

# A randomized controlled trial of the impact of protein supplementation on leg lean mass and integrated muscle protein synthesis during inactivity and energy restriction in older persons

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## ABSTRACT

**Background:** In older persons, muscle loss is accelerated during physical inactivity and hypoenergetic states, both of which are features of hospitalization. Protein supplementation may represent a strategy to offset the loss of muscle during inactivity, and enhance recovery on resumption of activity.

**Objective:** We aimed to determine if protein supplementation, with proteins of substantially different quality, would alleviate the loss of lean mass by augmenting muscle protein synthesis (MPS) while inactive during a hypoenergetic state.

**Design:** Participants (16 men, mean  $\pm$  SD age:  $69 \pm 3$  y; 15 women, mean  $\pm$  SD age:  $68 \pm 4$  y) consumed a diet containing  $1.6 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , with  $55\% \pm 9\%$  of protein from foods and  $45\% \pm 9\%$  from supplements, namely, whey protein (WP) or collagen peptides (CP): 30 g each, consumed 2 times/d. Participants were in energy balance (EB) for 1 wk, then began a period of energy restriction (ER;  $-500 \text{ kcal/d}$ ) for 1 wk, followed by ER with step reduction (ER + SR;  $<750 \text{ steps/d}$ ) for 2 wk, before a return to habitual activity in recovery (RC) for 1 wk.

**Results:** There were significant reductions in leg lean mass (LLM) from EB to ER, and from ER to ER + SR in both groups ( $P < 0.001$ ) with no differences between WP and CP or when comparing the change from phase to phase. During RC, LLM increased from ER + SR, but in the WP group only. Rates of integrated muscle protein synthesis decreased during ER and ER + SR in both groups ( $P < 0.01$ ), but increased during RC only in the WP group ( $P = 0.05$ ).

**Conclusions:** Protein supplementation did not confer a benefit in protecting LLM, but only supplemental WP augmented LLM and muscle protein synthesis during recovery from inactivity and a hypoenergetic state. This trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT03285737. *Am J Clin Nutr* 2018;108:1060–1068.

**Keywords:** muscle protein synthesis, older adults, whey protein, collagen peptides, step reduction

## INTRODUCTION

Periods of inactivity and muscle disuse, such as during bed rest and hospitalization or protracted illness, are more common in older persons (1). The decline in muscle mass and function during hospitalization can transiently accelerate sarcopenic decline, resulting in incomplete recovery, particularly for older persons (2). We have shown that periods in which fewer steps are taken, as a model of inactivity but not outright muscle disuse, result in reductions in anabolic sensitivity to protein (3, 4) and declines in leg lean mass (3). Such periods of inactivity are, we suggest, more common than bed rest and complete disuse, and may be deleterious in older persons particularly if frequent and incomplete recovery occurs.

In addition to reduced ambulation, hospitalization or illness can be accompanied by a decrease in appetite and food intake, which can lead to an energy deficit and muscle loss (5). Typically,  $\sim 25\%$  of body mass lost in an energy deficit can be attributed to fat-free mass (6), some of which is likely muscle (7). Hospitalization is also associated with energy and protein underfeeding that may further exacerbate muscle catabolism (8).

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Abbreviations used: APE, atomic percentage excess; CP, collagen peptide; CRP, C-reactive protein; CSA, cross-sectional area; DXA, dual-energy X-ray absorptiometry; EAA, essential amino acid; EB, energy balance phase; ER, energy-restricted phase; ER + SR, energy-restricted and step-reduction phase; LBM, lean body mass; LLM, leg lean mass; MPS, muscle protein synthesis; PASE, physical activity scale for the elderly; RC, recovery phase; RDA, Recommended Dietary Allowance; SR, step reduction; WP, whey protein.

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An increase in dietary protein intake may alleviate inactivity-induced muscle loss (9, 10). Whey protein has a high essential amino acid (EAA) content, particularly leucine, and its ingestion stimulates muscle protein synthesis (MPS) (11). Supplementing the diet with protein sources rich in EAA and leucine is known to enhance rates of MPS (12, 13), and may serve to offset losses in muscle mass and strength during periods of physical inactivity (14). Although studies have examined the influence of increased protein intake on muscle atrophy during immobilization (15), no study has examined the efficacy of increased protein consumption to offset loss of muscle mass during inactivity while hypoenergetic and to promote recovery in older adults.

We investigated whether providing healthy older adults with twice the Recommended Dietary Allowance (RDA) of protein ( $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) would attenuate the inactivity-induced loss of leg lean mass (LLM) and integrated rates of MPS while energy restricted. We also examined whether supplementation with proteins of different quality would affect muscle outcomes. Supplements were high-quality whey protein (WP) or lower-quality collagen peptides (CP). We selected collagen as a comparator as it provides an isonitrogenous and isoenergetic comparison (as opposed to carbohydrate, which is often used) (16), and as it has shown considerable and impressive anabolic properties in older adults (17) [which have been questioned (18)]. To our knowledge, no other study had compared WP and CP for their effect on MPS in older adults. We hypothesized that energy restriction and step reduction would result in reductions in LLM and MPS as primary outcomes. Further, we hypothesized that WP, but not CP, would mitigate declines in LLM and maintenance of MPS. As secondary outcomes, we believed that ER + SR would induce an increase in levels of systemic inflammation independent of supplement type. We also hypothesized that ER + SR would result in impaired glucose handling congruent with previous findings from our laboratory (4).

