International Dairy Journal 63 (2016) 35–41

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Understanding the sensitivity of muscle protein synthesis to dairy protein in middle-aged men

Cameron J. Mitchell [a, *], Randall F. D'Souza [a], Nina Zeng [a], Robin A. McGregor [b, d], Aaron C. Fanning [c], Sally D. Poppitt [b], David Cameron-Smith [a]

[a] Liggins Institute, University of Auckland, Private Bag 92 019, Victoria Street West, Auckland 1142, New Zealand
[b] School of Biological Sciences, University of Auckland, Private Bag 92 019, Victoria Street West, Auckland 1142, New Zealand
[c] Fonterra Research and Development Centre, Private Bag 11 029, Palmerston North 4442, New Zealand
[d] Cardiovascular and Metabolic Disease Centre, College of Medicine, Inje University, 633-165, Gaegeum-Dong, Busanjin-Gu 614-735, South Korea

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
19 July 2016
Accepted 21 July 2016
Available online 4 August 2016

ABSTRACT

Although the consumption of dairy protein results in a robust increase in muscle protein synthesis (MPS), the magnitude of the increase is dependent upon the dose of protein ingested and the age of the individual. The minimum dose of dairy protein that can stimulate MPS in middle age (50.1 ± 4.5 years), a period of life during which muscle mass is typically lost, is not known. Sixteen middle-aged men (45–60 years) were randomly assigned to consume beverages containing either 10 g of milk protein (MP10) or 6 g of MP plus 20 g of carbohydrate (MP6 + CHO). A primed constant infusion of $^{13}C_6$ phenylalanine was maintained and muscle biopsy samples were collected to calculate MPS. MP10 increased MPS above fasting levels for 90 min after ingestion, whereas MP6 + CHO did not increase MPS. This study demonstrates that between 6 and 10 g of MP is required to stimulate MPS in middle-aged men.

1. Introduction

Protein is an important macronutrient for many biological processes including the maintenance of muscle mass and the repair of damaged tissue (Wolfe, 2012). Dairy products are well established as providing a source of high quality dietary protein (Phillips, Tang, & Moore, 2009) and have been incorporated into a wide range of consumer products to support muscle function. Many of these products have been targeted towards middle-aged adults who are aiming to maintain optimal health (Lagrange, Whitsett, & Burris, 2015). Although a wide range of dairy preparations currently exists, the protein content is variable. To date, little information on the minimum amount of dairy protein that will provide an anabolic benefit in middle-aged adults is available.

In the post-absorptive state, muscle protein breakdown (MPB) predominates over muscle protein synthesis (MPS) (Biolo, Tipton, Klein, & Wolfe, 1997). Feeding with either protein or carbohydrate can suppress MPB, but protein is required to stimulate MPS and induce a period of net muscle anabolism (Glynn et al., 2010a). The dose of high quality protein required to maximally stimulate MPS has been shown to be 20 g or less in young men (Witard et al., 2014). With ageing, there appears to be a greater resistance to the anabolic actions of ingestion, such that the required dose to maximally stimulate protein synthesis is increased (Pennings et al., 2012; Yang et al., 2012). It is likely that middle age represents a transition period between the high degree of anabolic sensitivity observed in young adults and the anabolic resistance observed in older adults. Understanding the sensitivity of responsiveness and the lower limits of functionality is important for the crafting of recommendations on the required protein dose in a single meal. Such information is of benefit for the formulation of products that can be used to supplement meals with inadequate protein quality or quantity, as present in many parts of the developing world, which still experiences protein–energy malnutrition (Schönfeldt & Gibson Hall, 2012; Swaminathan, Vaz, & Kurpad, 2012). In other circumstances, protein distribution is uneven across the day, with recent evidence suggesting that a more even distribution of protein intake throughout the day can improve daily MPS rates and may help to promote muscle mass retention with ageing (Mamerow et al., 2014), especially during energy deficit (Murphy et al., 2015).

