

Short Communication

Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men

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Abstract

We aimed to determine the effect of consuming pure isolated micellar casein or pure whey protein isolate on rates of myofibrillar protein synthesis (MPS) at rest and after resistance exercise in elderly men. Healthy elderly men (72 (SEM 1) years; BMI 26.4 (SEM 0.7) kg/m²) were divided into two groups (*n* 7 each) who received a primed, constant infusion of L-[ring-¹³C₆]phenylalanine to measure MPS at rest and during 4 h of exercise recovery. Participants performed unilateral leg resistance exercise followed by the consumption of isonitrogenous quantities (20 g) of casein or whey. Blood essential amino acids and leucine concentration peaked 60 min post-drink and were greater in amplitude after whey protein ingestion (both, *P*<0.05). MPS in the rested leg was 65% higher (*P*=0.002) after ingestion of whey (0.040 (SEM 0.003)%/h) when compared with micellar casein (0.024 (SEM 0.002)%/h). Similarly, resistance exercise-stimulated rates of MPS were greater (*P*<0.001) after whey ingestion (0.059 (SEM 0.005)%/h) v. micellar casein (0.035 (SEM 0.002)%/h). We conclude that ingestion of isolated whey protein supports greater rates of MPS than micellar casein both at rest and after resistance exercise in healthy elderly men. This result is probably related to a greater hyperaminoacidaemia or leucinaemia with whey ingestion.

Key words: Sarcopenia: Muscle: Ageing: Leucine

The loss of muscle mass with ageing is rooted in an imbalance between the rates of muscle protein synthesis and muscle protein breakdown, with the former playing the dominant role in the regulation of muscle mass in healthy individuals⁽¹⁾. A ‘resistance’ of muscle protein synthesis to dietary protein, which describes a situation of a blunted muscle protein synthetic response to a given dietary protein load, in otherwise healthy elderly individuals is thought by some to be a major contributing factor to age-related muscle loss^(2,3). There is also evidence suggesting an attenuated myofibrillar protein synthetic response after resistance exercise in the elderly v. young⁽⁴⁾; however, adequate nutrition in the form of dietary amino acids can overcome this difference⁽⁵⁾.

Recent evidence has shown that whey protein has a superior capacity, by comparison with other proteins such as soya and casein, to stimulate rates of mixed muscle protein synthesis at rest in both young⁽⁶⁾ and elderly individuals⁽⁷⁾, as well as after exercise in the young⁽⁶⁾. Moreover, even when compared with an isonitrogenous mixture of equivalent constituent amino acids, whey protein stimulates a greater anabolic response⁽⁸⁾. The greater stimulatory effect of whey has been attributed to its high leucine content combined with a rapid rate of digestion, both of which lead to a rapid leucinaemia and aminoacidaemia, in general, for an optimal stimulation of muscle protein synthesis^(6,7). While these studies support the use of whey protein for stimulating

Abbreviations: EAA, essential amino acids; EX-FED, myofibrillar protein synthesis after resistance exercise; FED, myofibrillar protein synthesis at rest; t/Tr, tracer:tracee.

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mixed muscle protein synthesis, they do not provide insight into the synthesis of specific muscle protein subfractions. For example, myofibrillar (i.e. contractile) and non-myofibrillar muscle protein fractions do not always demonstrate concordant responses to nutrition and exercise⁽⁹⁾. Given the importance of maintaining an adequate quantity and quality of contractile proteins with age, we aimed to determine the effectiveness of rapidly digested isolated whey protein and slowly digested isolated micellar casein to stimulate rates of myofibrillar protein synthesis at rest (FED) and after resistance exercise (EX-FED) in elderly skeletal muscle. Since the postprandial rise in circulating amino acids in the blood appears to dictate the extent of stimulation of myofibrillar protein synthesis⁽⁶⁾, we hypothesised that the myofibrillar protein synthetic response would be greater, regardless of prior contractile activity, after ingestion of whey when compared with slowly digested micellar casein.

Experimental methods

Participants

A total of fourteen older men (72 (SEM 1) years; BMI 26.4 (SEM 0.7) kg/m²) were recruited to participate in the present study. Participants were non-smokers, non-diabetic and light-to-moderately active. All participants were deemed healthy based on their response to a routine medical screening questionnaire and examination of their baseline characteristics. All participants were informed of the purpose of the study, the experimental procedures involved and all the potential risks involved before obtaining written consent. The study was approved by the local Health Sciences Research Ethics Board of McMaster University and conformed to standards for the use of human subjects in research as outlined in the fifth Declaration of Helsinki and with current Canadian funding agency guidelines for use of human subjects in research⁽¹⁰⁾.

