

## Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men

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(Submitted 10 August 2011 – Final revision received 23 November 2011 – Accepted 14 December 2011 – First published online 7 February 2012)

### Abstract

Feeding stimulates robust increases in muscle protein synthesis (MPS); however, ageing may alter the anabolic response to protein ingestion and the subsequent aminoacidaemia. With this as background, we aimed to determine in the present study the dose–response of MPS with the ingestion of isolated whey protein, with and without prior resistance exercise, in the elderly. For the purpose of this study, thirty-seven elderly men (age 71 (SD 4) years) completed a bout of unilateral leg-based resistance exercise before ingesting 0, 10, 20 or 40 g of whey protein isolate (W0–W40, respectively). Infusion of L-[1-<sup>13</sup>C]leucine and L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine with bilateral *vastus lateralis* muscle biopsies were used to ascertain whole-body leucine oxidation and 4 h post-protein consumption of MPS in the fed-state of non-exercised and exercised leg muscles. It was determined that whole-body leucine oxidation increased in a stepwise, dose-dependent manner. MPS increased above basal, fasting values by approximately 65 and 90% for W20 and W40, respectively ( $P < 0.05$ ), but not with lower doses of whey. While resistance exercise was generally effective at stimulating MPS, W20 and W40 ingestion post-exercise increased MPS above W0 and W10 exercised values ( $P < 0.05$ ) and W40 was greater than W20 ( $P < 0.05$ ). Based on the study, the following conclusions were drawn. At rest, the optimal whey protein dose for non-frail older adults to consume, to increase myofibrillar MPS above fasting rates, was 20 g. Resistance exercise increases MPS in the elderly at all protein doses, but to a greater extent with 40 g of whey ingestion. These data suggest that, in contrast to younger adults, in whom post-exercise rates of MPS are saturated with 20 g of protein, exercised muscles of older adults respond to higher protein doses.

**Key words:** Sarcopenia: Protein metabolism: Hypertrophy

The sarcopenia of ageing occurs as a result of a gradual net loss of skeletal muscle protein due to an imbalance between the synthesis and breakdown of skeletal muscle proteins<sup>(1,2)</sup>. The response of muscle protein synthesis (MPS) to anabolic stimuli, such as resistance exercise<sup>(3)</sup> and protein ingestion<sup>(4–8)</sup>, appears blunted in older adults compared with their younger counterparts; although not all have observed this<sup>(8–10)</sup>. The failure of older muscle to mount a robust ‘youthful’ response to protein ingestion and exercise has been coined ‘anabolic resistance’<sup>(11)</sup>. The precise intracellular mechanisms underlying the blunted response of muscle protein synthesis to anabolic stimuli in aged skeletal muscle are not well defined.

Given the potency of the branched-chain amino acid leucine as a key regulator of MPS through activation of the mammalian target of rapamycin pathway<sup>(12,13)</sup>, we<sup>(14)</sup>, and

others<sup>(15)</sup>, have hypothesised the existence of a leucine ‘threshold’ that must be surpassed for protein ingestion to stimulate MPS. It appears that resistance exercise enhances sensitivity to amino acids and may well lower this threshold<sup>(14)</sup>. In support of this thesis, Katsanos *et al.*<sup>(7)</sup> reported that additional leucine was required to increase rates of MPS in the elderly to the same extent as in younger adults. Thus, the leucine ‘threshold’ and acute protein requirements may be higher in aged muscles; a conclusion also reached by others<sup>(15)</sup>. To date, the exact dose–response of myofibrillar MPS to graded protein ingestion in the elderly has not been investigated.

Studies have consistently demonstrated that resistance exercise increases MPS in older adults<sup>(5,10,16)</sup> although the response is blunted compared to that in younger adults<sup>(17)</sup>. It is well defined that resistance exercise potentiates the

**Abbreviations:** EAA, essential amino acids; FSR, fractional synthetic rate; MPS, muscle protein synthesis; SPPB, Short Physical Performance Battery; W0, 0 g of whey protein isolate; W10, 10 g of whey protein isolate; W20, 20 g of whey protein isolate; W40, 40 g of whey protein isolate.

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muscle synthetic response to protein ingestion<sup>(18–20)</sup>, such that a net gain in muscle protein occurs<sup>(21)</sup>. We have previously shown that ingestion of 5 and 10 g of dietary protein after resistance exercise was sufficient to stimulate rates of MPS above exercise alone in young adults<sup>(22,23)</sup>, a response that reaches a plateau with 20 g of protein ingestion post-exercise. The synergistic effect of resistance exercise plus protein feeding is also evident in skeletal muscle of older adults<sup>(9,10,24,25)</sup>. It is unclear whether the synergistic effects of prior resistance exercise and protein feeding on MPS in the elderly are influenced by the amount of protein ingested.