## METHODS

### Ethical approval

The 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](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. The trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT03285737.

### Participants

Thirty-two older adults were recruited from the greater Hamilton area, in response to local advertisements, to participate in this study. Potential participants were screened first by telephone to ensure they were nonsmokers, nondiabetic, and between the ages of 65 and 80 y. Exclusion criteria included significant loss or gain of body mass in the past 6 mo ( $>2 \text{ kg}$ ); regular use of: nonsteroidal anti-inflammatory drugs (with the exception of daily low-dose aspirin); use of simvastatin or atorvastatin; use of anticoagulants; the use of a walker, cane,

or assistive walking device; current or recently remised cancer; infectious disease; or gastrointestinal disease. **Figure 1** shows the Consolidated Standards of Reporting Trials (CONSORT) diagram for subject flow through the protocol.

### Study overview

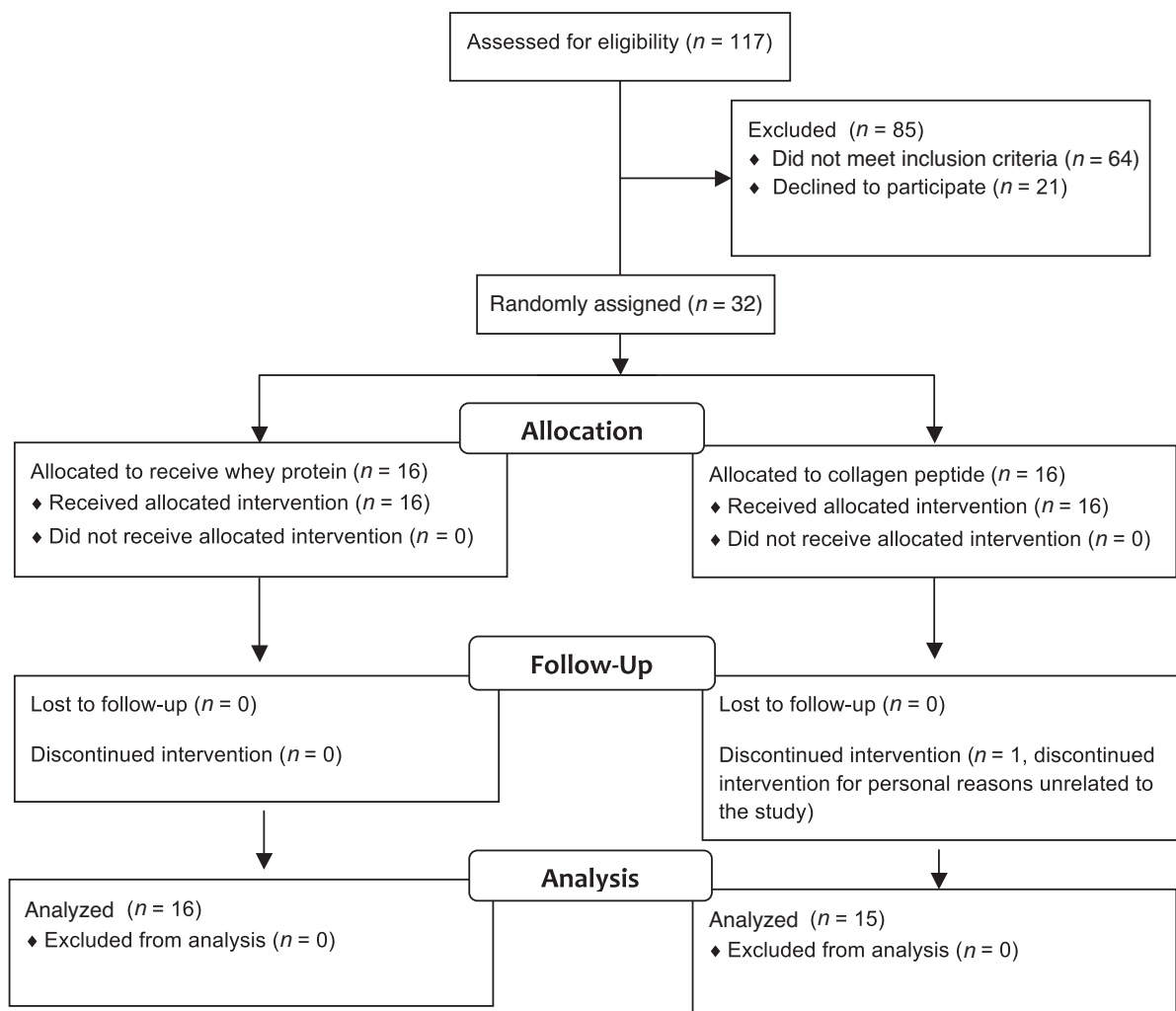
An overview of the study is shown in **Figure 2**. The study was a double-blind, parallel-group, randomized controlled trial. Eligible participants were allocated to consume 1 of 2 types of protein supplement: 30 g 2 times/d of WP or CP. Allocation was concealed from the participants and researchers for the duration of the study and until all analyses were complete. After baseline testing and familiarization with all study measures, participants commenced the 5-wk-long protocol during which they consumed a controlled diet provided by the study investigators. The protocol was divided into 4 distinct phases. The first phase was a week-long run-in phase in which subjects were in energy balance (EB) with protein intake equal to the RDA ( $0.8 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ). Subjects were then placed in an energy restriction phase (ER) for 1 wk where they consumed an energy-restricted diet ( $-500 \text{ kcal/d}$ ) and protein intake was increased to twice the RDA ( $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) by consumption of a twice-daily supplement (30 g/dose) of either WP or CP. Inactivity, as step reduction (SR), was superimposed on ER (ER + SR) for 2 wk. During the ER + SR phase, participants were instructed to reduce their daily step count to  $\leq 750$  steps/d, which is a daily step count similar to what is observed in older hospitalized patients (19). Participants monitored their daily step counts with the use of a waist-mounted pedometer (PiezoX, Deep River, ON, Canada) and recorded their steps at the end of each day on a log sheet that was checked at each visit. Energy intake during the ER + SR phase was adjusted to account for subjects' inactivity (20). Finally, during recovery (RC, 1 wk), participants returned to their habitual levels of activity (matching their average daily step count seen in EB and ER). During RC, participants maintained their high protein intake ( $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) while consuming the same supplements and an energy intake matching their activity levels during EB. Before and after each dietary phase, participants had blood collected for fasting serum and plasma. Before and at the end of each phase participants underwent a dual-energy X-ray absorptiometry (DXA) scan (GE-Lunar iDXA; Aymes Medical, Newmarket, ON, Canada).