Experimental protocol

Participants reported to the laboratory approximately 1 week before the experimental infusion trial to undergo preliminary testing. After familiarisation with the equipment, participants underwent strength testing on a leg extension machine to determine their unilateral ten repetition maximum. Body composition was assessed by dual-energy X-ray absorptiometry and participants were randomly assigned to the whey or micellar casein groups but counterbalanced for lean body mass. Participants were required to record all food or drink consumed in a diet log during a 3 d period (i.e. two weekdays and one weekend) before the start of the study to provide an estimate of habitual macronutrient intake as analysed by a commercially available software program (Nutritionist V, First; Data Bank, San Bruno, CA, USA). Reference lists for portion size estimates were provided to the participants. Based on the responses, the average daily energy and protein intakes were calculated (9.5 MJ/d and 1.0 g/kg per d, respectively). Moreover, 2 d preceding the infusion trial, participants were provided a standardised diet that contained a moderate

protein intake (1.0 g/kg per d) and energy at a level estimated according to the Harris–Benedict equation⁽¹¹⁾, with individual activity factors according to individual self-report logs (mean activity factor 1.5 (SEM 0.1)).

After having refrained from strenuous exercise for 3 d, participants arrived at the laboratory after an overnight fast and a baseline blood sample was drawn. Subsequently, participants performed an acute bout of unilateral resistance exercise with one leg (EX-FED) on a guided-motion knee extension machine (three sets at the participant's ten repetition maximum). The contralateral leg did not perform exercise but was under the influence of nutrition (FED).

A primed (2 μmol/kg), constant infusion (0.05 μmol/kg per min) of L-[ring-¹³C₆]phenylalanine (99 at%; Cambridge Isotopes, Andover, MA, USA) was initiated. Immediately after the start of the infusion, participants consumed a drink containing 20 g micellar casein (MCN-85; AMCO, Burlington, NJ, USA), which contained 8.2 g essential amino acids (EAA), 4.0 g branched-chain amino acids (BCAA) and 1.6 g leucine, or 20 g whey protein isolate (Alacen 352; Fonterra, Palmerston North, New Zealand), which contained 10.2 g EAA, 5.2 g BCAA and 2.8 g leucine, determined using methods described previously⁽¹²⁾. All drinks were dissolved in 400 ml of water. Drinks were enriched with a small amount of tracer in accordance with the phenylalanine content of approximately 4% of the micellar casein and whey protein⁽⁶⁾. This approach is necessary to prevent dilution of tracer enrichment, and thus maintain steady-state enrichment in the intracellular amino acid pool⁽¹³⁾, after introduction of unlabelled amino acids into the blood (i.e. the amino acids from the protein drink). Arterialised blood samples were collected every 0.5–1 h after the start of infusion and processed as described previously⁽¹⁴⁾. Biopsy samples were taken at 240 min from the *vastus lateralis* of the EX-FED and FED legs using a 5 mm Bergström needle that was custom-modified for manual suction under local anaesthesia (2% xylocaine). Biopsy samples were immediately blotted and freed of any visible blood, fat or connective tissue before immersion in liquid N₂ and storage at –80°C until further analysis.

Analytical procedures

Plasma [ring-¹³C₆]phenylalanine enrichments were determined as described previously⁽¹⁵⁾. Blood amino acid concentrations were measured by HPLC as described previously⁽¹⁶⁾. Blood glucose concentrations were analysed using a blood glucose meter (OneTouch Ultra 2; Lifescan, Inc., Milpitas, CA, USA) within 5 min of blood collection. Plasma insulin was measured using a commercially available immunoassay kit (Alpco Diagnostics, Salem, NH, USA). Myofibrillar-enriched protein fractions were isolated from approximately 30 mg of wet muscle as described previously⁽¹⁷⁾. A separate piece of wet muscle (approximately 15 mg) was used to extract the intracellular amino acids as described previously⁽¹⁸⁾.

Calculations

The fractional synthetic rates of myofibrillar proteins were calculated according to the standard precursor–product

equation, with intracellular phenylalanine enrichment as a surrogate for transfer RNA labelling⁽¹³⁾. The use of 'tracer-naive' participants allowed us to use the pre-infusion blood sample as the baseline enrichment for muscle protein; an approach we have recently validated and described in detail elsewhere⁽¹³⁾.

Statistics

A between-subject repeated-measures design was utilised for the present study. Data were analysed using a two-factor repeated-measures ANOVA. Tukey's *post hoc* test was performed to determine differences between means for all significant main effects and interactions. For all analyses, differences were considered significant at $P < 0.05$. Results are presented as means with their standard errors.

Results

Fasting plasma insulin concentrations were similar in the micellar casein 39.1 (SEM 7) pmol/l and whey protein (36.2 (SEM 5) pmol/l) groups. Whey ingestion transiently increased plasma insulin by approximately 179% above fasting at 60 min, whereas there was no effect of micellar casein (Table 1). Blood glucose remained stable across time for both whey and micellar casein groups (data not shown).

