Leucine supplementation enhances integrative myofibrillar protein synthesis in free-living older men consuming lower- and higher-protein diets: a parallel-group crossover study[1]

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ABSTRACT

Background: Leucine co-ingestion with lower-protein (LP)–containing meals may overcome the blunted muscle protein synthetic response to food intake in the elderly but may be effective only in individuals who consume LP diets.

Objective: We examined the impact of leucine co-ingestion with mixed macronutrient meals on integrated 3-d rates of myofibrillar protein synthesis (MyoPS) in free-living older men who consumed higher protein (HP) (1.2 g · kg^{-1} · d^{-1}) or LP (0.8 g · kg^{-1} · d^{-1}) in rested and resistance exercise (REX) conditions.

Design: In a crossover design, 20 healthy older men [aged 65–85 y] were randomly assigned to receive LP or HP diets while ingesting a placebo (days 0–2) and Leu supplement (5 g leucine/meal; days 3–5) with their 3 main daily meals. A bout of unilateral REX was performed during the placebo and Leu treatments. Ingested ^2H_2O and skeletal muscle biopsies were used to measure the 3-d integrated rate of MyoPS during the placebo and Leu treatments in the rested and REX legs.

Results: Leucinemia was higher with Leu treatment than with placebo treatment ($P < 0.001$). MyoPS was similar in LP and HP during both treatments ($P = 0.39$) but was higher with Leu treatment than with placebo treatment in the rested (pooled mean ± SD: Leu, 1.57% ± 0.11%/d; placebo, 1.48% ± 0.08%/d; main effect of treatment: $P < 0.001$) and REX (pooled mean: Leu, 1.87% ± 0.09%/d; placebo, 1.71 ± 0.10%/d; main effect of treatment: $P < 0.001$) legs.

Conclusions: Leu co-ingestion with daily meals enhances integrated MyoPS in free-living older men in rested and REX conditions and is equally effective in older men who consume daily protein intakes greater than or equal to the RDA. This trial was registered at clinicaltrials.gov as NCT02371278. *Am J Clin Nutr* 2016;104:1594–606.

Keywords: aging, deuterated water, dietary protein, leucine, muscle protein synthesis

INTRODUCTION

The progressive loss of skeletal muscle mass and function with advancing age, which is termed sarcopenia, contributes substantially to disability, physical dependence, and mortality in older adults (1–3). Aging is associated with an attenuated muscle protein synthetic response to protein ingestion compared with in younger persons (4, 5). This attenuation is particularly apparent with small-to-moderate meal-like quantities of dietary protein (~≤20 g protein/meal), which is a phenomenon termed anabolic resistance and is implicated as a key factor that underpins sarcopenia (4, 5). Although the anabolic resistance to protein feeding can be overcome with the ingestion of larger protein servings (~0.4 g protein · kg^{-1} · $meal^{-1}$, which is equivalent to ~30–40 g protein/meal), many older adults may find it challenging to consume these quantities of food-based protein on a per-meal basis (4). Consequently, there is considerable interest in potential strategies to augment the muscle protein synthetic response to lower protein (LP)[4] doses in the elderly.

A number of studies have reported that the acute, postprandial muscle protein synthetic response to a suboptimal protein dose is enhanced when the leucine content of the protein bolus is increased (6–8). However, longer-term intervention trials have been unable to show clear benefits of leucine supplementation on muscle mass and strength in older adults (9–11). A potential explanation for the disparity between the results from the acute (6–8) and longer term (9–11) studies is that the participants in the longer-term studies were already consuming protein intakes that were greater than the Recommended Dietary Allowance (RDA) (1.0–1.2 g · kg^{-1} · d^{-1}) (12, 13). Thus, when protein intake is greater than the RDA, the addition of leucine to the diet may have had a minimal impact in augmenting postprandial muscle protein synthesis (MPS) and ultimately muscle mass (10, 11). It is also possible that acute measurements of MPS do not adequately capture longer-term phenotypic (i.e., muscle-mass) outcomes. For example, the MPS studies were conducted over short time periods (~3–6 h) under controlled laboratory conditions (i.e., after an overnight fast or with participants typically confined to a laboratory bed), and leucine was co-ingested with either an isolated protein bolus (7) or an essential amino acid

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[4] Abbreviations used: AUC_{pos}, AUC above baseline; C_{max}, concentration maximum; EAA, essential amino acid; HP, higher protein; LP, lower protein; MPS, muscle protein synthesis; MyoPS, myofibrillar protein synthesis; RDA, Recommended Dietary Allowance; REE, resting energy expenditure; REX, resistance exercise.

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(EAA) bolus (6, 14) or as part of a mixed macronutrient meal that was provided in small aliquots over several hours (15). However, protein is most often consumed as whole foods and is co-ingested with carbohydrate and fat during meals within a discrete eating occasion. The food form (i.e., solid compared with liquid) and the addition of fats and carbohydrates can markedly alter the kinetics of intestinal amino acid absorption and subsequent aminoacidemia (16, 17), which is an important determinant of the MPS response (18). As such, further work is necessary to delineate whether the co-ingestion of leucine with normal, mixed macronutrient meals has the capacity to enhance the cumulative, longer-term MPS response in older adults in a free-living environment.

We examined the impact of 3 d of leucine co-ingestion with mixed macronutrient meals on the integrated rate of myofibrillar protein synthesis (MyoPS) in free-living older men who consumed daily protein intakes at the current RDA (0.8 g · kg^{-1} · d^{-1}) or at current recommendations for optimal protein intake in healthy older adults (1.2 g · kg^{-1} · d^{-1}) (12, 13) both at rest and when combined with resistance exercise (REX). We hypothesized that leucine co-ingestion with the main daily meals would enhance integrated MyoPS in both groups *1*) under resting conditions and *2*) when combined with performance REX.

METHODS

Ethical approval

This study was approved by the Hamilton Integrated Research Ethics Board and conformed to the standards for the use of human subjects in research as outlined by the Canadian Tri-Council Policy on the ethical use of human subjects in research (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). Each participant was informed of the purpose of the study, experimental procedures, and potential risks before written consent was provided. This trial was registered at clinicaltrials.gov as NCT02371278.

Participants

Older adult men were recruited from Hamilton, Ontario, to participate in the study through posters and local newspaper advertisements between April and July 2015. Inclusion criteria were as follows: men, 65–85 y of age, BMI (in kg/m^2) from 20 to 35, nonsmokers, and generally healthy according to responses to a standard health-screening questionnaire. Exclusion criteria included self-reported diabetes mellitus, cardiovascular disease, renal disease, gastrointestinal disease, neuromuscular disease, infectious disease, cancer, significant body mass changes in the 1-mo period before the study, vegetarianism, and the use of medications that are known to interfere with muscle metabolism including β-blockers, high-dose nonsteroidal anti-inflammatory drugs, corticosteroids, hormone-replacement therapy, warfarin, prescription-strength acne medications, oral hypoglycemic agents, and insulin. On the basis of previous leucine-supplementation studies, we calculated that $n = 18$ would be sufficient to detect a mean ± SD difference in integrated MyoPS between placebo- and leucine-supplementation periods of 0.12% ± 0.08%/d with an $\alpha = 0.05$ and $\beta = 0.20$.