We aimed to determine the dose–response curve of MPS in response to graded doses of whey protein that we propose represent the minimally (10 g) and maximally (20–40 g) effective doses in the elderly. The choice of whey protein ingestion in the present study is based on a number of studies highlighting the superior anabolic properties of whey compared to soya and casein proteins<sup>(26,27)</sup>, which probably stems from its rapid digestion kinetics and greater peak leucinaemia<sup>(26,28)</sup>. We hypothesised that resting rates of myofibrillar MPS would be increased above basal fasting values with the ingestion of ≥20 g of whey protein in the elderly, which is greater than the dose required to elevate MPS in the young<sup>(22)</sup>, as elderly ‘resistant’ muscle would have a higher leucine requirement in order to stimulate MPS rates<sup>(7)</sup>. Further, we hypothesised that a combination of resistance exercise with protein ingestion would be additive in stimulating MPS rates at all doses, compared with feeding alone. However, in contrast to the skeletal muscles of young adults, in which post-exercise MPS rates become saturated with ≥20 g of dietary protein, the stimulation of MPS in elderly muscles would be greater in amplitude after ingestion of 40 g of whey protein, compared with all other doses.

## Methods

### Participants

A total of thirty-seven older men (age 71 (SD 4) years, BMI 26 (SD 2.7) kg/m<sup>2</sup>) were recruited to complete the present study

and were randomly assigned to one of four treatment groups that were counterbalanced for body mass, age and physical activity levels. Participants were light-to-moderately active, non-smokers, non-diabetic and considered generally healthy. Participants taking medications controlling blood pressure were allowed in the study. The participant characteristics of each group are given in Table 1. Participants were informed about the experimental procedure to be used as well as the purpose of the study and all potential risks before obtaining written consent. This study was approved by the local Health Sciences Research Ethics Board of McMaster University and conformed to standards for the use of human participants in research as outlined in the 5th Declaration of Helsinki and with current Canadian funding agency guidelines for use of human participants in research<sup>(29)</sup>.

### General design

During the study, seven different groups of older men ingested 0, 10, 20 or 40 g of whey protein isolate as a beverage after performing an acute bout of unilateral resistance exercise. Employing a unilateral exercise model ensured that each participant served as their own resting control.

### Preliminary assessments

A week before the infusion trial, total body mass and body composition were obtained from dual-energy X-ray absorptiometry scans (Table 1). Physical performance was assessed using the Short Physical Performance Battery (SPPB) described in more detail elsewhere<sup>(30)</sup>, consisting of a 3–4 m walk test, chair stand and balance test (total SPPB score presented in Table 1). Health parameters were also assessed and included systolic and diastolic blood pressure, resting heart rate and blood parameters: fasting glucose, TAG, total cholesterol, HDL, LDL and ratio of total cholesterol-to-HDL. At least 1 week before the experimental infusion trial, participants underwent a maximum strength test to determine their unilateral ten repetition maximum on a standard leg extension machine as previously described<sup>(26)</sup>.

**Table 1.** Participant characteristics  
(Mean values and standard deviations)

| Parameter                | W0 (n 10) |      | W10 (n 7) |      | W20 (n 9) |      | W40 (n 10) |      |
|--------------------------|-----------|------|-----------|------|-----------|------|------------|------|
|                          | Mean      | SD   | Mean      | SD   | Mean      | SD   | Mean       | SD   |
| Age (years)              | 71        | 5    | 70        | 3    | 70        | 4    | 70         | 4    |
| Total body mass (kg)     | 78        | 13   | 79        | 16   | 80        | 7    | 81         | 12   |
| Fat-free mass (kg)       | 55        | 9    | 55        | 8    | 55        | 4    | 56         | 9    |
| Body fat (%)             | 26        | 5    | 26        | 6    | 24        | 4    | 27         | 8    |
| BMD (g/cm <sup>2</sup> ) | 1.19      | 0.11 | 1.29      | 0.06 | 1.20      | 0.11 | 1.23       | 0.11 |
| Height (m)               | 1.73      | 0.06 | 1.75      | 0.06 | 1.73      | 0.03 | 1.75       | 0.09 |
| BMI (kg/m <sup>2</sup> ) | 25.9      | 3.4  | 25.8      | 4.8  | 25.8      | 1.9  | 26.0       | 2.2  |
| Systolic BP (mmHg)       | 136       | 15   | 133       | 18   | 128       | 17   | 129        | 14   |
| Diastolic BP (mmHg)      | 80        | 10   | 71        | 6    | 75        | 6    | 78         | 5    |
| Total SPPB score*        | 11.7      | 0.5  | 12.0      | 0.1  | 11.6      | 0.5  | 11.6       | 0.7  |

W0, 0 g of whey protein isolate; W10, 10 g of whey protein isolate; W20, 20 g of whey protein isolate; W40, 40 g of whey protein isolate; BMD, bone mineral density; BP, blood pressure; SPPB, Short Physical Performance Battery.

\* Total SPPB score calculated as the sum of walk test, chair stand and balance tests.