Dairy proteins contain essential amino acids (AAs), particularly the AA leucine, which is known to stimulate MPS via activation of

* Corresponding author. Tel.: +64 9 923 6606.
E-mail address: cameron.mitchell@auckland.ac.nz (C.J. Mitchell).

http://dx.doi.org/10.1016/j.idairyj.2016.07.008
0958-6946/

the mammalian target of rapamycin (mTOR) pathway (Tang & Phillips, 2009). As mTOR functions in association with a number of other proteins, its activity is inferred from its downstream targets, such as ribosomal protein S6 (rpS6), which is involved in protein translation initiation (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009). Insulin is known to simulate MPS is the post-absorptive state through the activation of Akt (protein kinase B), which is an up stream positive regulator of mTOR (Timmerman et al., 2010). When amino acid availability is optimal additional insulin does not further stimulate MPS (Glynn et al., 2013; Greenhaff et al., 2008). It is unknown if a larger plasma insulin concentration will have any effects on MPS in the presence of a suboptimal protein dose.

This study examined whether a single bolus of 10 g of milk protein (MP) or 6 g of MP combined with carbohydrate in a formulated dairy product is sufficient to stimulate MPS above fasted levels in middle-aged men. We have previously shown that 20 g of MP stimulates MPS in this age group (Mitchell et al., 2015b). The middle-aged range was chosen as it is likely to represent the onset of anabolic resistance and is a life stage during which muscle loss starts to impact on muscular function (von Haehling, Morley, & Anker, 2010); as yet, few data in this age group are available. The study also examined the intracellular mechanisms of action by measuring the activation of the mTOR pathway.

2. Methods

2.1. Subjects

Sixteen middle-aged men participated in the study; they were free of metabolic and neuromuscular or associated diseases or pharmacotherapy at the time of the study. None of the subjects had previously been infused with a $^{13}C_6$ phenylalanine tracer. Baseline body composition and blood biochemistry were analysed as previously described (Mitchell et al., 2015b) The homeostatic model assessment insulin resistance (HOMA-IR) was calculated as described by (Hill, Levy, & Matthews, 2013; Matthews et al., 1985). Written consent was obtained before the commencement of study which was approved by the Northern Health and Disability Ethics Committee (New Zealand).

2.2. Study design

On the evening before to the infusion study, participants consumed a standard dinner (30% fat, 55% carbohydrate, 15% protein) containing one-third of their daily caloric requirements, as estimated by the Harris–Benedict equation (Roza & Shizgal, 1984). The meal was consumed prior to 10 p.m. and the participants fasted until they arrived at the laboratory the following morning at 7 a.m. A cannula was inserted into an arm vein, a baseline blood sample was then collected. A saline drip was employed to ensure the catheter was patent and blood samples were arterialised using a heating blanket. A primed constant infusion of L-[ring-$^{13}C_6$] phenylalanine (prime: 2 μmol kg^{-1}; infusion: 0.05 μmol kg^{-1} min^{-1}) was initiated via a second cannula in the contralateral arm and sustained until the final muscle biopsy was complete. Participants then rested quietly in a supine position for 3 h. After a blood sample, a muscle biopsy sample was then obtained from the vastus lateralis as described by (Tarnopolsky, Pearce, Smith, & Lach, 2011). Immediately following the biopsy, the participants consumed one of two study beverages (described in Section 2.3). Plasma samples were collected every 15 min for the first 90 min after beverage consumption and then 120, 180 and 210 min. Additional biopsies were obtained at 90 and 210 min after ingestion of the beverage.

2.3. Study beverages

Participants consumed either 10 g of MP (Milk Protein Concentrate 485; Fonterra Co-operative Group Ltd, Auckland, New Zealand) (MP10) or a formulated dairy product containing 6.4 g of MP plus 20 g of carbohydrate (MP6 + CHO). Both beverages were supplied by Fonterra Co-operative Group Ltd (Auckland, New Zealand). Each ingredient was dissolved in 350 mL of water (Table 1 gives the composition data). Group allocation was randomised and both participants and researchers were blinded to the identity of the beverages.

Table 1
Beverage composition.[a]

Component	MP10	MP6 + CHO
Energy	186	461
Carbohydrate	0.6	20.0
Fat	0.2	0.9
Total protein	10.0	6.4
Aspartic acid/asparagine	0.7	0.6
Threonine	0.4	0.4
Serine	0.5	0.3
Glutamic acid/glutamine	2.1	1.1
Proline	0.9	0.4
Glycine	0.2	0.1
Alanine	0.3	0.3
Valine	0.6	0.3
Isoleucine	0.5	0.4
Leucine	0.9	0.6
Tyrosine	0.5	0.2
Phenylalanine	0.5	0.2
Lysine	0.7	0.5
Histidine	0.3	0.1
Arginine	0.3	0.2
Cystine	0.1	0.1
Methionine	0.3	0.1
Tryptophan	0.2	0.1

[a] All values are g except for Energy that is given in kJ; total protein calculated as total nitrogen × 6.25.