Study overview

This was a randomized, single-blind, parallel-group, placebo-controlled crossover study that was conducted at McMaster University. An overview of the study design is shown in **Figure 1**. The project coordinator used a simple randomization procedure (a computer-generated random-number method) to allocate participants to 1 of 2 groups as follows: a group with higher protein (HP) intake (1.2 g · kg^{-1} · d^{-1}) or a group with LP intake (0.8 g · kg^{-1} · d^{-1}). Allocation was concealed from the participants for the duration of the study. After baseline testing, participants commenced a 9-d controlled diet that was designed to meet energy requirements for weight maintenance and to provide a daily protein intake according to each participant's group

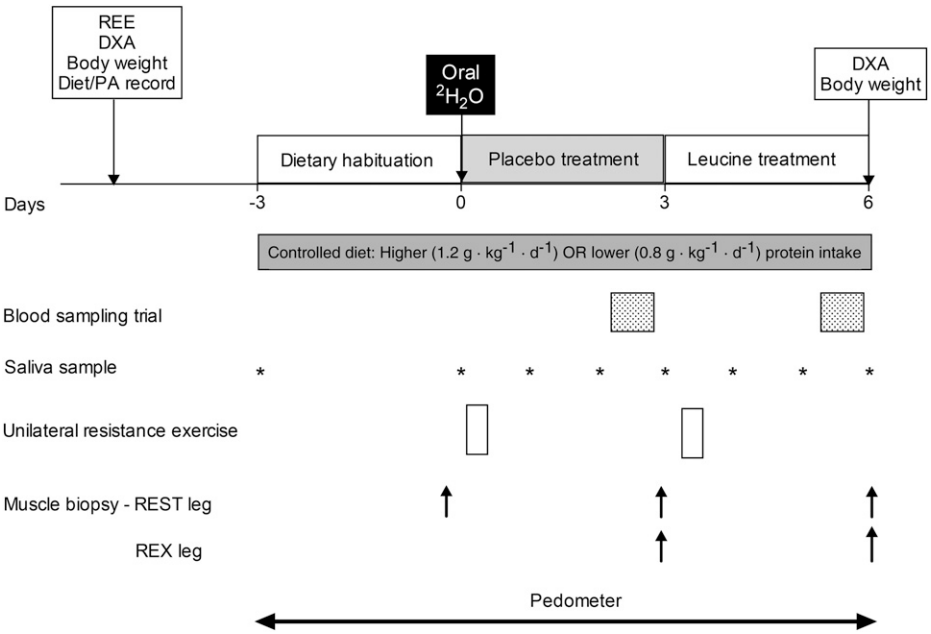

FIGURE 1 Schematic overview of study design. DXA, dual-energy X-ray absorptiometry; PA, physical activity; REE, resting energy expenditure; REST, rested; REX, resistance exercised.

allocation (i.e., 0.8 or 1.2 g · kg^{-1} · d^{-1}). Days −3 to −1 of the diet served as a prestudy dietary habituation period, and no supplements were consumed during this time. During days 0–2, participants consumed a placebo supplement with breakfast, lunch, and dinner, and during days 3–5, participants consumed a taste-matched supplement that contained 5 g leucine (Leu) with each of the 3 daily meals. The placebo- and Leu-supplement contents are provided in **Table 1**. Participants performed a bout of a unilateral leg-extension exercise at the start of the placebo treatment (day 0) and at the start of the Leu treatment (day 3). The integrated rate of MyoPS, which was the primary outcome of the study, was measured in the exercised and rested legs over the 3 d of placebo intake (day 0–2) and Leu intake (days 3–5) with the use of orally ingested ^2H_2O and muscle biopsies that were obtained from the vastus lateralis on the mornings of days 0, 3, and 6. The secondary outcomes of aminoacidemia, insulinemia, and glycaemia were determined in response to the coingestion of the supplements with the daily meals during the placebo treatment (day 2) and Leu treatment (day 5).

Baseline testing

Before study commencement, participants were asked to wear a pedometer (Piezo SC-StepX; StepsCount) and to complete a physical activity and weighed food record (Nutribase version 11.5; Cybersoft Inc.) for 3 d (2 weekdays and 1 weekend day) to assess habitual physical activity levels and dietary intakes. Approximately 1 wk before commencement of the study diet, participants reported to the laboratory at ∼0700 in a fasted state. Participants arrived by car and were instructed to minimize physical activity before the visit. Height was measured to the nearest 0.1 cm with the use of a stadiometer, and body mass was assessed to the nearest 0.1 kg with the use of a calibrated scale. A venous blood sample was obtained, and resting energy expenditure (REE) was measured with the use of a ventilated hood system (Moxus modular oxygen uptake system; AEI Technologies). REE testing was performed in a thermoneutral environment with participants lying quietly in a supine position. After a 10-min adaptation to the hood, oxygen uptake and carbon dioxide output were measured continuously for 30 min. After the REE measurement, participants underwent unilateral strength testing for the right-knee extensors with the use of a seated knee-extension device (C-605 unilateral leg extension; Atlantis). Participants performed a series of graded-load knee extensions to determine their 10-repetition maximum strength and the maximum load that they could lift for 15–24 repetitions.

TABLE 1
Nutritional composition of the supplements per single serving

	Supplement	
	Leu	Placebo
Leucine, g	5.0	0.0
Alanine, g	0.0	3.5
Glycine, g	0.0	1.5
Maltodextrin, g	3.0	3.0
Sucrose, g	7.0	7.0
Stevia, g	0.2	0.2
Citric acid, g	1.2	1.2
Flavoring, g	1.2	0.3
Totals		
∑TAAs,[1] g	5.0	5.0
Carbohydrate, g	10.0	10.0
Energy, kcal	60.0	60.0

[1] TAA, total amino acid.

Diets

Each participant's energy requirement to maintain an energy balance was determined with the use of the REE with the appropriate activity factor (19). Activity factors were determined for each participant on the basis of their baseline physical activity records and daily step counts. Each morning throughout the study, body mass was recorded before food or drink consumption, and a fasted dual-energy X-ray absorptiometry scan (GE-LUNAR iDXA; Aymes Medical) was performed immediately before and after the 9-d diet to confirm that participants maintained an energy balance throughout the intervention. For participants in the LP group, the diet provided a protein intake of 0.8 g · kg^{-1} · d^{-1}, which reflected the current RDA for protein in adults ≥19 y old (20). In the HP group, the diet provided 1.2 g protein · kg^{-1} · d^{-1} in line with the recent recommendations from a number of expert committees for optimal protein intake in healthy older adults (12, 13). The increased protein intake in the HP group was achieved by reducing the proportion of energy from carbohydrate, whereas the proportion of energy from fat (30%) was kept constant between the 2 groups. In both groups, protein was provided from a variety of plant- and animal-based sources. To emulate the skewed protein-intake pattern that is typically consumed by older adults (21, 22), protein was distributed across breakfast, lunch, dinner, and an evening snack in the proportions ∼15%:25%:50%:10%, respectively. Participants were required to abstain from alcohol for the duration of the 9-d diet.

To enhance compliance, study diets were designed by a research dietitian who met with each participant individually to customize meal plans according with each participant's personal food preferences. Participants were supplied with all of the food that was required for the duration of the study, which, in both groups, consisted of prepackaged, frozen meals (Copper County frozen foods) and other items that required minimal preparation (i.e., granola bars, fruit cups, and juices). Participants were asked to consume breakfast within 30 min of waking (with the exception of days 0, 3, and 6 when breakfast was provided immediately after biopsies that were obtained at 0900) and were instructed to space meals ∼4–6 h apart. This meal spacing was selected to reflect a traditional meal pattern, and in an attempt to ensure that a muscle-full effect that persisted from the previous meal did not hamper the MyoPS response to the subsequent meal (23, 24). To assess compliance, participants were instructed to record the time of day that meals were eaten and the percentage of their food items that were consumed in a log. Participants were strongly encouraged to consume all of the food provided to them and to avoid eating food that was not provided by the study, but if this additional food was consumed, the participant was asked to record these deviations in the meal-plan log.

Supplementation

All participants were provided with identically flavored (lemon) supplemental beverages (Infinit Nutrition) and were instructed to

consume 1 beverage midway through each main meal (breakfast, lunch, and dinner) from days 0–5 of the intervention diet. No supplement was consumed with the evening snack. During days 0–2, participants consumed a placebo beverage that contained 5 g nonessential amino acids (alanine and glycine), and during days 3–5, the beverage contained 5 g crystalline leucine (Table 1). The order of the supplementation periods (i.e., placebo followed by Leu) was not randomized but was always provided as the placebo before Leu to avoid a potential carryover effect of previous leucine supplementation on the MyoPS response (25). Individual servings of the supplement were packaged into a sealed pouch by Infinit Nutrition in powder form, and participants were asked mix this serving with 250 mL H_2O at home before consumption. Beverages were provided in a single-blind manner and were isonitrogeous, energy-matched, and indistinguishable in odor, color, and taste (Table 1).

Physical activity

Each participant's habitual daily step count was determined on the basis of the mean of his pedometer counts that were measured for 3 d at baseline. To ensure that activity levels remained consistent throughout the intervention, participants were asked to maintain their daily step count ≤10% of their mean value. These levels were monitored with the use of a pedometer and were recorded by the participants daily in their meal plan logs. Participants were asked to abstain from any REX other than the exercises performed as part of the intervention.