### Dietary control

Participants were required to complete their dietary records before the start of the study to provide an estimate of habitual macronutrient intake as analysed using a commercially available software program (Nutritionist V; First Data Bank). Reference lists for portion size estimates were provided to participants who were instructed to record all food or drink consumed in a diet log during a 3 d period (i.e., two weekdays and one weekend day). Based on the responses, the average daily energy and protein intakes were 10.2 MJ/d and 1.0 g/kg per d, respectively (Table S1, supplementary material for this article can be found at <http://www.journals.cambridge.org/bjn>). Then, 2 d before the trial, participants were supplied with pre-packaged diets that provided a moderate protein intake (1.0 g/kg per d). Energy requirements for the controlled diets were estimated according to the Harris–Benedict equation and were adjusted using an activity factor calculated for each individual subject from their activity logs (1.65 (SD 0.1)). Body mass was monitored over the course of the controlled diet period to ensure that participants were in energy balance. Additionally, participants were instructed to abstain from any strenuous exercise until after completion of the trial.

### Infusion protocol

Participants reported to the laboratory at approximately 07.00 hours in a 10 h post-absorptive state. Upon arriving at the laboratory, a baseline breath sample was collected for determination of  $^{13}\text{CO}_2$  enrichment by isotope ratio MS (BreathMat Plus; Finnigan MAT GmbH). A plastic catheter was then inserted in an antecubital arm vein and a baseline blood sample was drawn before a 0.9% saline drip was started to keep the catheter patent for repeated blood sampling. After baseline breath and blood samples had been obtained, a unilateral resistance exercise bout was performed on a guided-motion knee extension machine. The exercise bout involved three sets of unilateral knee extension, using a pre-determined load based on each participant's ten repetition maximum, shown previously to increase rates of MPS<sup>(17,31)</sup>. Each set was completed within approximately 25 s with a rest period of 2 min between each set. Immediately after exercise, blood and breath samples were obtained and a second catheter was inserted into a contralateral antecubital arm vein to prime the bicarbonate pool with  $\text{NaH}^{13}\text{CO}_2$  (2.35  $\mu\text{mol/kg}$ ). Thereafter, priming doses of [1- $^{13}\text{C}$ ]leucine (7.6  $\mu\text{mol/kg}$ ) and L-[ring- $^{13}\text{C}_6$ ]phenylalanine (2  $\mu\text{mol/kg}$ ; 99 atom percent; Cambridge Isotopes) were introduced, before a continuous infusion of L-[1- $^{13}\text{C}$ ]leucine (7.6  $\mu\text{mol/kg}$  per h) and L-[ring- $^{13}\text{C}_6$ ]phenylalanine was initiated (0.05  $\mu\text{mol/kg}$  per min). Arterialised blood samples were obtained by wrapping the forearm in a heating blanket (45°C) for the duration of the infusion; a procedure that we have found completely arterialises venous blood sampled from a hand vein. Blood samples were processed as previously described<sup>(22)</sup>. Immediately after post-exercise blood and breath samples had been obtained, participants consumed a drink containing 0, 10, 20 or 40 g of whey protein (W0, W10, W20 and W40,

respectively) dissolved in 400 ml water. The whey protein was generously donated by PGP International (IWPI 9500). The amino acid profiles of the different doses of whey protein are provided in Table S2 (supplementary material for this article can be found at <http://www.journals.cambridge.org/bjn>). On the basis of a leucine content of 10% and a phenylalanine content of 3–5% in whey protein, drinks were enriched to 8% with [1- $^{13}\text{C}$ ]leucine and 8% with [ring- $^{13}\text{C}_6$ ]phenylalanine to minimise disturbances in isotopic steady state; an approach that we have validated<sup>(32)</sup>. Complete drink consumption was considered  $t = 0$  min and the isotopic infusion was continued until  $t = 240$  min. During the remainder of the infusion, arterialised blood and breath samples were obtained to confirm steady state and measure leucine oxidation and MPS as previously described<sup>(22,28)</sup>. At the end of the infusion ( $t = 240$  min), muscle biopsies were obtained (described next).

### Muscle biopsy sampling

Muscle biopsy samples were taken from the *vastus lateralis* of the thigh from exercised and non-exercised legs using a 5-mm Bergstrom needle (modified for manual suction), under 2% local anaesthesia by xylocaine. Muscle biopsies were freed from any visible blood, fat and connective tissue and rapidly frozen in liquid  $\text{N}_2$  for further analysis.

### Blood analyses

Plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments were determined as previously described<sup>(33)</sup>. Blood amino acid concentrations were analysed by HPLC as previously described<sup>(34)</sup>. Blood glucose concentrations were analysed using a blood glucose meter (OneTouch Ultra 2; Lifescan, Inc.) within 5 min of blood collection. Plasma insulin was measured using a commercially available immunoassay kit (ALPCO Diagnostics) following the manufacturer's instructions.