2.4. Fractional synthetic rate

Myofibrillar fractional synthetic rate was calculated as previously described (Burd et al., 2010b; Mitchell et al., 2015b). Briefly muscle samples were homogenised in a buffer containing a protease/phosphatase inhibitor cocktail. Centrifugation then separated the myofibrillar pellet that was used of fractional synthetic rate analysis from the homogenate that was used for western blotting. The pellet was then hydrolysed overnight, the free amino acids were purified using an ion exchange column prior to conversion to their *N*-acetyl-n-propyl ester derivatives. A combustion–isotope ratio mass spectrometer was then used to measure isotopic enrichments in the samples. The precursor product method was used to calculate factional synthetic rate (FSR; Burd et al., 2010a) with plasma phenylalanine enrichments acting as the precursor pool (Mitchell et al., 2015b). The fasting FSR was calculated using an average pre-infusion protein-bound enrichment from previous studies (Mitchell et al., 2015b, 2014) because of technical difficulties with the baseline plasma enrichment measurement from isotope ratio mass spectrometry. This method results in slightly more variable fasted rates (Smith et al., 2010); however, the fasted rates we report are very similar to those in our previous studies and do not alter the conclusions or main findings of the paper.

2.5. Western blotting

The muscle homogenate supernatant (described above) was used to determine total protein content of each sample via

bicinchoninic acid assay. Western blotting was performed using as previously described (D'Souza et al., 2014) using sodium dodecyl sulphate-polyacrylamide gel electrophoresis then transferred to a polyvinyl fluoride membrane. Primary antibodies and dilutions are described in (Mitchell et al., 2015a,b). Imaging and spot densitometry were performed as previously reported (D'Souza et al., 2014); glyceraldehyde 3-phosphate dehydrogenase was used to normalise for protein loading.

2.6. Plasma analysis

Ultra-high pressure liquid chromatography was used to determine plasma AA concentration as previously described (Milan et al., 2015). Standard curves for each AA were used to calculate AA concentrations. Insulin and blood biochemistry were analysed as described in Mitchell et al. (2015b). Whole body rate of phenylalanine appearance (R_a) was calculated as described by Mikkelsen et al. (2015) and expressed relative to body mass.

2.7. Statistical analysis

Differences in the characteristics of the subjects at baseline and area under the curve (AUC) measurements were assessed using T-tests. Differences in MPS, plasma AA concentration and anabolic signalling were assessed with two-way repeated measures analysis of variance with time as a within-subject factor and group as a between-subject factor. Sidak's post hoc method was used to assess between-group differences. All data are reported as mean ± standard deviation in the text and tables and as mean ± standard error of the mean (SEM) in the figures. Significance was set at alpha ≤0.05. All statistical analyses were conducted using SPSS version 20.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Subjects

The characteristics of the subjects are shown in Table 2. Participants had a mean age of ~50 years and a body mass index of ~27, and were confirmed as healthy based on screening blood biochemistry. There were no differences in any anthropometric characteristics or biochemistry at baseline.

3.2. Glucose and insulin

The plasma glucose concentration was significantly elevated above baseline at 30, 45 and 60 min after the ingestion of MP6 + CHO but not MP10 (time × beverage, $P < 0.001$, Fig. 1A). The glucose AUC was smaller in the MP10 group [27 ± 62 arbitrary units (AU)] than in the MP6 + CHO group (108 ± 26 AU; $P = 0.020$). The plasma insulin concentration was significantly elevated above baseline between 30 and 45 min after the ingestion of MP6 + CHO and was not increased following MP10 ingestion when corrected for multiple comparisons ($P = 0.001$, Fig. 1B). The insulin AUC was lower in the MP10 group (3381 ± 2613 AU) than in the MP6 + CHO group (7596 ± 3288 AU; $P = 0.013$).