Blood sampling trials

To characterize the typical patterns of aminoacidemia, insulinemia, and glycaemia in response to the daily meals and supplements in the placebo and Leu phases of the study, participants underwent a blood sampling trial on the final day of placebo intake (day 2) and on the final day of Leu intake (day 5). Participants reported to the laboratory after an overnight fast at ∼0630. On arrival, a catheter was inserted into an antecubital vein for blood sampling, and participants were provided with their individualized breakfast, lunch, and dinner meals in the laboratory. Breakfast was provided at 0700, lunch was provided 240 min after the completion of breakfast (∼1130), and dinner was provided 300 min after the completion of lunch (∼1700). Blood samples were obtained before breakfast and 20, 40, 60, 90, 120, 180, and 240 min after the ingestion of each meal as well as 300 min after the consumption of lunch (**Figure 2**). On day 2 (placebo trial), the placebo supplement was consumed, and on day 5 (leucine trial), the Leu supplement was consumed midway through each meal. To attenuate the suppressive effects of inactivity on MyoPS and to simulate normal daily movement during the trials, participants were asked to walk around the University campus between meals. Steps were monitored with the use of a pedometer, and participants were required to walk until they reached their habitual daily step counts.

Isotope protocol

Oral consumption of ^2H_2O (70 atom%; Cambridge Isotope Laboratories) was used to label newly synthesized myofibrillar proteins as previously described (26). Participants reported to the laboratory in the fasted state on day 0, and after collection of a saliva sample and a muscle biopsy from the vastus lateralis, participants consumed a single 100-mL oral bolus of ^2H_2O at ∼0900. Immediately after consumption of the ^2H_2O bolus, participants performed a bout of a unilateral leg-extension exercise that consisted of 3 sets at the previously determined maximum load that they could lift for 15–24 repetitions (∼50% of a one-repetition maximum) until volitional failure. The exercise was performed with the use of the left leg and with a rest interval of 2 min between sets. This unilateral study design meant that the rested leg was exposed to the effect of feeding and supplementation alone (rested), whereas the exercised leg was exposed to the combination of feeding and supplementation and REX. On day 3, participants performed an identical REX session with the exception that the exercise was performed on the right leg and that repetitions were clamped at the same number of repetitions per set that were achieved on day 0. The exercise was performed on different legs in the placebo and Leu treatments in view of previous work that suggested that the acute transcriptional (27) and protein synthetic (28) responses to an initial bout of REX may vary substantially from the responses to subsequent bouts in untrained individuals. Therefore, varying the exercise leg allowed us to examine the MyoPS response to REX in an exercise-naive leg during both the placebo and Leu treatments. The exercised leg was not randomized (i.e., the left leg was always exercised during the placebo treatment, and the right leg was always exercised during the Leu treatment) to avoid an additional complicating factor that would increase risk of human error. After both exercise sessions, participants consumed their individualized breakfasts (with the placebo beverage on day 0 and the Leu beverage on day 3) in the laboratory before returning home. Bilateral muscle biopsies were obtained before the exercise on day 3 (end of placebo treatment) and on day 6 (end of Leu treatment). All muscle biopsies were obtained with the use of a 5-mm Bergström needle that was adapted for

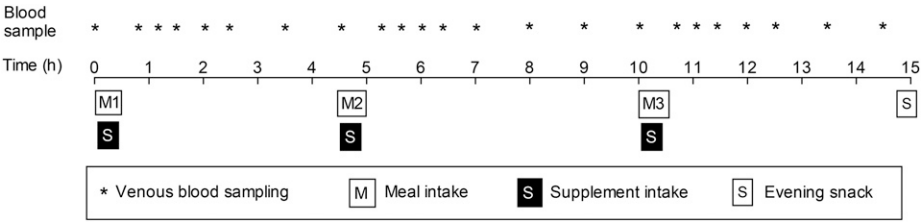

FIGURE 2 Blood sampling trial protocol. Note that each meal was consumed over ∼30 min as reflected by the width of the meal-intake boxes. Placebo and Leu supplements were consumed midway through each meal during the placebo and Leu trials, respectively. Participants consumed their evening snacks after returning home after the trial.

manual suction under 2% xylocaine local anesthesia. Tissue samples were freed from visible fat and connective tissue, frozen immediately in liquid nitrogen, and stored at −80°C for further analysis. All muscle biopsies were obtained in the fasted state between 0900 and 1000.

Total body water ^{2}H enrichment can be used as a surrogate for plasma alanine labeling (28) and was determined from saliva swabs that were collected by participants at ∼0900 every morning before and after the ingestion of the ^2H_2O bolus (28). Participants were instructed not to eat or drink anything for 30 min before saliva sampling, and samples were stored at −20°C before analysis.

Analytic methods

Plasma glucose concentrations were measured with the use of the glucose oxidase method (YSI 2300; YSI Life Sciences). Plasma insulin concentrations were measured with the use of the dual-site chemiluminescent method (Immulite 2000 immunoassay system; Siemens). Plasma amino acid concentrations were analyzed with the use of gas chromatography–mass spectrometry (Hewlett-Packard) with the use of the EZfaast amino acid analysis kit (Phenomenex) per the manufacturer's instructions.

Myofibrillar-enriched proteins were isolated from the muscle biopsies as previously described (29). The incorporation of deuterium (^{2}H) into protein-bound alanine was determined with the use of Delta V isotope-ratio mass spectrometry (ThermoFisher Scientific) coupled to a Trace Ultra Gas Chromatograph (ThermoFisher Scientific) as previously described (30). Saliva samples were analyzed for ^{2}H enrichment via cavity ring-down spectroscopy with the use of a Liquid Water Isotope Analyzer with an automated injection system (Los Gatos Research) as previously described (30).

Calculations

The fractional synthetic rate of myofibrillar protein was determined from the incorporation of deuterium-labeled alanine into protein with the use of the enrichment of body water (corrected for the mean number of deuterium moieties incorporated per alanine: 3.7) as the surrogate precursor labeling between subsequent biopsies. In brief, the following standard equation was used:

$$\mathrm{FSR}(\%/d) = [(\mathrm{APE_{Ala}})] \div [(\mathrm{APE_p}) \times t] \times 100 \qquad (1)$$

where atom percent excess $(\mathrm{APE})_{\mathrm{Ala}}$ is the deuterium enrichment of protein bound alanine, $\mathrm{APE_P}$ is the mean precursor enrichment over the time period, and t is the time between biopsies.

Statistical analyses

All analyses were performed with the use of SPSS software (version 22.0; SPSS Inc.). Exercise and dietary intervention variables were analyzed with the use of a 2-factor mixed-model (group × treatment) ANOVA. The AUC and concentration maximum (C_{max}) for plasma concentrations (glucose, insulin, and amino acids) were examined with the use of a 3-factor [group (LP compared with HP) × treatment (placebo compared with Leu) × meal (breakfast compared with lunch compared with dinner)] mixed-model ANOVA. MyoPS was analyzed with the use of a 3-factor [group × treatment × condition (rested compared with REX)] mixed-model ANOVA. Tukey's post hoc test was performed whenever a significant interaction was shown to isolate specific differences. Post hoc power calculations revealed that we had 93% power to detect a group × treatment interaction and 69% power to detect a group × treatment × condition interaction. Significance was accepted when P was ≤0.05. For data that did not pass normality, values were transformed with the ln of the value. The statistical analysis was performed on transformed data, but nontransformed data are presented in graphic or tabular form for clarity. Results are presented as means ± SD unless otherwise specified with the exception of in Figure 6 (mean ± range) and Figure 7.

RESULTS

Participants

Figure 3 shows a flowchart of the progress from recruitment through completion of the study. Twenty-two older men were recruited to take part in the study. Two participants in the LP group did not perform the exercise protocol correctly and were excluded from the analysis. Characteristics of the 20 participants who were included in the final analysis (n = 10/group) are shown in **Table 2**. Participants' habitual dietary intakes that were assessed at baseline are shown in **Table 3**.

Physical activity and exercise variables

Participants maintained their habitual daily step counts throughout the intervention (P = 0.28), and step counts were not different between groups (placebo: LP, 9159 ± 3601; HP, 12,116 ± 6131; Leu: LP, 9870 ± 3168; HP, 12,194 ± 5378) (P = 0.23). The volume [load (kilograms) × number of repetitions] of the leg-extension exercise performed was similar during the placebo and Leu phases (P = 0.61).