### Baseline testing

Before study commencement, participants were asked to complete a physical activity and weighed food record (Nutribase version 11.5; Cybersoft Inc., Phoenix, AZ) for 3 d (2 weekdays and 1 weekend day) to assess habitual physical activity levels and dietary intakes.

### Diets

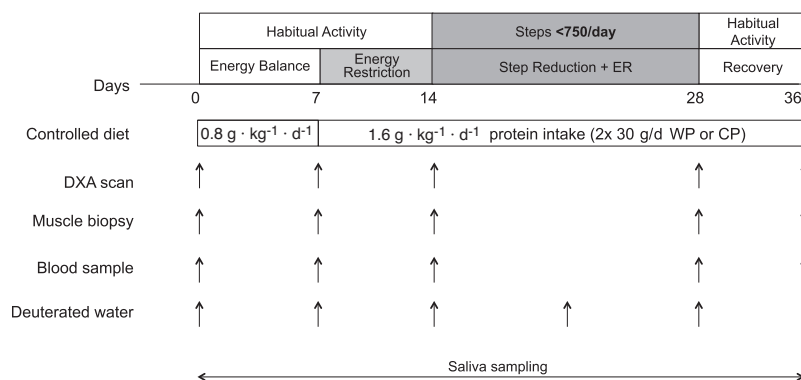
Each participants' energy requirement was determined with the use of the Oxford prediction equations for basal metabolic rate (21) using height and body mass for men and women aged  $>60 \text{ y}$  (20). Activity factors were determined for each participant on the basis of their baseline physical activity records, daily step



**FIGURE 1** CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

counts, and Physical Activity Scale for the Elderly questionnaire (PASE) (22) for energy intake during the EB, ER, and RC phases. During the ER + SR phase, a reduced activity factor (of 1.3) was applied to the basal metabolic rate in order to match caloric intake to activity level. During the ER and ER + SR phases, a reduction in total energy intake of 500 kcal/d below the estimated

energy requirement was applied to the diet to simulate a moderate energy restriction that is common during hospitalization (23). During the EB phase of the study, participants were provided with a protein intake of  $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , which reflects the current RDA for protein in adults  $\geq 19 \text{ y}$  (24). For the ER, ER + SR, and RC phases, participants were provided with a



**FIGURE 2** Schematic of study design. CP, collagen peptide supplement; DXA, dual-energy X-ray absorptiometry; WP, whey protein supplement.

protein intake of  $1.6 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , in line with recommendations from a number of expert committees for optimal protein intake for older adults who are hospitalized (25). Increasing protein intake during the ER, ER + SR, and RC phases of the study was achieved by reducing the proportion of food energy provided from carbohydrates, whereas the proportion of energy from fat was maintained at  $\sim 25\%$  of total energy across all phases. Dietary protein was derived via a combination of plant- and animal-based protein sources throughout the 5-wk trial. During phases ER, ER + SR, and RC, participants were provided with their prepackaged protein supplements (either WP or CP) to be consumed 2 times/d, once in the morning prior to breakfast and once in the evening  $\sim 1\text{--}2$  h before sleep.

Participants were prescribed a customized meal plan according to food preferences and food was supplied at the beginning of each week. Food consisted of prepackaged frozen meals (Heart to Home Frozen Meals, Brampton, ON) and items that required minimal preparation. Participants were provided with a dietary log where they were to indicate the percentage of the provided food consumed during the day and were strongly encouraged to consume only the study diet. If food outside of the provided diet was consumed, additions were recorded in the dietary log. Overall, compliance with the prescribed diets and supplements was excellent with subjects consuming  $98\% \pm 2\%$  of what was provided.

### Supplementation

Supplements contained WP isolate (Whey Protein Isolate 895, Nealanderes International Inc., Mississauga, ON, Canada), or hydrolyzed collagen peptide (Gelita, Eberbach, Germany). Individual servings were identically flavored and packaged by Infinit Nutrition (Windsor, ON, Canada) in powdered form. Participants were instructed to mix each package with 300 mL of water before ingestion, and were asked to consume the beverage within a 5-min period. Supplements were isonitrogenous and energy-matched; their contents appear in **Table 1**.