EAA in the blood peaked at 60 min after micellar casein ingestion ($P < 0.001$; Table 1) before returning to fasting EAA concentrations at 120 min post-drink ($P = 0.07$; Table 1). A prolonged elevation in blood leucine concentrations was detected after micellar casein ingestion with a peak at 60 min post-drink before returning to baseline by 240 min ($P = 0.24$; Table 1). Whey protein ingestion induced an acute rise in blood EAA and leucine concentrations at 60 min post-drink that was greater in amplitude than micellar casein ingestion (both, $P < 0.05$; Table 1).

Linear regression analysis indicated that the slopes of the plasma enrichments were not significantly different from zero (both, $P < 0.05$) in the micellar casein or the whey group, indicating that an isotopic plateau was achieved (data

not shown). Intracellular free phenylalanine enrichments in the casein group were 0.043 (SEM 0.001) tracer:tracee (t/Tr) ratio and 0.044 (SEM 0.001) t/Tr in the FED and EX-FED conditions, respectively. Intracellular free phenylalanine enrichments in the whey group were 0.040 (SEM 0.003) t/Tr and 0.041 (SEM 0.001) t/Tr in the FED and EX-FED conditions, respectively. Resistance exercise-induced rates of myofibrillar protein synthesis were greater ($P < 0.001$) than those observed after feeding alone in both micellar casein and whey protein groups (Table 1). However, whey protein ingestion stimulated myofibrillar protein synthetic rates to a greater degree than micellar casein at rest in both FED ($P = 0.002$) and EX-FED ($P < 0.001$) conditions (Fig. 1).

Discussion

We report for the first time that whey protein ingestion stimulates rates of myofibrillar protein synthesis greater than the equivalent protein ingested as micellar casein in healthy elderly men not only at rest, but also after resistance exercise. The practical application of this finding, matching that of Penning *et al.*⁽⁷⁾, is that isolated whey protein is a more effective source of protein for augmenting postprandial muscle protein synthesis rates in older men. Moreover, the greater stimulatory effect on myofibrillar protein synthesis with whey was also observed during post-exercise recovery where the myofibrillar protein synthetic rate was approximately 60% greater than that seen after ingestion of micellar casein. Collectively, these results are consistent with a greater stimulatory effect of whey protein ingestion *v.* other, more slowly digested high-quality dietary proteins of similar amino acid concentrations, such as micellar casein, on mixed muscle protein synthesis rates previously observed in young⁽⁶⁾ and elderly men⁽⁷⁾.

The present data are at odds with the results of Reitelsheder *et al.*⁽¹⁹⁾ in young men and Dideriksen *et al.*⁽²⁰⁾ in older men and women demonstrating that ingestion of whey protein compared with calcium caseinate resulted in similar rates of myofibrillar protein synthesis. Certainly, methodological differences including different types of protein (i.e. micellar casein *v.* calcium caseinate), the timing of the synthetic

Table 1. Blood amino acid and plasma insulin concentrations after ingestion of 20 g micellar casein or whey protein in elderly men (Mean values with their standard errors (n 7))

	Time after drink (min)													
	0		30		60		90		120		180		240	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Micellar casein														
EAA‡ (µM)	615	44	832	112	969*	75	940*	15	866	47	723	19	695	38
Leu (µM)	103	4	188*	30	202*	21	212*	15	181*	14	187*	17	150.4	17
Insulin (pmol/l)	39.1	7	–	–	55.1	6	–	–	30.7	3	33.0	4	22.0	2
Whey														
EAA‡ (µM)	584	75	1015*†	84	1189*†	80	1062*	60	873*	70	740	63	647	74
Leu (µM)	109	13	233*	16	296*†	20	245*	16	177*	18	158	15	133	16
Insulin (pmol/l)	36.2	5	–	–	101.2*†	17	–	–	36.5	7	29.2	5	32.6	6

EAA, essential amino acids.

* Mean values were significantly different from the 0 time point ($P < 0.05$).

† Mean values were significantly different from those of the micellar casein group ($P < 0.05$).

‡ EAA (sum of Leu, Ile, Val, Phe, Lys, His, Met and Thr; note that Trp was not measured).