Body composition, diet, and supplementation

There was no change in body mass (day −3: LP, 85.2 ± 10.0 kg; day 6: LP, 85.3 ± 10.2 kg; day −3: HP, 81.4 ± 9.1 kg; day 6: HP, 81.3 ± 9.1 kg) (P = 0.76) or in any of the body-composition variables over the course of the study (all $P > 0.05$; data not shown), which indicated that participants in both groups were maintained in a state of energy balance throughout the intervention. The intervention diet provided energy from protein, carbohydrate, and fat in the proportions of ∼10%:60%:30%, respectively, in the LP group and 15%:55%:30%, respectively, in the HP group. There was a high level of reported compliance with the prescribed intervention diet with no difference between groups (placebo: LP, 98% ± 3%; HP, 98% ± 2%; Leu: LP, 98% ± 3%; HP, 95% ± 8%) (P = 0.32). The reported adherence to supplementation was also high with no group differences (placebo: LP, 100% ± 0%; HP, 99% ± 3%; Leu: LP, 100% ± 2%; HP, 99% ± 3%) (P = 0.46). Combined intakes from diet and supplements throughout the intervention are summarized in **Table 4**. Total leucine intake from the diet plus supplements during Leu treatment (LP: ∼0.24 ± 0.02 g · kg^{-1} · d^{-1}; HP: ∼0.28 ± 0.03 g · kg^{-1} · d^{-1}) remained substantially lower than the upper tolerable and safe limit in humans of 0.53 g · kg^{-1} · d^{-1} (31). No adverse effects of the leucine supplement were reported.

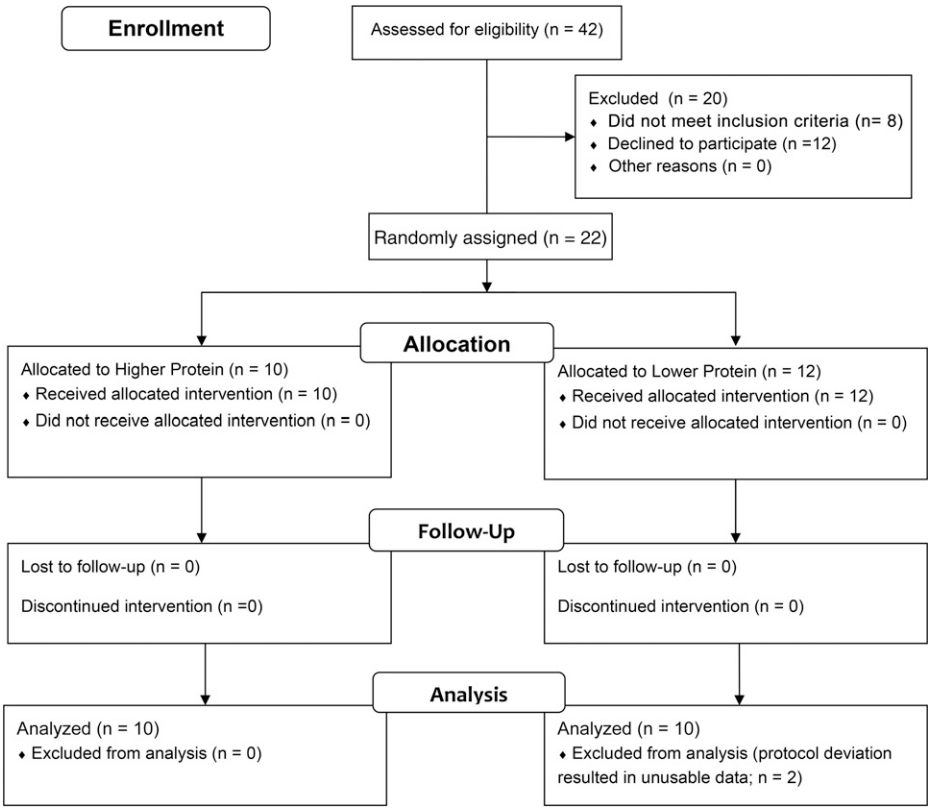

FIGURE 3 Participant flowchart.

Plasma glucose, insulin, and amino acid concentrations

Fasting glucose and insulin concentrations as well as the HOMA-IR (placebo: LP, 2.0 ± 0.2; HP, 2.0 ± 0.2; Leu: LP, 2.2 ± 0.2; HP, 2.0 ± 0.2) were similar in LP and HP groups with both treatments ($P > 0.05$). The glucose AUC (**Figure 4**A) and C_{max} after breakfast, lunch, and dinner did not differ between groups or treatments ($P > 0.05$). The insulin AUC (Figure 4B) after the meals did not differ between groups ($P = 0.965$) or treatments ($P = 0.707$). However, there was a meal × treatment interaction for the insulin C_{max} such that it was higher after dinner with the Leu treatment (40.2 ± 9.7 μIU/mL) than with the placebo treatment (32.2 ± 9.6 μIU/mL) ($P = 0.003$) with no difference between groups ($P = 0.928$; data not shown).

Plasma concentrations over time for the sum of the EAAs and leucine are shown in **Figure 5**. There were no differences in fasting concentrations of plasma leucine and ∑EAAs between groups or treatments. There was a treatment × group interaction for fasting plasma Ile concentrations ($P < 0.001$) and a trend for a treatment × group interaction for fasting plasma Val concentrations ($P = 0.050$). Tukey's post hoc tests revealed that the fasting plasma Ile concentration was lower in the LP group during Leu treatment than during placebo treatment (placebo: 65 ± 9 nmol/mL; Leu: 46 ± 12 nmol/mL; $P = 0.002$), whereas the fasting Ile concentration was higher during Leu treatment than during placebo treatment in the HP group (placebo: 46 ± 9 nmol/mL; Leu: 60 ± 3 nmol/mL; $P = 0.023$). Fasting plasma Val was similar with placebo and Leu treatments in the LP group (placebo 141 ± 20 nmol/mL; Leu: 143 ± 33 nmol/mL; $P = 0.786$) but were higher with Leu treatment in the HP group (placebo: 123 ± 20 nmol/mL; Leu: 153 ± 36 nmol/mL; $P = 0.005$).

The AUC above baseline (AUC_{pos}) and C_{max} were analyzed for plasma ∑EAAs, leucine, Ile, and Val for each meal and are shown for ∑EAAs and leucine in **Table 5**. For the plasma leucine AUC_{pos}, there was a treatment × group interaction ($P = 0.020$) such that the leucine AUC_{pos} was higher in HP than LP groups with placebo treatment but not with Leu treatment ($P = 0.015$) (Figure 5). The plasma leucine C_{max} was higher with Leu treatment than with placebo treatment ($P < 0.001$) with no difference between groups ($P = 0.690$). The AUC_{pos} for ∑EAAs was higher in the HP group than in the LP group ($P < 0.01$) and was higher

TABLE 2
Baseline participant characteristics[1]

	LP	HP
Age, y	72 ± 4	73 ± 6
Height, m	1.7 ± 0.1	1.7 ± 0.1
Body mass, kg	85.2 ± 9.9	81.4 ± 9.1
BMI, kg/m^2	28.2 ± 2.5	26.7 ± 3.1
Fat mass, kg	26.3 ± 6.3	24.1 ± 7.1
FFM, kg	55.7 ± 4.2	54.0 ± 5.3
Fasting blood glucose, mmol/L	5.5 ± 0.4	5.6 ± 0.3
Fasting insulin , μU/mL	7.7 ± 2.3	7.1 ± 2.1
HbA1c, %	5.4 ± 0.4	5.3 ± 0.2
LDL, mmol/L	3.0 ± 0.7	2.5 ± 0.5
HDL, mmol/L	1.2 ± 0.2	1.2 ± 0.3
Triglyceride, mmol/L	0.97 ± 0.29	1.04 ± 0.52
Steps per day, *n*	9065 ± 3598	11,860 ± 6329

[1] All values are means ± SD. *n* = 10/group. FFM, fat-free (bone-free) mass; HbA1c, glycated hemoglobin; HP, higher-protein group; LP, lower-protein group.

