in the LEAA group, this was not consistent in all individuals and did not reach significance. However, MBF increased following FED-EX in both the LEAA and WP groups (LEAA +91 ± 22%, WP +229 ± 23%, \( P < 0.05 \); Fig. 3, B and D).

**Effects of WP and LEAA on APS and MPS.** Increases in plasma APS were observed in response to WP, whereas a trend existed for LEAA only at 0–2 h (LEAA basal to FED 0.19 ± 0.02 to 0.23 ± 0.03%/h, WP basal to FED 0.17 ± 0.01 to 0.26 ± 0.04%/h, \( P < 0.1 \) and \( P < 0.05 \), respectively; Fig. 4). APS remained elevated in both groups at 0–4 h (LEAA, FED increased to 0.25 ± 0.02%/h, WP, FED increased to 0.29 ± 0.02%/h, \( P < 0.05 \)). In response to WP and LEAA, MPS was increased at 0–2 h (LEAA BASAL to FED 0.071 ± 0.004 to 0.089 ± 0.008%/h, WP BASAL to FED 0.066 ± 0.006 to 0.082 ± 0.009%/h, both \( P < 0.05 \)); this anabolic effect abated rapidly, returning to postabsorptive rates (0–4 h, \( P > 0.05 \); Fig. 5A). In the exercised leg, MPS also increased at 0–2 h (LEAA BASAL to FED-EX 0.071 ± 0.004 to 0.085 ± 0.014%/h, WP BASAL to FED-EX 0.066 ± 0.006 to 0.095 ± 0.013%/h, both \( P < 0.05 \)), but unlike with FED, MPS remained elevated over the entire 4-h study period (LEAA BASAL to FED-EX 0.089 ± 0.005%/h, WP BASAL to FED-EX 0.093 ± 0.007%/h, both \( P < 0.05 \); Fig. 5B). Increases in MPS in response to both FED and FED-EX were indistinguishable between the WP and LEAA groups (\( P > 0.05 \)).

**Effects on muscle anabolic signaling.** Muscle anabolic signals as determined by measuring the phosphorylation of protein kinase B (Akt), the mechanistic target of rapamycin complex 1 (mTORc1) translational initiation substrates ribosomal protein S6 kinase (p70 S6K1) and 4E-BP1, and the elongation factor eEF2 (Fig. 6, A–F) were not increased detectably in FED. Only in response to FED-EX could we detect significant increases (+133 ± 22%, \( P < 0.01 \); Fig. 6D) in
argue against optimizing muscle anabolic responses to nutrition as being paramount in older age. We show here that increasing the quantity of EAA consumed does not necessarily augment MPS over and above the ingestion of ~1.2 g LEU (plus a small 1.8-g mixed EAA) in older women. Therefore, providing greater quantities of AA as meals or supplements to older women may not bolster MPS beyond, e.g., LEAA. Nonetheless, since anabolic resistance may be overcome by providing larger doses of WP to older men (~40 g; see Ref. 43), it does remain entirely plausible that neither 20 g of WP nor 3 g of LEAA maximally stimulates MPS in skeletal muscles of older women; further studies are needed to define this. Yet despite this, these findings highlight that a small dose of LEAA (of low caloric value) provides adequate EAA substrate to promote robust enhancement of MPS and thereby could perhaps promote better maintenance of muscle mass in older women.

Although the stimulation of MPS by EAA has long been defined, it is becoming increasingly clear that protein or AA dose response studies belie the importance of certain highly anabolic EAA. For example, LEU is a major driver of MPS (61) to the extent that enrichment of “suboptimal” doses of protein or EAA with LEU robustly stimulates MPS (10), as does the provision of just ~3 g of LEU to ~70 kg in younger men entirely in the absence of other exogenous EAA (61). Herein, we extend these findings by demonstrating that LEAA stimulates MPS in older women with equal efficacy to a large (20 g) bolus of WP despite the latter providing more than three times more EAA per kilogram of LBM; we anticipate that LEU was instrumental in this response given its unique potent anabolic properties (7, 11, 61). This is supported somewhat by the fact that despite WP having greater EAA and total AA content, both WP and LEAA showed the same peak in LEU concentration (Fig. 2E), and despite the WP maintaining significantly raised LEU concentrations for longer than the LEAA, there was no additional stimulation of MPS with WP. On this basis, research is needed to define the effects of LEU in the context of AA requirements for muscle health in old age (7, 8, 15, 19, 46, 55, 58).