### Isotope protocol

Oral consumption of  $^2\text{H}_2\text{O}$  (70 at.%; 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 the collection of a saliva sample (26) and a muscle biopsy from the vastus lateralis, participants consumed a single 100-mL oral bolus of  $^2\text{H}_2\text{O}$ . This process was repeated at the beginning of each dietary phase of the study. An additional 100 mL dose of  $^2\text{H}_2\text{O}$  was provided to participants at the beginning of the second week of ER + SR. Total body water enrichment of  $^2\text{H}$  was used as a surrogate of the precursor for plasma alanine labeling, which remains in a constant ratio of  $\sim 3.7$  with water. This has been confirmed in our laboratory (data not shown) and by others (26–28), and was determined from saliva swabs that were collected by participants between  $\sim 0700$  and  $0900$  each morning.

All muscle biopsies were obtained with the use of a 5-mm Bergström needle that was adapted for manual suction under 1% xylocaine local anesthesia. Muscle tissue samples were freed from any visible connective and adipose tissue, rapidly frozen in

**TABLE 1**

Amino acid composition of protein supplements<sup>1</sup>

	WP supplement		CP supplement	
	g/100 g	g/30 g	g/100 g	g/30 g
Alanine	5.7	1.7	8.6	2.6
Arginine	3.0	0.9	7.3	2.2
Aspartic acid	12.5	3.8	5.8	1.7
Cystine	4.0	1.2	0	0
Glutamic acid	17.6	5.3	10.2	3
Glycine	1.8	0.5	22.2	6.7
Histidine	2.0	0.6	1.0	0.3
Proline	4.5	1.4	12.7	3.8
Serine	4.5	1.4	3.2	1.0
Tyrosine	4.2	1.3	0.8	0.2
Tryptophan	2.4	0.7	0	0
Isoleucine	6.3	1.9	1.4	0.4
Leucine	14.3	4.3	2.7	0.8
Lysine	11.2	3.4	3.6	1.1
Methionine	2.4	0.7	0.9	0.3
Phenylalanine	3.8	1.1	2.1	0.6
Threonine	5.3	1.6	1.8	0.5
Valine	5.6	1.7	2.4	0.7
$\Sigma$ EAAAs	51.3	15.4	14.9	4.5
$\Sigma$ NEAAAs	59.8	17.9	71.8	21.5

<sup>1</sup>CP, collagen peptide; EAA, essential amino acid; NEAA, nonessential amino acid; WP, whey protein.

liquid nitrogen for measurement of MPS, and mounted in optimal cutting temperature medium for immunohistochemistry; samples were then stored at  $-80^\circ\text{C}$  for further analysis.

### Analytic methods

Myofibrillar proteins were isolated from the muscle biopsies as previously described (29). The incorporation of deuterium into protein-bound alanine was determined and rates of protein synthesis were calculated as detailed previously (30).

Saliva samples were analyzed for  $^2\text{H}$  enrichment by cavity ring-down spectroscopy with the use of a liquid isotope analyzer (Picarro L2130-i analyzer, Picarro, Santa Clara, CA) with an automated injection system. The water phase of saliva was injected 6 times and the average of the last 3 measurements was used for data analysis (coefficient of variation  $\leq 0.5\%$ ). Standards were measured before and after each participant run. The  $^2\text{H}$  isotopic enrichments for muscle and saliva initially expressed as  $\delta^2\text{H} \text{‰}$  were converted to atomic percentage excess (APE) using standard equations (27).

### Body composition

Body composition was assessed following an overnight fast ( $\sim 12$  h). DXA measurements were conducted using a GE Lunar iDXA total body scanner (GE Medical Systems Lunar, Madison, WI) and analyzed (Lunar enCORE version 14.1, GE Medical Systems) in medium-scan mode. The machine was calibrated each testing day with a 3-compartment Universal Whole Body DXA Phantom (Oscar, Jr; Orthometrix, Naples, FL). The analysis regions were standard regions where the head, torso, arms, and legs were subdivided by the software, but were subsequently



checked manually, in a blinded manner, by a single investigator (SYO).

### Blood metabolites and hormones

Serum glucose concentrations were measured with the use of the glucose oxidase method (YSI 2300; YSI Life Sciences, Yellow Springs, OH). Plasma insulin concentrations were measured with the use of the dual-site chemiluminescent method (Immulite 2000 immuno-assay system; Siemens, Germany). High-sensitivity C-reactive protein (CRP) levels were measured with an Express Plus autoanalyzer (Chiron Diagnostics Co, Walpole, MA) and using a commercially available high-sensitivity CRP-Latex kit (Pulse Scientific, Burlington, ON). IL-6 and TNF- $\alpha$  levels were measured with a Bio-Plex reagent kit on a Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA). Intra-assay coefficients of variation were all <5% for all blood analyses.

### Calculations

The fractional synthetic rate of myofibrillar protein was determined from the incorporation of deuterium-labeled alanine into protein with the use of enrichment of body water, corrected for the mean number of deuterium moieties incorporated per alanine (27), as the surrogate precursor labeling between subsequent biopsies. In brief, the following standard equation was used:  $FSR(\%/d) = [(APE_{Ala})]/[(APE_p) \times t] \times 100$  where FSR is the fractional synthetic rate,  $APE_{Ala}$  is the deuterium enrichment of protein-bound alanine,  $APE_p$  is the mean precursor enrichment over the time period, and  $t$  is the time between biopsies.

The HOMA-IR was calculated with fasted glucose and insulin levels using the standard equation  $[(\text{glucose} \times \text{insulin})/22.5]$  (31).