TABLE 3
Baseline dietary intake measured by 3-d weighed diet record[1]

	LP	HP
Energy, kcal/d	2143 ± 359	2133 ± 417
Fat		
g/d	87 ± 23	75 ± 22
Energy intake, % of total	37 ± 6	33 ± 7
CHO		
g/d	237 ± 48	257 ± 61
Energy intake, % of total	41 ± 6	44 ± 6
Protein		
g/d	97 ± 20	89 ± 23
g · kg BM^{-1} · d^{-1}	1.2 ± 0.3	1.1 ± 0.4
Energy intake, % of total	19 ± 2	17 ± 2
Alcohol, energy intake, % of total	3 ± 5	6 ± 5

[1] All values are means ± SD. n = 10/group. BM, body mass; CHO, carbohydrate; HP, higher-protein group; LP, lower-protein group.

in both groups during Leu treatment than during placebo treatment ($P < 0.05$) (Figure 5). The AUC_{pos} for Ile and Val did not differ between groups or treatments ($P > 0.05$; data not shown). There was a group × treatment interaction for the Ile C_{max} and Val C_{max} such that they were similar with placebo and Leu treatments in the LP group but were higher with Leu treatment than with placebo treatment in the HP group ($P < 0.05$). No formal statistical analyses were performed on the AUC below baseline or the time of maximum concentration data, but means for the plasma leucine AUC below baseline and the time of maximum concentration were lower with Leu treatment than with placebo treatment with no obvious difference between groups (Table 5).

Body water enrichment

Figure 6 shows the mean body water enrichment over the 6-d MyoPS measurement period. A single bolus of 100 mL of 70% ^2H_2O led to a body water enrichment of 0.131% ± 0.013% 24 h postingestion (range: 0.114–0.171%). Body water enrichment followed an exponential decay pattern (r^2 = 0.99) whereby it decaying slowly over the 6-d period and reached 0.084% ± 0.008% (range: 0.073–0.105%) at the end of the study.

MyoPS

MyoPS was similar between LP and HP groups (P = 0.39); however, there were main effects for the treatment ($P < 0.001$) and condition ($P < 0.001$) as well as a treatment × condition interaction (P = 0.016). Tukey's post hoc test revealed that the myofibrillar fractional synthetic rate was higher with Leu treatment than with placebo treatment in the rested condition (pooled mean: Leu, 1.57% ± 0.11%/d; placebo, 1.48% ± 0.08%/d; $P < 0.001$) (**Figure 7**A) as well as in the REX condition (pooled mean: Leu, 1.87% ± 0.09%/d; placebo, 1.71% ± 0.10%/d;

TABLE 4
Dietary intake during the placebo (days 0–2) and Leu (days 3–5) treatment periods[1]

Treatment, group, and meal	Energy, kcal/kg BM	Protein			Fat		CHO		Fiber, g	Leucine,[2] g
		g	g/kg BM	g/kg FFM	g	g/kg BM	g	g/kg BM		
Placebo										
LP										
Breakfast	8 ± 1	13 ± 3	0.15 ± 0.03	0.23 ± 0.04	16 ± 6	0.2 ± 0.1	126 ± 19	1.5 ± 0.1	7 ± 2	1.0 ± 0.2
Lunch	9 ± 1	17 ± 3	0.20 ± 0.03	0.31 ± 0.05	31 ± 5	0.4 ± 0.1	110 ± 22	1.3 ± 0.2	8 ± 2	1.4 ± 0.3
Dinner	11 ± 1	31 ± 4	0.36 ± 0.04	0.55 ± 0.05	35 ± 9	0.4 ± 0.1	123 ± 18	1.5 ± 0.2	10 ± 2	2.4 ± 0.3
ES	5 ± 1	6 ± 2	0.07 ± 0.01	0.11 ± 0.02	12 ± 3	0.1 ± 0.1	68 ± 19	0.8 ± 0.2	4 ± 1	0.5 ± 0.1
Total	33 ± 3	67 ± 9	0.79 ± 0.03	1.20 ± 0.09	94 ± 16	1.1 ± 0.1	428 ± 60	5.0 ± 0.5	29 ± 5	5.4 ± 0.7
HP										
Breakfast	7 ± 1	18 ± 3	0.22 ± 0.03	0.34 ± 0.07	19 ± 3	0.2 ± 0.0	85 ± 20	1.1 ± 0.3	6 ± 1	1.5 ± 0.3
Lunch	10 ± 2	27 ± 3	0.34 ± 0.03	0.51 ± 0.07	28 ± 5	0.4 ± 0.1	111 ± 13	1.4 ± 0.3	9 ± 1	2.2 ± 0.3
Dinner	13 ± 2	49 ± 6	0.61 ± 0.04	0.92 ± 0.11	32 ± 4	0.4 ± 0.1	126 ± 12	1.6 ± 0.3	10 ± 2	3.9 ± 0.5
ES	5 ± 2	7 ± 2	0.09 ± 0.03	0.13 ± 0.04	14 ± 4	0.2 ± 0.5	74 ± 22	0.9 ± 0.3	6 ± 4	0.5 ± 0.1
Total	35 ± 6	102 ± 11	1.26 ± 0.06	1.90 ± 0.21	92 ± 10	1.2 ± 0.2	395 ± 51	4.9 ± 1.0	30 ± 5	8.2 ± 0.9
Leu										
LP										
Breakfast	8 ± 1	13 ± 3	0.15 ± 0.03	0.23 ± 0.04	16 ± 6	0.2 ± 0.1	126 ± 19	1.5 ± 0.1	7 ± 2	6.0 ± 0.2
Lunch	9 ± 1	17 ± 4	0.20 ± 0.03	0.31 ± 0.05	31 ± 6	0.4 ± 0.1	110 ± 23	1.3 ± 0.2	8 ± 2	6.4 ± 0.3
Dinner	11 ± 1	31 ± 4	0.36 ± 0.04	0.55 ± 0.05	34 ± 8	0.4 ± 0.1	123 ± 15	1.5 ± 0.2	10 ± 2	7.4 ± 0.3
ES	5 ± 1	6 ± 2	0.07 ± 0.01	0.11 ± 0.03	12 ± 3	0.1 ± 0.0	71 ± 18	0.8 ± 0.2	4 ± 1	0.6 ± 0.2
Total	33 ± 3	67 ± 9	0.79 ± 0.03	1.21 ± 0.09	93 ± 16	1.1 ± 0.1	429 ± 61	5.0 ± 0.5	30 ± 5	20.4 ± 0.7
HP										
Breakfast	7 ± 1	18 ± 3	0.22 ± 0.03	0.34 ± 0.07	19 ± 3	0.2 ± 0.3	85 ± 20	1.0 ± 0.3	6 ± 1	6.5 ± 0.3
Lunch	10 ± 2	27 ± 4	0.33 ± 0.05	0.50 ± 0.07	28 ± 6	0.4 ± 0.1	111 ± 17	1.4 ± 0.3	9 ± 2	7.1 ± 0.3
Dinner	12 ± 3	46 ± 8	0.57 ± 0.08	0.85 ± 0.09	30 ± 6	0.4 ± 0.1	120 ± 24	1.5 ± 0.4	9 ± 1	8.7 ± 0.6
ES	5 ± 1	7 ± 2	0.09 ± 0.03	0.13 ± 0.04	14 ± 4	0.2 ± 0.1	70 ± 18	0.9 ± 0.3	6 ± 4	0.6 ± 0.2
Total	35 ± 7	98 ± 13	1.21 ± 0.12	1.82 ± 0.16	90 ± 14	1.1 ± 0.2	386 ± 64	4.8 ± 1.1	30 ± 6	22.9 ± 1.0

[1] All values are means ± SDs. n = 10/group. Intakes were recorded by participants in meal-plan food logs and included foods and supplements. Participants were randomly assigned to either the LP or the HP group and remained in their allocated groups through both placebo and Leu treatments. No statistical tests were performed on the data. BM, body mass; CHO, carbohydrate; ES, evening snack; FFM, fat-free (bone-free) mass; HP, higher protein; LP, lower protein.

[2] Dietary leucine intake was estimated with the assumption that, on average, leucine accounts for ~8% of the amino acid content of protein.