Physical activity in the form of RE (9, 26, 29, 35, 42) or even maintaining habitual movement (57) remains the best-known countermeasure against sarcopenia. Nonetheless, aging is associated with impaired anabolic responses to exercise, meaning that it is essential to study how exercise and nutrition can be best combined to yield maximal MPS. For example, increases in MPS in response to exercise (18, 34, 35) and the combination of exercise and nutrition (18) are blunted in older men, as is muscle hypertrophy in both men (32) and women (23). In the present study, we report that older women exhibit prolonged MPS responses to FED-EX (vs. FED) and that responses are equal between LEAA and WP. Therefore, intake of low doses of EAA enriched with LEU robustly stimulates MPS following exercise in older women, similar to what has been shown in younger men at rest (10) and after exercise (11). It is noteworthy in the latter study that WP bolus, but not LEAA, led to sustainment of the anabolic effects of the nutrient/exercise combination. In contrast, we found similarly prolonged anabolic responses to FED-EX after both LEAA and WP; this is in agreement with what has been observed in older men with analogous LEAA supplementations post-RE (13). We speculate that the lack of difference in MPS between WP and LEAA...
might reflect blunted hypertrophy in older vs. young individuals (23, 37, 60), i.e., being manifested as diminished uptake of excess AA substrate into muscle proteins. This is also substantiated by the lack of robust increases in anabolic signaling [as reported in older men (34) and women (50)] and leg/muscle blood flow, facets that may underlie the moderate anabolic response to raised AA availability after FED and FED-EX in older women.

When studying leg and muscle blood flow, we hypothesized that WP may elicit greater blood flow responses compared with LEAA due to the vasodilatory properties of arginine and greater insulin responses associated with higher AA concentrations, i.e., through promoting secretory effects of AA upon pancreatic β-cells. Nonetheless, whereas plasma insulin (and arginine) concentrations were expectedly higher in the WP group (Fig. 2F), there were no differences in LBF in line with work we completed recently in older men (39, 44); that is, older people exhibit “vascular resistance” to nutritional intake. In contrast, there were significant increases to both WP and LEAA in MBF, but only in the FED-EX leg (Fig. 3), suggesting that this may have some role in the sustainment of MPS beyond 2 h in the FED-EX leg. Indeed, despite the fact that we have shown previously that pharmacological enhancement of leg and muscle blood flow above the response to FED does not enhance anabolism (45), when superimposed onto a metabolic background of a prior bout of RE, our observed sustainment in MBF with FED-EX may facilitate sustaining anabolic responses beyond 2 h.

An important discussion point is to what extent, and under what conditions, such WP or LEAA supplements yield efficacy, e.g., on muscle mass/function. Since protein supplements have significant effects to positively influence muscle hypertrophy in response to resistance exercise training (28, 63), we speculate based on the information provided by the present study that low-dose LEAA would be a similarly effective supplement (at least for older women) without the need for more satiating higher protein doses that may act as meal replacements, perhaps negatively impacting overall energy/protein intake. Moreover, most studies have focused on the effects of supplements on muscle gains; however, given the
incipient nature of sarcopenia, longer-term studies are needed to
determine the efficacy of supplements in offsetting losses in
muscle mass/function. Nonetheless, those studies, which have
been conducted, have indeed shown promise (30). Additionally,
although caution should be applied in terms of the extrapolation
of one-off metabolic studies to the chronic scenario, we contend
that these acute metabolic readouts of protein anabolism form a solid platform for identifying candidate interventions for testing in longer-term supplement studies.

In addition to the muscle-specific effects observed in the
present study, the influence of nutrition on APS is important
particularly with age due to the known associations between
low-serum albumin, health, morbidity, and mortality (12). Indeed, albumin concentrations decrease with age (31), and females have slower rates of APS (56). In this context, we have demonstrated that LEAA led to significant stimulation of APS equivalent to a large dose of WP, which is sustained for ≤4 h postfeeding. This suggests that the LEAA supplement (and also the larger WP bolus) may also have implications for sustaining APS in older women. Given the known links to muscle and general health, this could transpire to be an important observation.

Potential study limitations warrant comment. First, we were unable to define the specific importance of LEU in mediating the efficacy of LEAA. Previous work by one of the current authors using intravenous flooding dose techniques revealed anabolic effects of other EAA in our LEAA feed (i.e., valine, phenylalanine) in man (52). Nonetheless, given potent mTORc1 signaling actions attributed to LEU (3, 21, 25), it is speculated LEU was central. Second, we measured neither muscle protein breakdown nor whole body protein turnover; i.e., age-related differences in splanchnic metabolism (40) and insulin handling may also impact muscle and whole body responses to feeding/exercise, particularly because of the anti-proteolytic effect of insulin on muscle protein breakdown (33). Also, although 20 g of WP led to a significant stimulation of MPS, larger amounts of protein (>40 g) have been shown to assist in potentiating MPS responses in older men (63), and therefore, the MPS response to WP in the current group of older women may be suboptimal. That said, our aim was to assess the efficacy of a low-dose, lower-satiating EAA nutritional supplement rather than test the absolute dose of WP for maximal MPS stimulation. Finally, with regard to anabolic signaling (in which responses were negligible), we may have missed the “peak” best reflecting MPS responses; nonetheless, we designed the studies to acquire biopsies at times most appropriate to our primary outcome measurement of MPS. Moreover, given “peak signaling,” event(s) may differ between distinct feeding regimens such as those applied herein (20 g of WP or 3 g of EAA) in addition to dissociations between anabolic signaling and MPS (2, 22); this is relatively uncontrollable. To conclude, our findings show that low-dose LEAA supplements have potential alone or combined with exercise as strategies for older women to enhance muscle maintenance.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

Hisaamine Kobayashi is an employee of Ajinomoto Co., Inc. The authors declare no other conflicts of interest.

AUTHOR CONTRIBUTIONS

REFERENCES