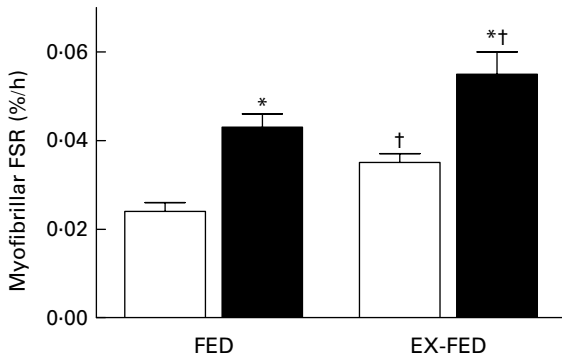


Fig. 1. Myofibrillar protein synthesis (fractional synthetic rate (FSR)) after ingestion of 20 g whey protein isolate (■) or micellar casein (□) at rest (FED) and after resistance exercise (EX-FED). Values are means with their standard errors represented by vertical bars (n 7). *Mean values were significantly different from those of the micellar casein group for the same condition ($P < 0.05$). †Mean values were significantly different from those of the FED group ($P < 0.05$).

measurements (i.e. 4 *v.* 6 h of recovery) and/or the tracer infused (i.e. [¹³C]leucine *v.* ring-[¹³C₆]phenylalanine) may account for the contrasting findings between their work^(19,20) and ours⁽⁶⁾. The main difference, however, probably relates to the characteristics of micellar casein, the pure native component found in milk, having different absorption kinetics from that of calcium caseinate. This point is not trivial, as it is clear that the increased solubility induced by reacting casein with an alkali of Ca increases the rate of digestion and speeds the resultant aminoacidaemia after calcium caseinate ingestion^(19,20) to a markedly faster rate of that seen with micellar casein⁽⁶⁾. The longer tracer incorporation time (i.e. approximately 6 h) may have precluded the researchers' ability to detect the potential benefits of whey protein, since hyperaminoacidaemia after whey ingestion is rapid and relatively transient^(19,20). In fact, Reitelseder *et al.*⁽¹⁹⁾ observed a trend towards a greater muscle protein synthetic rate up to 3 h of recovery after ingestion of whey when compared with calcium caseinate in young men⁽¹⁹⁾. The same researchers also observed a sharp decline in rates of myofibrillar protein synthesis between 3 and 6 h following whey ingestion after resistance exercise, which is inconsistent with our data of a sustained elevation in myofibrillar protein synthesis rates during this time⁽⁹⁾.

The present results in elderly men are entirely consistent with our recent observation of a greater post-exercise rise in myofibrillar protein synthesis with a bolus ingestion of whey when compared with small repeated feedings mimicking that of casein ingestion⁽²¹⁾ and with those of Pennings *et al.*⁽⁷⁾. However, determining myofibrillar protein synthesis rates, as we did in the present study, will generally result in rates that are approximately 2–3-fold lower than mixed muscle protein synthesis rates, which was the protein fraction studied by Pennings *et al.*⁽⁷⁾. It is noteworthy that the degree of insulinaemia was greater after whey protein consumption (Table 1). Although this result may have had little influence on the observed myofibrillar protein synthesis rates⁽²²⁾, a higher insulinaemia after meal consumption may be

favourable in terms of suppressing proteolysis in ageing muscle⁽²²⁾. Regardless, we propose that the ingestion of a leucine-rich and rapidly digested protein, such as whey, has the best potential to maximally stimulate rates of myofibrillar protein synthesis during the acute recovery period after exercise in aged muscle. It should also be highlighted that the present results are consistent with recent reports in elderly men⁽⁵⁾ demonstrating that casein protein is still able to support increased rates of muscle protein synthesis, albeit at lower rate than whey, when ingested after exercise.

In the 'real world', it is common for individuals, regardless of age, to consume mixed meals that contain all macronutrients. Indeed, insulin resistance of muscle protein metabolism in ageing muscle is a recurring theme^(22–24). Thus, the co-ingestion of carbohydrate, and the associated greater insulinaemia, with dietary protein may be beneficial for the feeding-induced stimulation of muscle protein synthesis rates in the elderly. However, we would expect little additional benefit in terms of stimulating muscle protein synthesis with co-ingestion of carbohydrate with a saturating dose of whey protein in older participants, which is what we observed in younger men⁽²⁵⁾. Indeed, the optimal dose of whey protein to consume remains to be defined in ageing muscle, either at rest or after resistance exercise. Moreover, carbohydrate co-ingestion may also be beneficial for the stimulation of muscle protein synthesis rates after ingestion of a protein source which results in a prolonged aminoacidaemia, such as micellar casein. Given all this, it is clear that further research is necessary to confirm any speculations.

The present study reveals that in elderly men, a rapid increase in EAA and leucine after ingestion of isolated whey protein stimulated greater FED and EX-FED when compared with a prolonged leucinaemia that occurs with micellar casein ingestion. This finding continues to support a growing body of evidence suggesting that the amplitude and possibly the rate of postprandial rise in circulating amino acids in the blood dictates the extent of stimulation of myofibrillar protein synthesis. The present data may have a practical ramification for formulations of protein feedings designed to maximise muscle protein synthesis rates, which would appear to be most beneficial when consisting of whey protein.

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