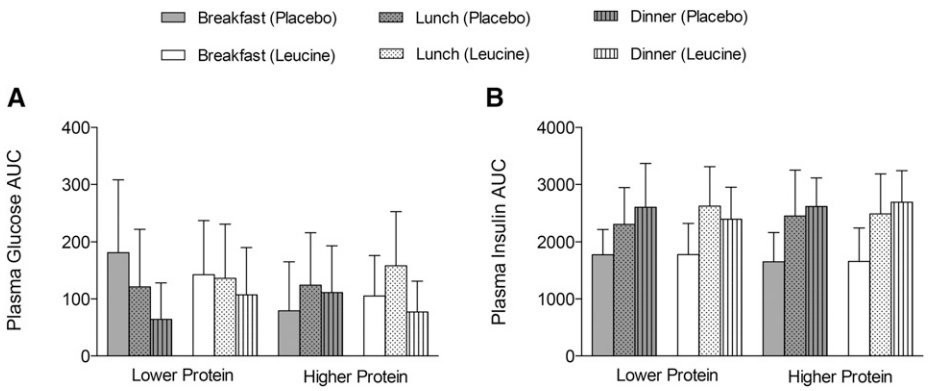

FIGURE 4 Mean ± SD AUCs of postprandial plasma glucose (mmol/L · 4 h for breakfast and dinner; mmol/L · 5 h for lunch) (A) and insulin (μIU/mL · 4 h for breakfast and dinner; μIU/mL · 5 h for lunch) (B) concentrations in response to the co-ingestion of placebo and Leu supplements with breakfast, lunch, and dinner in the LP group (0.8 g protein · kg^{-1} · d^{-1}) or HP group (1.2 g protein · kg^{-1} · d^{-1}). Data were analyzed with the use of a 3-factor [group (LP compared with HP) × treatment (placebo compared with Leu) × meal (breakfast compared with lunch compared with dinner)] mixed-model ANOVA. Analyses revealed no main effects for group or treatment or interactions. n = 10/group. HP, higher protein; LP, lower protein.

$P < 0.001$) (Figure 7B). MyoPS was higher in the REX leg than in the rested leg with both placebo and Leu treatments ($P < 0.001$).

DISCUSSION

To our knowledge, this study provides novel data showing that the co-ingestion of 5 g leucine with normal daily meals enhanced integrated MyoPS in free-living older men. The addition of leucine was equally effective in men who consumed daily protein intakes at the RDA for protein (0.8 g · kg^{-1} · d^{-1}) and at more-optimal amounts of protein (1.2 g · kg^{-1} · d^{-1}) (12, 13), which has implications for protein (or leucine) requirements in older men. Furthermore, we report that, in addition to enhancing integrative MyoPS under rested (nonexercised) conditions, leucine co-ingestion with meals further potentiated the stimulatory effect of REX on integrative MyoPS. To our knowledge, these are the first data to show, under free-living conditions and across days, that leucine supplementation of meals has a marked stimulatory effect on MyoPS regardless of protein intake and external muscle loading.

Because of numerous age-related issues, many older adults are likely unable to achieve protein intakes (∼0.4 g · kg^{-1} · $meal^{-1}$) that are required to optimally stimulate MyoPS at each of the daily meals (12, 13, 22). Furthermore, dietary restrictions that are imposed to slow the progression of certain chronic diseases (i.e., chronic renal disease) may prevent older adults from achieving protein intakes required to attenuate muscle loss (32). In the current study, we showed that a practical strategy of leucine co-ingestion (as a liquid supplement) with mixed meals can augment MyoPS in older adults without increasing the total amount of protein consumed. We provided leucine in a low fluid volume (250 mL) that was well tolerated and did not affect the ability of participants to finish meals. Because leucine is also readily available and relatively inexpensive, our results indicate that leucine co-ingestion could represent a viable strategy to enhance MyoPS in older adults without compromising food intake and broader nutrition goals.

Our integrated rates of MyoPS during placebo supplementation translated to acute rates of ∼0.062%/h and 0.071%/h in rested and REX conditions, respectively. These values are in line with those that have been generally reported in short-term (i.e., hours) tracer-infusion studies in the rested, postprandial state (∼0.04–0.07%/h) (4, 33, 34) and after REX (33, 35) and feeding (∼0.05–0.08%/h) for men of a similar age. Moreover, our findings are consistent with studies that have shown that increasing the leucine content of LP-containing meals enhances short-term MPS in older adults at rest (6–8) and after REX (36). In the current study, leucine supplementation increased MyoPS in the rested condition ∼6%, which was slightly lower than the ∼12% acute increase that was observed by Katsanos et al. (6) when the leucine content of an EAA drink was increased from 26% to 41%. This difference is perhaps not surprising because the integrated nature of the D_2O measurement, which incorporates the prolonged overnight fast, means that the impact of leucine on MyoPS will likely be diluted compared with acute postprandial measurements. The rates of MyoPS in the current study reflect rates that were measured over several days in free-living older adults who consumed normal diets and performed their usual daily activities. Thus, we have expanded on previous studies in which MPS was measured over several hours under controlled laboratory conditions. Our data provide MyoPS rates that are more representative of real-world MyoPS and support a robust effect of leucine supplementation in an applied setting. Indeed, the absolute rates of MyoPS (determined by estimating myofibrillar protein mass from the results of dual-energy X-ray absorptiometry) in the current study showed that leucine supplementation resulted in the synthesis of an additional ∼3.2 and ∼5.5 g myofibrillar protein/d in the rested and REX legs, respectively, compared with the effect of placebo treatment. With the assumption of no increase in muscle protein breakdown and an (even partially) sustained effect of leucine on MyoPS over time, the data provide support for the idea that leucine supplementation could translate to a meaningful impact on muscle-mass maintenance over prolonged periods. Our findings also provide broader support for increased leucine requirements in older persons to maintain muscle mass, which is a concept that is congruent with HP requirements in older persons (37–39).

Few longer-term (3–6-mo) randomized controlled trials have examined the influence of chronic leucine supplementation on indexes of muscle mass or function in older adults (9–11). Of