### Muscle analyses

Myofibrillar-enriched protein fractions were isolated from approximately 30 mg of wet muscle as described previously<sup>(35)</sup>. Intracellular amino acids were isolated from a separate piece of wet muscle (approximately 25 mg) as previously described<sup>(31)</sup>.

### Calculations

The fractional synthetic rates (FSR) of myofibrillar proteins were calculated using the standard precursor-product method:

$$\text{FSR (\%/h)} = E_{p2} - E_{p1}/E_{ic} \times 1/t \times 100,$$

where  $E_{p2}$  and  $E_{p1}$  are the protein-bound enrichments from muscle biopsies at 240 min and baseline plasma proteins, respectively. The difference represents the change in bound protein enrichment between two time points;  $E_{ic}$  is the mean intracellular phenylalanine enrichment from the biopsies taken from both legs at  $t = 240$  min;  $t$  is the tracer incorporation time. The utilisation of 'tracer-naive' subjects allowed

us to use the pre-infusion blood sample (i.e. mixed plasma protein fraction) as a surrogate baseline enrichment of muscle protein; an approach we have previously validated<sup>(31)</sup>. Previously, others have used a pre-infusion muscle biopsy and found equivalent rates of muscle protein synthesis and shown such an approach<sup>(36)</sup> to be valid; we have found baseline plasma enrichment to be equivalent to that of pre-infused muscle (NA Burd and SM Phillips, unpublished results).

Leucine oxidation was calculated as described in our previous publications<sup>(22,28)</sup> from the appearance of the <sup>13</sup>C-label in expired CO<sub>2</sub> using the reciprocal pool model with fractional bicarbonate retention factors of 0.7 and 0.83 for fasted (0 g protein) and fed (10–40 g whey protein) states, respectively<sup>(37)</sup>. The area under the leucine oxidation by time curve was calculated using GraphPad Prism 5 as an estimate of total leucine oxidation<sup>(22,28)</sup>. In addition, the area under the plasma insulin concentration by time curve was also calculated using the same graphing software.

### Statistical analyses

Differences among the groups were analysed using ANOVA. Following the observation of a significant *F* ratio by ANOVA, Bonferroni-adjusted *t* tests were used for *post hoc* analyses. Time × group differences for serum insulin response were analysed using multiple ANOVA due to non-spherical data based on the Mauchly's test of sphericity and that the Greenhouse–Geisser epsilon was less than 0.7. Localisation of significant interaction was then determined using simple main effect analyses. Significance was set at *P* ≤ 0.05. All statistical analyses were performed using SPSS 17 for Windows.

## Results

### Participant characteristics

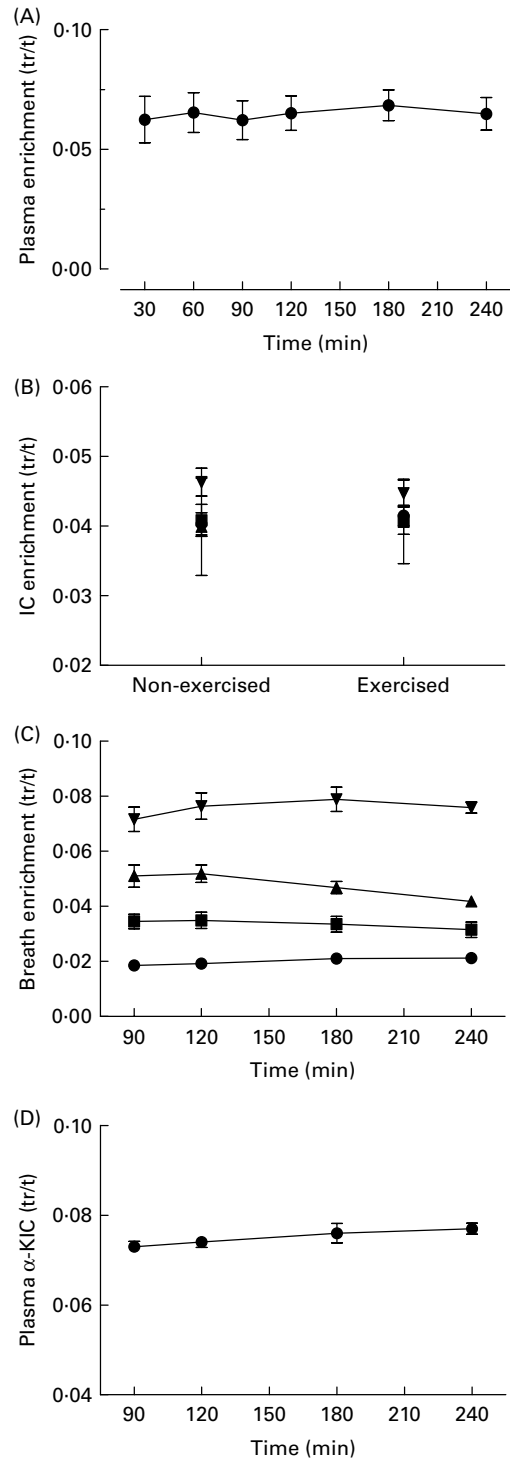
There was no between-group difference in age, body weight and composition, SPPB or the health parameters outlined previously (Table 1). Dietary intake in the 2 d run-in to the study was similar for all groups (Table S1, supplementary material for this article can be found at <http://www.journals.cambridge.org/bjn>).