### Histologic staining

Muscle cross-sections, 7- $\mu$ m thick, were prepared from optimal cutting temperature medium-embedded samples and allowed to air-dry for ~30 min before being stored at -80°C. Tissue sections were thawed and fixed as previously described (32). Primary antibodies used were A4.951 (MHCI; slow isoform; neat; DSHB); myosin heavy-chain type II (MHCII; fast isoform; 1:1000; ab91506; Abcam, Cambridge, MA); laminin (1:500; ab11575; Abcam). Secondary antibodies used were MHCI (Alexa Fluor 488 anti-mouse, 1:500); MHCII (Alexa Fluor 647 anti-rabbit, 1:500), and laminin (Alexa Fluor anti-rabbit 488, 1:500). Slides were refixed with 4% PFA in between the MHCII and laminin staining steps to limit cross-reactivity. Nuclei were labeled with 4',6-diamidino-2-phenylindole (DAPI, 1:20,000; Sigma-Aldrich) before slides were coverslipped with fluorescent mounting media (DAKO, Burlington, ON, Canada). Images were observed with a Nikon Eclipse 90i microscope and captured with a Photometrics Cool SNAP HQ2 fluorescent camera (Nikon Instrument, Melville, NY). Analysis was completed per our previous work (32–35), fiber typing was conducted using  $\geq 300$  fibers, and cross-sectional area (CSA) was based on  $\geq 50$  fibers/fiber type. Muscle fibers on the periphery of muscle cross-sections were not used in the analysis.

**TABLE 2**  
Participants' characteristics<sup>1</sup>

	WP supplement (n = 16, 8F)	CP supplement (n = 15, 7F)
Age, y	69 $\pm$ 4	68 $\pm$ 2
Height, m	1.71 $\pm$ 0.09	1.70 $\pm$ 0.10
Body mass, kg	92.4 $\pm$ 14.2	82.0 $\pm$ 17.9
BMI, kg/m <sup>2</sup>	31.2 $\pm$ 5.2	28.0 $\pm$ 4.8
Body fat, %	41.1 $\pm$ 8.6	37.2 $\pm$ 8.6
LBM, kg	52.3 $\pm$ 9.7	49.3 $\pm$ 11.0
Steps/d	6237 $\pm$ 2890	8392 $\pm$ 4290
Knee extensor MVC, Nm	143 $\pm$ 62	146 $\pm$ 43
Chair stands, stands/30 s	15 $\pm$ 3	16 $\pm$ 3
TUG, s	7.8 $\pm$ 2.0	7.1 $\pm$ 1.2
6MWT distance, m	542 $\pm$ 99	562 $\pm$ 67
Gait speed, m/s	1.5 $\pm$ 0.3	1.6 $\pm$ 0.2

<sup>1</sup> Values are means  $\pm$  SDs. CP, collagen peptide; LBM, lean body mass; MVC, maximum voluntary contraction; TUG, timed up and go test; WP, whey protein; 6MWT, 6-min walk test.

### Statistics

Data were compared using a 2-way mixed-model ANOVA with between (group) and within (time, EB, ER, ER + SR, and RC) factors. The ANOVA revealed no interaction between group and sex, and thus groups were collapsed across sex for all measures. All significant interaction terms for the ANOVA were further tested with the use of Tukey's post hoc test. Significance was set at  $P < 0.05$ . All statistical analyses were completed using SPSS (IBM SPSS Statistics for Mac, version 21; IBM Corp., Armonk, NY). Data in tables are presented as means  $\pm$  SDs. Graphical representation of data is in box and whisker plots with the box representing the IQR, the line indicating the median and the cross indicating the mean, and the whiskers indicate the maximum and minimum values.

## RESULTS

### Subjects' characteristics

Subjects' characteristics are presented in **Table 2**. There were no significant differences between groups for any variable. The baseline step counts of participants were  $6237 \pm 2890$  and  $8392 \pm 4290$  in the WP and CP groups, respectively and 2 wk of ER + SR resulted in a decrease in average daily steps by ~84% and ~90% ( $P < 0.001$ ). Subjects returned to taking similar steps during RC ( $6336 \pm 2348$  and  $8473 \pm 3586$ ) in both groups, showing no difference during RC compared with EB ( $P > 0.05$ ).

### Diet

There were no significant differences in any dietary variable between groups at any time points ( $P > 0.05$ ) (**Table 3**). All required supplements were consumed by each participant and recorded in a dietary log. Supplemental protein accounted for  $45\% \pm 9\%$  of total protein intake in ER, ER + SR, and RC with the remaining  $55\% \pm 9\%$  derived from food sources.