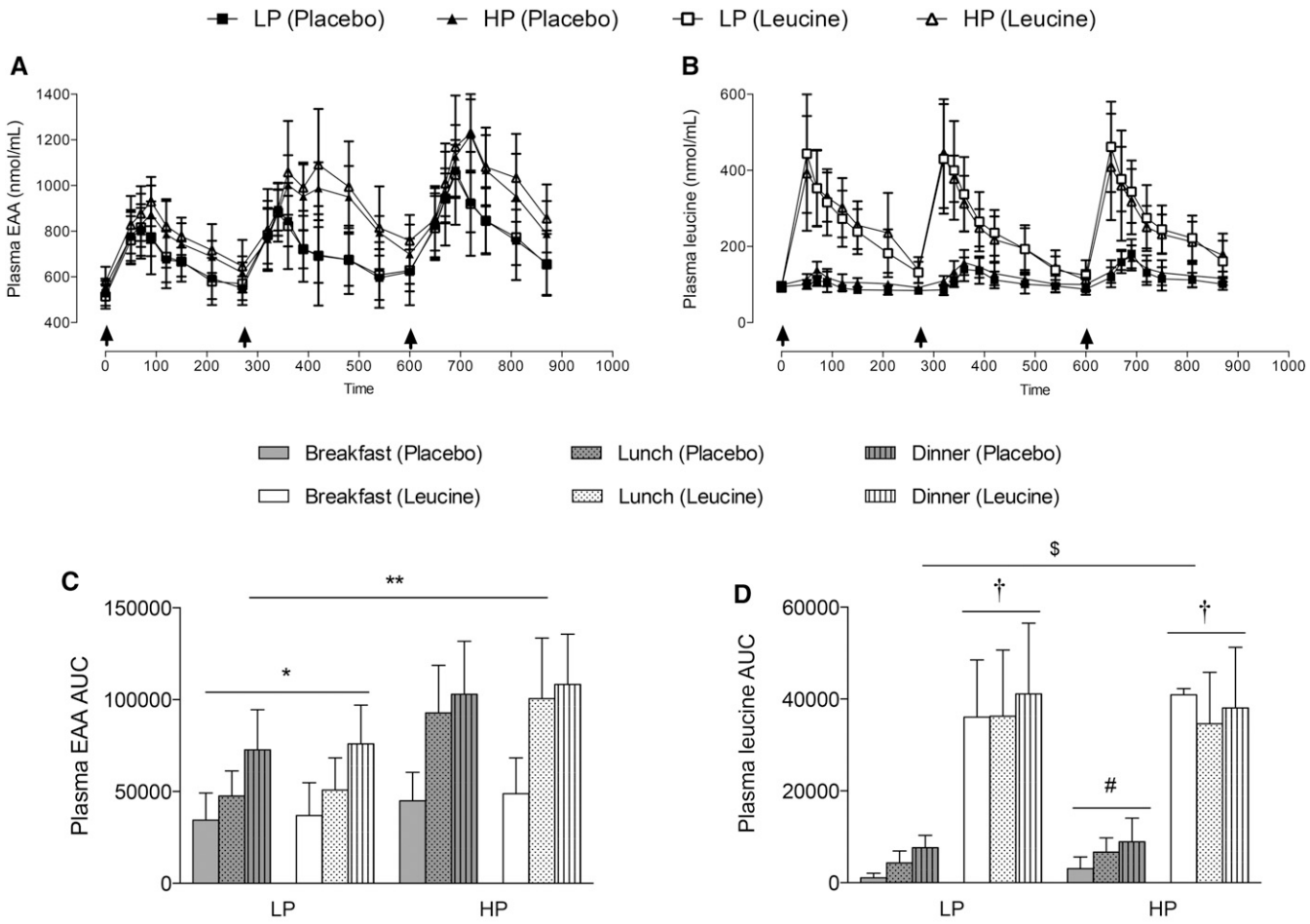

FIGURE 5 Mean ± SD plasma concentrations over time of EAA (nmol/mL) (A) and leucine (nmol/mL) (B), the EAA AUC_{pos} (nmol/mL · 4 h for breakfast and dinner; nmol/mL · 5 h for lunch) (C), and the leucine AUC_{pos} (nmol/mL · 4 h for breakfast and dinner; nmol/mL · 5 h for lunch) (D) in response to the co-ingestion of placebo and leucine supplements with breakfast, lunch, and dinner in the LP group (0.8 g protein · kg^{-1} · d^{-1}) or HP group (1.2 g protein · kg^{-1} · d^{-1}). Arrows indicate a meal that was co-ingested with a supplement (placebo or Leu). No statistical analysis was performed on the concentration-over-time data. AUC_{pos} data were analyzed with the use of a 3-factor [group (LP compared with HP) × treatment (placebo compared with Leu) × meal (breakfast compared with lunch compared with dinner)] mixed-model ANOVA with Tukey's post hoc test when there was a significant interaction. *Main effect for group; **main effect of treatment; $^{\$}$group × treatment interaction; $^{\#}$different from the LP group in placebo treatment; †different from placebo treatment within the same group. n = 10/group. AUC_{pos}, AUC above baseline; EAA, essential amino acids; HP, higher protein; LP, lower protein.

the 2 studies that did not include exercise, 1 study involved 3 mo of leucine co-ingestion with daily meals (3 meals/d × 2.5 g leucine/meal) in healthy elderly men (10), and the other study lasted 6 mo with the same dosing regimen in older men with type 2 diabetes (11). Neither study showed an effect of leucine supplementation on lean mass or function (10, 11). Speculation as to why longer-term leucine supplementation failed to enhance muscle mass (10, 11) was due to the habitual protein intake of ∼1.0 g · kg^{-1} · d^{-1} of the participants, which was closer to a hypothesized optimal protein intake in healthy older adults (12, 13). Our data argue against this hypothesis and showed that leucine co-ingestion with meals was equally as effective in augmenting integrated MyoPS in subjects who consumed lower-protein–containing diets (0.8 g · kg^{-1} · d^{-1}) or higher protein–containing diets (1.2 g · kg^{-1} · d^{-1}). An alternative explanation for the inability of leucine to affect muscle mass may be that 3–6 mo would not allow for the detection of an effect of leucine supplementation on changes in lean mass with the use of the available methods (40). It is also possible that the per-meal leucine dose (2.5 g/meal) that was provided in the studies by Verhoeven et al. (10) and Leenders et al. (11) was suboptimal to enhance MPS when provided alongside mixed meals. Although acute studies in older adults have shown an increase in MPS with the addition of small amounts of leucine (1.1–2.5 g) to meal-like doses of EAA and isolated protein (6, 7), the higher dose that was provided in the current study (5 g leucine/meal) may be required to create hyperleucinemia to the level required to augment MPS when leucine is co-ingested with a solid-food matrix. For example, we observed that the ingestion of 5 g crystalline leucine in a liquid form with mixed meals resulted in a peak plasma leucine concentration ∼450 nmol/mL, which was below the concentrations that were observed with the addition of just 2.5 g leucine to a 20-g liquid bolus of isolated casein (∼650 nmol/mL) (7) or the ingestion of a leucine-enriched (2.8 g leucine) EAA beverage (∼700 nmol/mL) (6).

It has been well established that REX is a potent stimulus for MPS, and resistance training is still considered the most effective and safe treatment for improving muscle mass, strength, and

TABLE 5
Plasma leucine and ∑EAA responses to breakfast, lunch, and dinner with placebo and Leu supplementation[1]

	Placebo		Leu	
Meal	LP	HP	LP	HP
Leucine				
AUC_{pos} [#]				
Breakfast	1078 ± 1010	3050 ± 2554	36,052 ± 12,458	40,927 ± 13,230
Lunch	4346 ± 2586	6662 ± 3115	36,280 ± 14,380	34,638 ± 11,187
Dinner	7544 ± 2683	8920 ± 5166	41,137 ± 15,386	38,098 ± 13,154
C_{max}, [†] μM				
Breakfast	118 ± 22	141 ± 18	452 ± 154	449 ± 107
Lunch	152 ± 25	168 ± 25	442 ± 134	479 ± 104
Dinner	190 ± 23	182 ± 21	462 ± 119	456 ± 84
T_{max}, [a] min				
Breakfast	50 ± 11	50 ± 25	28 ± 14	53 ± 51
Lunch	73 ± 24	91 ± 57	26 ± 10	28 ± 14
Dinner	56 ± 8	60 ± 23	20 ± 0	24 ± 8
AUC_{neg} [a]				
Breakfast	1372 ± 849	682 ± 854	0 ± 0	0 ± 0
Lunch	564 ± 546	431 ± 450	90 ± 285	0 ± 0
Dinner	247 ± 420	343 ± 737	0 ± 0	0 ± 0
∑EAA				
AUC_{pos} [*,†]				
Breakfast	34,435 ± 14,710	44,915 ± 15,532	36,921 ± 17,837	48,832 ± 19,424
Lunch	47,590 ± 13,594	92,867 ± 25,774	50,874 ± 17,391	100,530 ± 33,046
Dinner	72,688 ± 21,907	102,968 ± 28,867	75,952 ± 21,065	108,287 ± 27,460
C_{max}, [*,†] μM				
Breakfast	871 ± 91	911 ± 59	861 ± 95	975 ± 83
Lunch	949 ± 75	1114 ± 187	961 ± 90	1207 ± 235
Dinner	1098 ± 81	1271 ± 199	1104 ± 83	1287 ± 164
T_{max}, [a] min				
Breakfast	38 ± 18	64 ± 32	47 ± 19	64 ± 32
Lunch	69 ± 84	93 ± 41	55 ± 49	102 ± 40
Dinner	59 ± 14	87 ± 22	59 ± 14	84 ± 24
AUC_{neg} [a]				
Breakfast	974 ± 2494	392 ± 687	36 ± 113	513 ± 1082
Lunch	709 ± 1912	213 ± 674	144 ± 455	48 ± 152
Dinner	0 ± 0	117 ± 370	36 ± 113	0 ± 0

[1] All values are means ± SDs. $n = 10$/group. Data were analyzed with the use of a 3-factor [group (LP compared with HP) × treatment (placebo compared with Leu) × meal (breakfast compared with lunch compared with dinner)] mixed-model ANOVA. Participants were randomly assigned to the LP or HP and remained in their allocated groups through both placebo and Leu treatments. Plasma amino acids concentrations were measured immediately before and 20, 40, 60, 90, 120, 180, and 240 min after all meals and 300 min after lunch. *Main effect for group, [†]main effect of treatment, [#]treatment × group interaction; $P < 0.05$, [a]no formal statistical tests performed. AUC_{neg}, AUC below baseline; AUC_{pos}, AUC above baseline; C_{max}, maximum concentration; EAA, essential amino acid; HP, higher-protein group; LP, lower-protein group; T_{max}, time of maximum concentration.

function in older adults (41). Our observation that leucine coingestion with meals further enhanced the stimulatory effect of REX on MyoPS indicated that the combination of leucine supplementation and REX may be a particularly effective strategy for the preservation of muscle mass in older adults. In support of this thesis, Trabal et al. (9) recently reported a trend for greater improvements in maximal voluntary contraction and certain markers of functional performance after 12-wk of resistance training in older adults who consumed 5 g leucine (compared with a placebo) 1 h after lunch and dinner. Nevertheless, attrition was high in the latter study, and additional studies should explore the potential for leucine supplementation to potentiate resistance training–induced improvements in strength and function in older adults.