### Plasma insulin

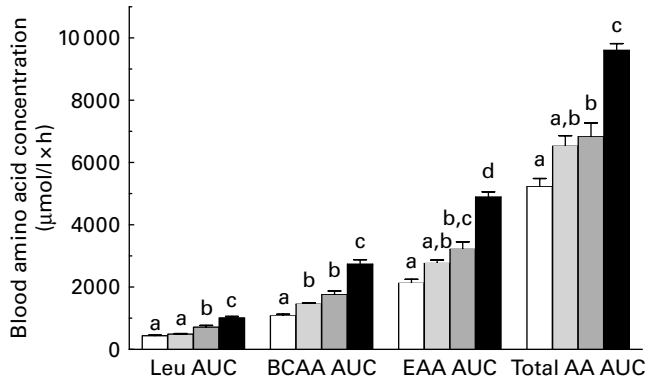
Plasma insulin concentration was similar for all groups at 0, 3 and 4 h post-drink. At 1 h post-drink, insulin concentration had increased by approximately 2.6- and 4-fold for W20 and W40, respectively, and was greater than W0 and W10 (*P* < 0.01). At 2 h post-drink, W40 maintained a higher insulin concentration compared with W0 and W10 (*P* < 0.05), whereas W20 was not different compared with all other doses (Fig. S1, supplementary material for this article can be found at <http://www.journals.cambridge.org/bjn>).

### Isotopic enrichments

Plasma <sup>13</sup>C<sub>6</sub> phenylalanine enrichment was stable over 30 to 240 min of tracer incorporation (Fig. 1(A)). These data



**Fig. 1.** Enrichment of <sup>13</sup>C<sub>6</sub> phenylalanine in (A) plasma and (B) intracellular (IC) pools (●, W0; ■, W10; ▲, W20; ▼, W40), <sup>13</sup>CO<sub>2</sub> enrichment in (C) breath (●, W0; ■, W10; ▲, W20; ▼, W40) and (D) plasma α-[<sup>13</sup>C]ketoisocaproate acid (α-KIC). Average plasma <sup>13</sup>C<sub>6</sub> and α-KIC enrichment for all groups over 240 min post-exercise are presented. IC <sup>13</sup>C<sub>6</sub> enrichments were determined from muscle biopsies at 240 min post-exercise in non-exercised and exercised legs. Breath <sup>13</sup>CO<sub>2</sub> enrichment was determined at 90, 120, 180 and 240 min post-exercise. Data are expressed as tracer-to-tracee ratio (tr/t). Values are means, with their standard errors represented by vertical bars. W0, 0 g of whey protein isolate; W10, 10 g of whey protein isolate; W20, 20 g of whey protein isolate; W40, 40 g of whey protein isolate



**Fig. 2.** Area under the blood amino acid concentration time curves (AUC) 0, 10, 20 and 40 g of whey protein (W0 (□), W10 (▨), W20 (▩) and W40 (■), respectively). Data presented for leucine (Leu), summed total of branched-chain amino acids (BCAA), summed total of the essential amino acids (EAA) and summed total of all amino acids (total AA). Values are means, with their standard errors represented by vertical bars. <sup>a,b,c</sup> Mean values with unlike letters were significantly different ( $P < 0.05$ ).

demonstrate that the additional tracer added to the drinks did not appear in the circulation more rapidly than the amino acids from the protein and that all measurements were made at isotopic equilibrium. There was no difference in intracellular <sup>13</sup>C<sub>6</sub> phenylalanine enrichment between groups or between non-exercised and exercised legs (Fig. 1(B)). Breath <sup>13</sup>CO<sub>2</sub> enrichment was stable over 30 to 240 min of tracer incorporation for all groups (Fig. 1(C)). Plasma α-[<sup>13</sup>C]ketoisocaproate acid enrichment was at plateau in all conditions and was not different between groups (Fig. 1(D)).

### Plasma amino acids

Peak plasma leucine concentration occurred between 1 and 1.5 h post-drink for all whey protein doses, but was greater with W40 compared with all other doses (Fig. S2, supplementary material for this article can be found at <http://www.journals.cambridge.org/bjn>). Peak leucine concentrations were greater for W20 compared with W10 and W0 ( $P < 0.05$ ). Area under the curve for essential amino acid (EAA) and total amino acid (Fig. 2) was greater for W40 compared with all other groups ( $P < 0.05$ ). Area under the curve for leucine and branched-chain amino acid was greater for W40 compared with W0, W10 and W20 ( $P < 0.05$ ).