**TABLE 3**

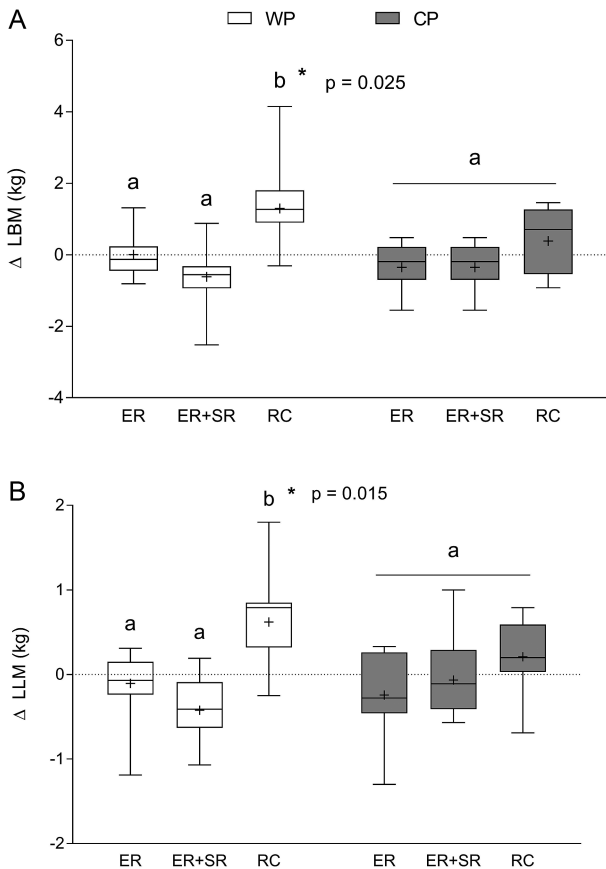
Nutrient intake during each dietary phase of the study<sup>1</sup>

	WP supplement				CP supplement			
	EB	ER	ER + SR	RC	EB	ER	ER + SR	RC
Intake, kcal	2535 ± 305	1986 ± 353	1406 ± 244	2337 ± 350	2442 ± 431	1989 ± 466	1394 ± 343	2193 ± 457
Intake, kcal/kg	30 ± 4	22 ± 4	16 ± 3	27 ± 4	29 ± 5	21 ± 5	15 ± 4	26 ± 5
Protein, g/kg	0.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Protein, g	75 ± 12	87 ± 20	86 ± 21	87 ± 20	67 ± 15	71 ± 29	71 ± 29	71 ± 29

<sup>1</sup>There were no significant differences in protein or calorie intake between WP and CP at any phase. Values are means ± SDs. CP, collagen peptide; EB, energy balance phase; ER, energy-restricted high-protein diet phase; ER + SR, energy-restricted high-protein diet and step-reduction phase; RC, habitual activity and caloric consumption, combined with high-protein intake recovery phase; WP, whey protein.

**Body composition**

Total lean body mass (LBM) was significantly reduced in ER + SR in comparison to EB ( $P < 0.001$ ). In RC, there was an increase in LBM, but only in WP (Figure 3A). Losses in LLM mimicked losses in LBM with a significant reduction at ER + SR



**FIGURE 3** Changes in (A) LBM between sequential study phases, and (B) LLM between sequential study phases. The box plot shows the median (line) and mean (+), with the box representing the IQR, and the whiskers representing the maximum and minimum values. Data were analyzed with 2-factor ANOVA with group as a between factor, and repeated measures for phase. WP ( $n = 16$ ), CP ( $n = 15$ ). Means that do not share a letter are significantly different within the group,  $P < 0.05$ . \*Differences between groups at that time point,  $P < 0.05$ .  $P$  value indicates the interaction of WP and CP at RC. CP, collagen peptide; ER, energy-restricted phase; ER + SR, energy-restricted and step-reduction phase; LBM, lean body mass; LLM, leg lean mass; RC, recovery phase; WP, whey protein.

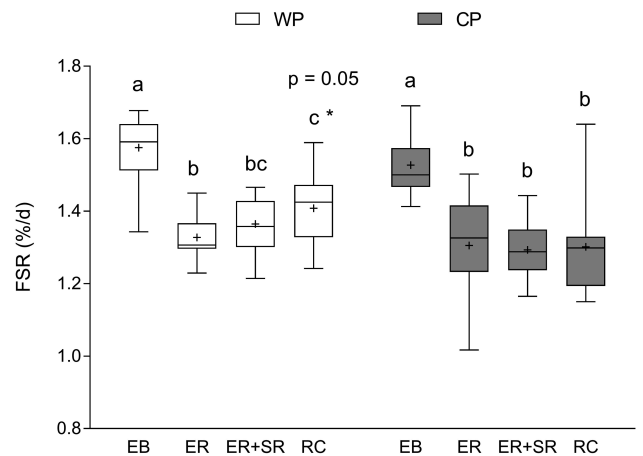
in comparison to ER that was increased at RC compared with other times in WP, but not in CP (Figure 3B).

**Myofibrillar protein synthesis**

There were no significant differences between basal rates of myofibrillar MPS between groups during EB ( $P > 0.05$ ). ER resulted in a significant reduction in fractional synthetic rate in both groups ( $P < 0.001$ ). MPS was significantly elevated from ER at RC in the WP group and in comparison to the CP group ( $P = 0.05$ ). Rates of MPS remained suppressed at ER + SR ( $P < 0.001$ ), and RC ( $P < 0.001$ ) from EB in the CP group (Figure 4).

**Glycemic control and inflammation**

There was a significant increase in fasted blood glucose in ER + SR compared with EB and ER that remained elevated at RC ( $P < 0.001$ ) (Table 4). There were no significant differences



**FIGURE 4** Rates of integrated myofibrillar muscle protein synthesis (%/d) during EB, ER, ER + SR, and RC. The box plot shows the median (line) and mean (+), with the box representing the IQR and the whiskers representing the maximum and minimum values. Data were analyzed with 2-factor ANOVA with group as a between factor and repeated measures for phase. WP ( $n = 16$ ), CP ( $n = 15$ ). Means that do not share a letter are significantly different within the group,  $P < 0.05$ . \*Differences between groups at that time point,  $P < 0.05$ .  $P$  value indicates the interaction of WP and CP at RC. CP, collagen peptide; EB, energy balance; ER, energy restriction; ER + SR, energy restriction and step reduction; RC, recovery; WP, whey protein.