An unexpected observation of the current study was the lack of a difference between LP and HP groups with respect to MyoPS in both the rested and REX conditions. Accumulating evidence supports that, although the current RDA ($0.8\ g \cdot kg^{-1} \cdot d^{-1}$) may be sufficient to maintain nitrogen balance in healthy people >65 y old (42), the protein requirement to support muscle mass and optimal physiologic function is likely higher (37, 38, 43–45). However, we did not observe a greater rate of MyoPS in the group who consumed 1.2 g protein $\cdot kg^{-1} \cdot d^{-1}$ than in the group who consumed 0.8 g protein $\cdot kg^{-1} \cdot d^{-1}$, but both groups saw a rise in MyoPS with Leu treatment, which suggested that protein intakes even $>1.2\ g \cdot kg^{-1} \cdot d^{-1}$ may be required to enhance MyoPS in healthy older adults. In support of this hypothesis, Kim et al. (46) reported a greater 24-h mixed MPS in older adults who consumed $1.5\ g \cdot kg^{-1} \cdot d^{-1}$ than in those who consumed $0.8\ g \cdot kg^{-1} \cdot d^{-1}$.

That we observed an increase in MyoPS when leucine was coingested with meals in the group who consumed $1.2\ g \cdot kg^{-1} \cdot d^{-1}$ further suggests that consumption of daily protein (or, more specifically, leucine) greater than the amount specified in recent

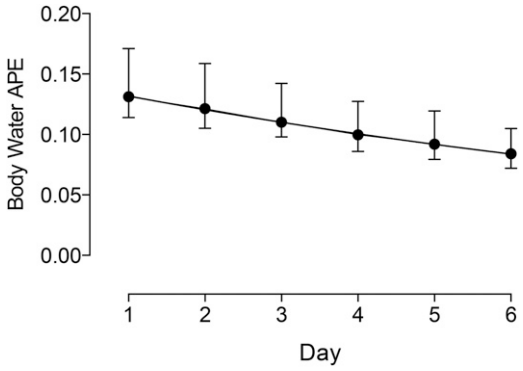

FIGURE 6 Mean ± range exponential time course of body water enrichment over 6 d after consumption of an oral bolus of 100 mL ^2H_2O (70 atom%) in all participants combined. n = 20. APE, atom percent excess.

recommendations (12, 13) is required to enhance the MyoPS response and would, perhaps, confer a greater benefit in terms of muscle-mass retention in older adults. Nevertheless, note that other factors, in addition to total daily protein intake per se, such as protein quality and timing, may influence MyoPS and muscle mass at a given protein intake (47). For example, the protein in the diets provided in the current study came from a variety of plant- and animal-based sources and, therefore, the diets were of mixed protein quality. The estimated leucine content of the LP and HP diets were 5.4 and 8.1 g · kg^{-1} · d^{-1}, respectively (Table 4). Therefore, it is conceivable that, at the same level of total protein intake, the replacement of lower-quality, lower-leucine protein sources with leucine-rich sources could enhance MyoPS [i.e., replacing wheat protein (6.8% leucine) and potato protein (5.2% leucine) with skimmed milk protein (10.9% leucine) and beef protein (8.8% leucine) (48)]. Another potentially important factor may be the distribution of protein throughout the day (47). The diets in the current study provided protein (and leucine) in a skewed pattern across the daily meals to emulate the manner in which protein is typically consumed in older adults (22). This meant that, even in the HP group, per-meal protein intakes were below the dose of 0.4 g/kg body mass that has been reported to maximally stimulate MyoPS in older adults at every meal except for dinner (4). As such, it is possible that a more even distribution of daily protein (and leucine) across the meals in the HP group (which would have resulted in the consumption of ∼0.4 g protein/kg body mass and ∼2.7 g leucine/meal) or the replacement of lower-leucine foods with leucine-rich sources could have improved the integrated MyoPS response with the result that leucine supplementation may have had no effect on MyoPS or that MyoPS would have been higher in the HP group than in the LP group. However, such a thesis requires further examination in future studies.

In the current study, the order of the supplementation periods (i.e., placebo followed by Leu) was not randomized, and the placebo was always given before Leu. This decision was made to avoid a potential carryover effect of previous leucine supplementation on the MyoPS response (25). Although no evidence exists, to our knowledge, to suggest that the order in which MyoPS measurements were conducted would have affected the response, we could not rule this possibility out as a potential confounding factor. In addition, although habitual daily step counts were successfully maintained throughout the study in each individual participant, there was an ∼30% difference (albeit nonsignificant) in step counts between the groups. Nonetheless, the clinical importance of this difference was likely minimal because, to the best of our knowledge, there is no evidence that higher daily steps can improve MyoPS at the moderately high levels of physical activity that were present in both groups in the current study (i.e., ∼9000–12,000 steps/d). Indeed, if such an improvement occurred, we would have expected higher MyoPS in the HP group than in the LP group, which we did not find.

In conclusion, our results show that the co-ingestion of 5 g leucine with daily meals enhances integrative MyoPS over a 3-d period in free-living older men and is equally effective for men who consumed daily protein intakes greater than (1.2 g · kg^{-1} · d^{-1}) and at (0.8 g · kg^{-1} · d^{-1}) the protein RDA. Furthermore, we show that leucine co-ingestion further enhances the anabolic effect of REX. Although further work is required to determine whether the increase in MyoPS is maintained with prolonged leucine supplementation and translates into a long-term functional response, we propose that leucine co-ingestion has the potential to be a simple dietary strategy to mitigate muscle-mass loss in older adults. Our data suggest that this treatment could be an effective strategy in elderly individuals in whom protein intake is habitually low or restricted because of comorbid conditions, as well as

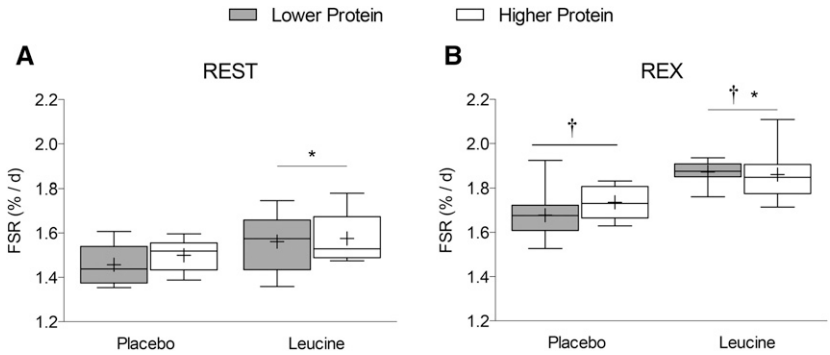

FIGURE 7 The integrated myofibrillar FSR (percentage per day) in the REST leg (A) and REX leg (B) in older men who consumed lower protein (0.8 g protein · kg^{-1} · d^{-1}) or higher protein (1.2 g protein · kg^{-1} · d^{-1}) who underwent 3 d of placebo supplementation and 3 d of Leu supplementation with meals. Boxes represent 25th to 75th percentiles. Horizontal lines and crosses within boxes represent medians and means, respectively. Whiskers represent minimums and maximums. n = 10/group. Data were analyzed with the use of a 3-factor [group (lower protein compared with higher protein) × treatment (placebo compared with Leu) × condition (rested compared with REX)] mixed-model ANOVA with Tukey's post hoc test after a treatment × condition interaction (P = 0.016). *Different from placebo treatment, $P < 0.001$. †Different from rested in same treatment, $P < 0.001$. FSR, fractional synthetic rate; REST, rested condition; REX, resistance exercise condition.

in those who consume apparently adequate protein intakes, particularly when combined with REX training.

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The authors' responsibilities were as follows—CHM and SMP: conceived and designed the research, analyzed the data, interpreted the results, and wrote the manuscript; and all authors: collected the data, revised the manuscript, and read and approved the final version of the manuscript. None of the authors reported a conflict of interest related to the study.

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