### Whole-body leucine oxidation

**Whey.** Whole-body leucine oxidation area under the curve increased in a stepwise manner with graded doses of whey protein ingestion (W40 > W20 > W10 > W0), such that whole-body leucine oxidation was greater for W40 compared with all other whey doses ( $P < 0.05$ ; Fig. 3).

### Myofibrillar protein fractional synthetic rate

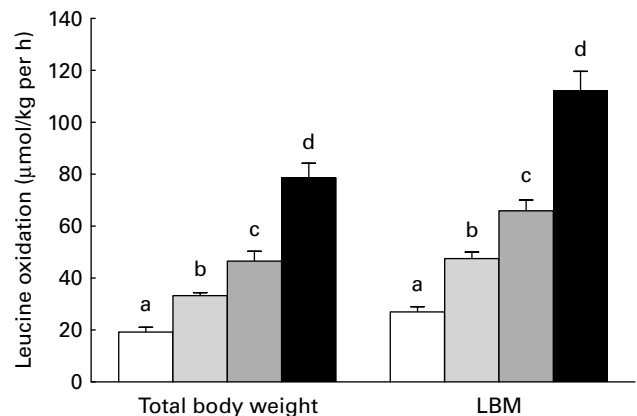
Myofibrillar FSR in the non-exercised leg (fed only) was significantly increased with W20 and W40 compared with W0 ( $P < 0.05$ ; Fig. 4), with no significant difference observed

between W20 and W40. Myofibrillar FSR for W40, but not W20, was greater than W10 ( $P < 0.05$ ). Myofibrillar FSR was greater in the exercised leg compared to the non-exercised leg for all whey doses ( $P < 0.05$ ); however, in the W20 and W40 exercised legs, myofibrillar FSR was statistically elevated above the W0 and W10 exercised legs ( $P < 0.01$ ). Furthermore, myofibrillar FSR for W40 was approximately 32% greater than for W20 ( $P = 0.02$ ).

### Discussion

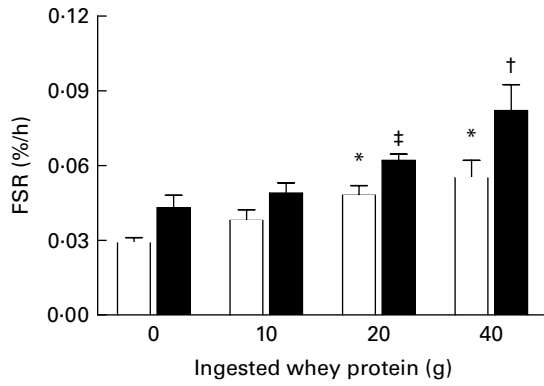
The present study is the first to determine the dose–response relationship between myofibrillar MPS and the ingestion of isolated whey protein in non-frail, older men. In addition, we investigated whether resistance exercise altered the response of myofibrillar MPS to graded whey protein feeding. Our findings demonstrate that ingestion of 20 g of whey protein was necessary to increase myofibrillar MPS above basal fasting rates and ingestion of 40 g of whey protein did not potentiate this response. In addition, we showed that the combined effect of resistance exercise with protein ingestion increased rates of MPS to a greater extent than feeding alone for all protein doses. However, 20 g of whey protein ingestion after resistance exercise was required to elevate rates of myofibrillar MPS above exercise alone, or exercise followed by 10 g of whey ingestion. Interestingly, while 40 g of whey protein ingestion did not enhance basal fasting rates of myofibrillar MPS above 20, 40 g of whey ingested after resistance exercise increased myofibrillar MPS above all other doses.

In support, and expanding on, previous studies in young adults<sup>(5,22)</sup>, our findings highlight that in the basal state, 20 g of whey protein ingestion is maximally effective in stimulating myofibrillar MPS above rest in the elderly. Furthermore, whole-body leucine oxidation increased in a stepwise manner with graded doses of whey protein ingestion. Thus, a dose of 20 g of whey protein appears necessary for the stimulation of myofibrillar MPS. Although we did not perform a direct comparison between older and younger adults, it has



**Fig. 3.** Whole-body leucine oxidation for 0, 10, 20 and 40 g of whey protein (W0 (□), W10 (▨), W20 (▩) and W40 (■), respectively). Data are expressed relative to total body weight and to lean body mass (LBM). Values are means, with their standard errors represented by vertical bars. <sup>a,b,c,d</sup> Mean values with unlike letters were significantly different ( $P < 0.05$ ).