**TABLE 4**Fasted glucose, insulin, and levels of systemic inflammation during each dietary phase of the study<sup>1</sup>

	WP supplement				CP supplement			
	EB	ER	ER + SR	RC	EB	ER	ER + SR	RC
Glucose, mM	4.9 ± 0.5 <sup>a</sup>	5.0 ± 0.6 <sup>a</sup>	6.2 ± 1.2 <sup>b</sup>	5.6 ± 0.9 <sup>b</sup>	4.6 ± 0.6 <sup>a</sup>	5.1 ± 0.6 <sup>a</sup>	6.2 ± 0.9 <sup>b</sup>	5.7 ± 0.8 <sup>b</sup>
Insulin, uIU/mL	9.9 ± 2.3 <sup>a</sup>	6.9 ± 1.5 <sup>b</sup>	9.2 ± 3.6 <sup>a,c</sup>	8.0 ± 2.3 <sup>b,c</sup>	10.0 ± 1.8 <sup>a</sup>	6.5 ± 1.7 <sup>b</sup>	7.9 ± 2.8 <sup>a,c</sup>	8.0 ± 2.6 <sup>b,c</sup>
HOMA-IR	2.2 ± 0.5 <sup>a</sup>	1.5 ± 0.4 <sup>b</sup>	2.5 ± 1.1 <sup>a</sup>	2.0 ± 0.7 <sup>a</sup>	2.1 ± 0.5 <sup>a</sup>	1.6 ± 0.4 <sup>b</sup>	2.2 ± 0.9 <sup>a</sup>	2.1 ± 0.8 <sup>a</sup>
TNF- $\alpha$ , pg/mL	15.1 ± 3.9 <sup>a</sup>	16.8 ± 2.9 <sup>a</sup>	23.5 ± 5.9 <sup>b</sup>	16.17 ± 3.8 <sup>a</sup>	16.3 ± 3.6 <sup>a</sup>	16.9 ± 3.6 <sup>a</sup>	22.8 ± 6.1 <sup>b</sup>	17.3 ± 4.8 <sup>a</sup>
IL-6, pg/mL	7.4 ± 1.6 <sup>a</sup>	6.1 ± 1.7 <sup>b</sup>	12.2 ± 4.8 <sup>c</sup>	8.2 ± 3.3 <sup>a</sup>	7.4 ± 1.7 <sup>a</sup>	6.9 ± 1.2 <sup>b</sup>	11.9 ± 5.7 <sup>c</sup>	8.7 ± 3.4 <sup>a</sup>
CRP, mg/L	9.0 ± 3.2 <sup>a</sup>	10.2 ± 2.9 <sup>a,b</sup>	12.7 ± 4.9 <sup>b,c</sup>	13.9 ± 4.6 <sup>c</sup>	9.1 ± 2.4 <sup>a</sup>	11.3 ± 2.5 <sup>a,b</sup>	12.4 ± 4.4 <sup>b,c</sup>	11.4 ± 4.3 <sup>c</sup>

<sup>1</sup>Data were analyzed with 2-factor ANOVA with repeated measures on time. There were no significant differences between the WP and CP groups at any time point. Values are mean ± SD. Means that do not share a letter are significantly different within the group,  $P < 0.05$ . CP, collagen peptide; CRP, c-reactive protein; EB, energy balance phase; ER, energy-restricted high-protein diet phase; ER + SR, energy-restricted high-protein diet and step-reduction phase; RC, habitual activity and caloric consumption, combined with high protein intake recovery phase; WP, whey protein.

between groups for any measures of insulin sensitivity or inflammation ( $P > 0.05$ ).

Plasma insulin concentration decreased significantly from EB at ER ( $P < 0.001$ ) but returned to levels similar to EB at ER + SR ( $P = 0.03$ ) before decreasing again at RC ( $P = 0.001$ ).

The calculation of HOMA-IR demonstrated a similar trend to fasted insulin levels where HOMA-IR was significantly decreased from EB at ER ( $P < 0.001$ ), but then was significantly elevated from ER at ER + SR, similar to EB ( $P = 0.02$ ) and then remained at levels no different to those of EB and ER + SR at RC ( $P > 0.05$ ).

Concentrations of TNF- $\alpha$  at ER + SR were significantly elevated from all other phases. Levels of IL-6 were significantly decreased at ER from EB ( $P = 0.049$ ) and elevated from all other time points at ER + SR (EB and ER  $P < 0.0001$ , ER + SR  $P = 0.018$ ). Levels of CRP were significantly elevated at ER + SR from EB ( $P = 0.034$ ), and remained elevated at RC from EB ( $P = 0.003$ ) and ER ( $P = 0.029$ ).

### Type I and II fiber CSA

There were no significant changes in either type I or type II fiber CSA from EB to ER + SR ( $P > 0.05$ ) with no significant differences between groups ( $P > 0.05$ ; **Table 5**).

## DISCUSSION

The novel finding of the present investigation was that 2 wk of physical inactivity (step reduction, <750 steps/d) in combination with a mild energy deficit (-500 kcal/d) resulted in a significant reduction in LLM in older men and women consuming a protein

intake twice the RDA. Importantly, we observed that consuming a WP supplement, in comparison to the consumption of a CP supplement, resulted in an increase in integrated MPS with return to habitual levels of physical activity. To our knowledge, this study is the first to examine the impact of protein supplementation with different protein sources during simulated hospitalization and convalescence concurrent with a state of energy restriction in older men and women.