**Fig. 4.** Myofibrillar protein fractional synthetic rate (%/h) for all whey groups. There were main effects for dose ( $P < 0.001$ ) and condition ( $P < 0.001$ ). There was also a significant dose  $\times$  leg interaction ( $P < 0.003$ ). Values are means, with their standard errors represented by vertical bars. \* Mean values were significantly different from non-exercised leg (□) in 0 g dose ( $P < 0.005$ ). † Mean values were significantly different from exercised leg (■) in 0, 10 and 20 g dose ( $P < 0.05$ ). ‡ Mean values were significantly different from exercised leg in 0 and 10 g dose ( $P = 0.05$ ).

been reported by others<sup>(5)</sup>, that as little as 2.5 g of crystalline EAA (typically contained in approximately 5–6 g of whey protein), can increase rates of myofibrillar MPS above basal fasting values in both the young and the elderly<sup>(22)</sup>. Congruent with Cuthbertson *et al.*<sup>(5)</sup>, we show the increase in myofibrillar MPS plateaus after ingestion of approximately 9 g of EAA (contained in 20 g of whey). However, our findings demonstrate a blunted sensitivity of myofibrillar MPS in elderly muscles to low doses of EAA (contained in 10 g intact whey protein). The discordance between the present study and that of Cuthbertson *et al.*<sup>(5)</sup> is unclear. However, in support of the preposition that muscles in the elderly are less sensitive to low doses of amino acids, Katsanos *et al.*<sup>(4)</sup> have reported that ageing results in a diminished accretion of muscle protein after ingestion of a low dose (6.7 g) of EAA. Thus, given the evidence that anabolic resistance in elderly skeletal muscle makes an impact on the responsiveness<sup>(5)</sup> and duration<sup>(10)</sup> of MPS to protein feeding, it seems intuitive that resistant elderly muscle would also be less sensitive to low doses of amino acids compared with young adults<sup>(22)</sup>. In summary, it appears that ingestion of  $\geq 20$  g of high-quality protein (containing approximately 10 g of EAA) is required to stimulate a rise in rates of myofibrillar MPS in older muscle<sup>(8)</sup>. However, not all studies suggest that the elderly need to ingest a higher dose of protein to maximise myofibrillar MPS<sup>(9,25)</sup>.

Despite recent evidence that older adults do not require more dietary protein than the young to achieve nitrogen balance<sup>(38)</sup>, the findings of the present study and those of others<sup>(39)</sup> suggest that meal-by-meal patterns of protein consumption, rather than overall daily requirements are more important than previously realised, as some have suggested<sup>(39)</sup>. In short, older adults, to achieve a maximal stimulation of protein synthesis, should distribute dietary protein intake equally across their daily meals. For example, with a breakfast-lunch-and-dinner eating pattern, each meal should consist of at least 20 g of high-quality protein, in order to acutely and repeatedly increase rates of MPS above basal fasting values. For

example, we speculate that a 75 kg individual consuming approximately 60 g of protein daily (based on the RDA of 0.8 g/kg) should endeavour to consume at least 20 g of protein with each meal, as opposed to the normal feeding regimen, in which the elderly typically ingest smaller amounts of protein with breakfast (approximately 8 g) and lunch (approximately 12 g) and the maximum of dietary protein with dinner (approximately 40 g)<sup>(40)</sup>. The impact of manipulating dietary protein intakes on daily muscle protein balance in the elderly has yet to be examined, but is clearly an area for future research.

The mechanism underpinning the blunted sensitivity of elderly muscles to low doses of EAA has yet to be elucidated, but may involve a greater ‘threshold’ to anabolic stimuli that must be surpassed in order to stimulate a rise in MPS compared with the young. In particular, the branched-chain amino acid, leucine, a potent activator of mRNA translation via the mammalian target of rapamycin signalling pathway, has been touted as a key metabolic regulator of MPS<sup>(12,41)</sup>. Whereas MPS in young adults appears responsive to a very low dose of leucine<sup>(4,5,42)</sup>, our data suggest that at least  $\geq 2$  g of leucine contained in 20 g of whey protein may be required to surpass the ‘leucine threshold’ and increase rates of myofibrillar MPS in the elderly. Support for this thesis has been demonstrated by Katsanos *et al.*<sup>(7)</sup> who reported that as little as 6.7 g of crystalline EAA increased mixed MPS above rest in the elderly provided the proportion of leucine was increased from approximately 1.7 g (26%) to 2.8 g (41%). In addition, studies have failed to detect anabolic resistance to feeding with ageing<sup>(9,43)</sup>, with the studies reporting that plasma amino acid concentrations increase to a greater extent after protein ingestion in the elderly, compared with younger adults. Thus, the greater appearance of circulating amino acids, specifically leucine, in older adults who ingest protein, may be critical for mitigating the age-induced resistance of MPS to protein feeding. Taken together, these data provide further support for the existence of a higher leucine ‘threshold’ in the elderly that, we report, can be overcome by ingesting a  $\geq 20$  g of rapidly digested whey protein or, according to another work<sup>(7)</sup>, a lower dose of whey protein enriched with leucine.

In comparison to the acute metabolic response we show herein, the efficacy of long-term leucine supplementation in the treatment of sarcopenia is unclear. Recent longitudinal studies<sup>(44,45)</sup> suggest that leucine supplementation in healthy<sup>(45)</sup> and type 2 diabetic<sup>(44)</sup> elderly men does not increase lean mass, strength or improve glycaemic control. However, these longitudinal studies did not utilise resistance exercise training concurrently with leucine supplementation. Thus, the discordance between acute<sup>(7,15)</sup> and chronic leucine supplementation studies<sup>(44,45)</sup> may be due to the relatively subtle phenotypic adaptations that occur in a feeding-only, non-exercised trial. That is, the summation, over time, of muscle protein synthetic responses to feeding-only stimuli would be relatively small. We posit that while leucine is an important ‘trigger’ in activating mRNA translational signalling machinery<sup>(12,13)</sup>, other EAA are required to facilitate

a robust muscle protein synthetic response and long-term hypertrophy<sup>(46)</sup> and strength<sup>(47)</sup> in the elderly.

The synergistic effect of resistance exercise in combination with EAA ingestion on rates of MPS has been well documented in the young<sup>(21,23,34,35,48)</sup> and the elderly<sup>(10,49–51)</sup>. Thus, utilising amino acid or protein feeding and resistance exercise concurrently will promote an optimal anabolic environment in elderly muscles compared with either stimulus alone. In support of this paradigm, the present data show that resistance exercise potentiates feeding-induced rates of MPS at all protein doses. Compared with exercise without feeding, rates of MPS were approximately 13, 44 and 91% greater with post-exercise ingestion of 10, 20 and 40 g of whey protein, respectively. These data are in contrast to our previous publications in young adults in which we show that ingesting as little as 5–10 g of dietary protein stimulates a robust increase in MPS following resistance exercise<sup>(22,23)</sup> and that with 40 *v.* 20 g of protein, there was no further increase in MPS. Thus, in line with our observation that the elderly require a greater dose of protein than the young in order to elevate basal rates of MPS, we also show that a greater dose is required to potentiate exercise-stimulated rates of MPS. In the aforementioned study of young adults<sup>(22)</sup>, post-exercise rates of MPS increased in a dose-dependent manner up to 20 g dietary protein, after which there was no further increase in MPS with 40 g. In contrast, we show herein that ingesting 40 g of whey protein does increase exercise-stimulated rates of MPS above 20 g in the elderly. Given the evidence that the muscle protein synthetic response following resistance exercise is blunted in aged muscle<sup>(17)</sup>, our data and that of others<sup>(9)</sup> suggest that consuming a relatively high amount of dietary protein after resistance exercise may, potentially, increase rates of MPS in the elderly to the same extent as in young adults. The feasibility of ingesting higher doses of protein in the context of resistance exercise needs to be examined more closely, particularly given the evidence that elevating circulating insulin concentrations, for example, by co-ingesting carbohydrate with protein, does not potentiate the anabolic response<sup>(52–54)</sup>.

In summary, we report that 20 g of intact whey protein ingestion is required to increase myofibrillar MPS above basal fasting rates in older adults, a response that does not increase further with 40 g of whey. The pronounced rise in blood leucine after  $\geq 20$  g of whey ingestion may be a critical factor for stimulating rates of MPS above rest in the elderly. Thus, basal-state anabolic resistance to protein feeding in the elderly could, potentially, be overcome provided sufficient doses of rapidly digested proteins are consumed. Resistance exercise increased the fasted-state rates of myofibrillar MPS in the elderly, and augmented the fed-state rates of myofibrillar MPS. However, although ingestion of 20 g of whey after exercise was required to increase myofibrillar MPS above exercise alone, post-exercise ingestion of 40 g of whey elevated rates of myofibrillar MPS to an even greater extent. These data highlight the finding that the combination of resistance exercise and consumption of a rapidly digested, leucine-rich protein may be an effective strategy for older adults to offset muscle loss due to sarcopenia.

## Acknowledgements

The authors are grateful to Todd Prior and Tracy Rerecich for their technical expertise and assistance during data collection. The present study was funded by a research award from the US Dairy Research Institute to S. M. P., Grants from the Canadian Natural Science and Engineering Research Council (NSERC) to S. M. P., and a graduate scholarship to T. A. C.-V., and from the Canadian Institutes for Health Research (CIHR) to S. M. P., and a graduate scholarship to A. R. J. The contributions of the authors to this study were as follows: Y. Y., N. A. B. and S. M. P. designed the research; Y. Y., N. A. B., A. J. H., T. A. C.-V., A. R. J., M. A. T. and S. M. P. conducted the research; Y. Y., L. B., N. A. B., A. J. H. and S. M. P. analysed the data; Y. Y., L. B., N. A. B. and S. M. P. wrote and edited the manuscript; S. M. P. had primary responsibility for the final content. All authors read and approved the final manuscript. Y. Y., L. B., N. A. B., A. J. H., T. A. C.-V., A. R. J., M. A. T. and S. M. P. have no conflicts of interest, financial or otherwise, to declare.

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