Consistent with previous reports (36, 37), we show that a reduction in energy intake induced a decline of ~16% in integrated myofibrillar MPS, and that during ER + SR there was no further decline. The reduction in rates of MPS in the present investigation are similar to those from our previous study in which 2 wk of inactivity alone resulted in an ~13% decline in integrated rates of myofibrillar MPS in older men and women (4). Thus, it appears that energy restriction and reduced activity do not synergistically lower rates of myofibrillar MPS, and that a lower limit exists to which MPS can decline in these scenarios, at least in healthy older adults. Importantly, in the present investigation, we report that twice-daily supplementation with WP was effective at increasing rates of MPS from ER + SR during RC in comparison to the consumption of a CP supplement. Interestingly, rates of MPS in the CP group remained suppressed following return to habitual activity. This finding is particularly relevant as our previous work (4) showed that a return to habitual activity in the absence of intervention was insufficient in restoring rates of MPS following 2 wk of return to habitual activity.

Another important finding of the present study was that the introduction of SR, in addition to a period of ER, did not result in a further decrease in LBM or LLM in comparison to ER alone when participants consumed twice the RDA for dietary protein intake.

**TABLE 5**Fiber CSA of type I and type II fibers at EB and ER + SR<sup>1</sup>

	WP supplement		CP supplement	
	EB	ER + SR	EB	ER + SR
Type I CSA, $\mu\text{m}^2$	5570 ± 1987	6479 ± 2912	5501 ± 940	4533 ± 1699
Type II CSA, $\mu\text{m}^2$	4377 ± 1758	4334 ± 1897	4533 ± 1699	4375 ± 1588

<sup>1</sup>Data were analyzed with 2-factor ANOVA with repeated measures on time. There were no significant differences in type I or II CSA at EB or ER + SR or between WP and CP. Values are means ± SDs. CP, collagen peptide supplement; CSA, cross-sectional area; EB, energy balance phase; ER + SR, energy restricted high protein diet and step reduction phase; WP, whey protein.

We also show that supplementation with WP increased LLM and LBM from ER + SR during RC. However, supplementation with CP did not result in increases in LLM or LBM during RC. Previously, daily supplementation with WP, albeit at what we would consider a suboptimal dose for an older adult population (38), has been shown to be ineffective at reducing losses in skeletal muscle with immobilization (15). Congruent with these findings, our changes in LLM and LBM do not show a significant benefit of supplementation to offset muscle loss during a period of reduced activity; however, this is the first study, to our knowledge, to show an increase in LLM and LBM with WP, but not CP, supplementation during recovery. The pronounced increase in LLM and LBM with WP supplementation provides support for increasing protein intake in older adults in an effort to overcome the heightened anabolic resistance to protein feeding that occurs with age (38).

Consistent with our work from our laboratory (4), we showed that 2 wk of reduced daily activity resulted in a significant impairment in glycemic control following ER + SR that did not fully recover at RC in both groups. We report that ER alone is capable of inducing a favorable reduction in plasma insulin concentration in older adults; however, the addition of inactivity during ER + SR resulted in the elevation of plasma insulin and impairment of glucose handling. Mirroring changes in fasted blood glucose, we found that levels of TNF- $\alpha$ , IL-6, and CRP were significantly elevated following ER + SR; however, ER alone did not result in marked changes in systemic inflammation. These findings are congruent with existing literature using a bed-rest model in which the authors found a significant mediating effect of bed rest on increases in systemic inflammation following 35 d of inactivity (39).

The progression of dietary and activity phases in the present study was a strength of this protocol as it allowed us to determine the effects of ER alone, and in combination with ER + SR with high protein intake, on muscle metabolism in older adults. However, there are some limitations of the current investigation that we acknowledge. First, we did not directly measure rates of muscle protein breakdown, and therefore the relative contributions of MPS and muscle protein breakdown to changes in LBM and LLM are unknown. Second, all the participants in the current study were healthy and free of any chronic condition, thus limiting the applicability of the intervention to older persons with clinically prevalent chronic conditions. However, if the detrimental effects of ER and SR on skeletal muscle are significantly pronounced in a cohort of healthy older adults, we propose that losses in muscle mass and impairments in glycemic control would be worsened in a compromised older adult population as we have reported (4).

In conclusion, we show here that 2 wk of inactivity resulted in the loss of LLM and a decrement in MPS. Importantly, we show that WP was able to stimulate recovery of MPS and increase LLM in 1 wk of return to habitual activity that was not seen in men or women supplemented with CP. Congruent with previous literature, we show that protein supplementation alone was insufficient to offset the absolute loss of muscle mass with acute inactivity, but that supplementation with WP may be protective on LLM from a bout of inactivity combined with a hypocaloric diet and even enhance recovery following return to habitual activity.

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The authors' responsibilities were as follows—SYO, CM, and SMP: conceived and designed the study, and drafted the manuscript; and all authors: participated in some aspect of data collection and/or analysis, provided content and/or editorial corrections, and read and approved the final version of the manuscript. SMP has received competitive research funding, travel expenses, and honoraria for speaking from the US National Dairy Council. The remaining authors had no conflicts of interest to declare.

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