Table 2
Baseline characteristics of subjects in the fasted state.[a]

Parameter	MP10 ($n = 8$)	MP6 + CHO ($n = 8$)	P
Age (years)	48.9 ± 4.4	51.2 ± 4.6	0.324
Body mass (kg)	86.8 ± 9.2	82.7 ± 9.0	0.384
Height (cm)	179.1 ± 7.0	178.5 ± 6.2	0.860
Fat mass (kg)	21.0 ± 4.4	17.3 ± 5.3	0.153
Lean mass (kg)	62.3 ± 7.3	61.7 ± 6.2	0.862
Body fat (%)	25.2 ± 4.0	21.7 ± 4.7	0.133
Glucose (mmol L^{-1})	5.53 ± 0.90	5.38 ± 0.72	0.719
Insulin (μU mL^{-1})	6.15 ± 2.49	5.52 ± 2.30	0.608
HOMA	1.58 ± 0.74	1.34 ± 0.61	0.492
Triglycerides (mmol L^{-1})	1.45 ± 0.61	1.20 ± 0.38	0.309
LDL-C (mmol L^{-1})	3.44 ± 1.17	3.68 ± 1.04	0.672
Cholesterol (mmol L^{-1})	5.45 ± 1.39	5.73 ± 1.10	0.662
HDL-C (mmol L^{-1})	1.12 ± 0.22	1.29 ± 0.41	0.319

[a] Abbreviations are: HOMA, homeostasis model assessment; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; values are means ± standard deviations, P values are the result of independent sample T tests.

3.3. Plasma amino acids

There was a main effect for the total plasma AA concentration to increase above the fasting baseline at 30 min following ingestion of the study beverage ($P < 0.001$), but the time × beverage interaction did not reach significance ($P = 0.115$; Fig. 2A); hence, there was no difference over time between the two dairy treatments. The total AA AUCs were 39,681 ± 30,868 AU and 23,163 ± 13,909 AU in the MP10 and MP6 + CHO groups, respectively, with no difference between the groups ($P = 0.190$).

The essential amino acid (EAA) concentration was elevated above baseline following ingestion of the study beverage to a greater extent in the MP10 group than in the MP6 + CHO group (time × beverage, $P = 0.003$, Fig. 2B). The EAA concentration was significantly higher in the MP10 group than in the MP6 + CHO group at 60, 75 and 150 min post beverage ingestion. The EAA AUCs were 24,905 ± 11,221 AU and 10,953 ± 4313 AU in the MP10 and

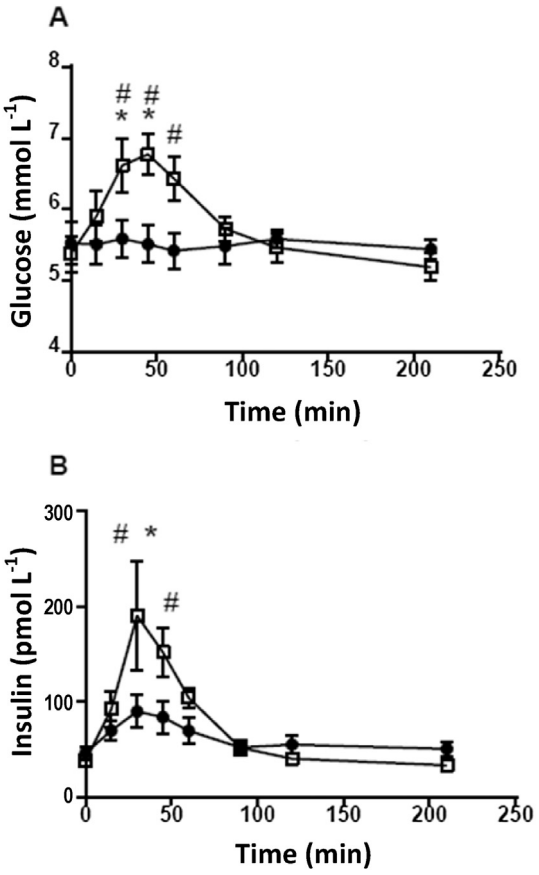

Fig. 1. Plasma glucose (A) and insulin (B) concentrations in response to consumption of MP10 (■) and MP6 + CHO (□). Data are means ± SEM; * denotes difference between MP10 and MP6 + CHO at the same time point, $P < 0.05$; # denotes difference from time 0 within the same group, $P < 0.05$.

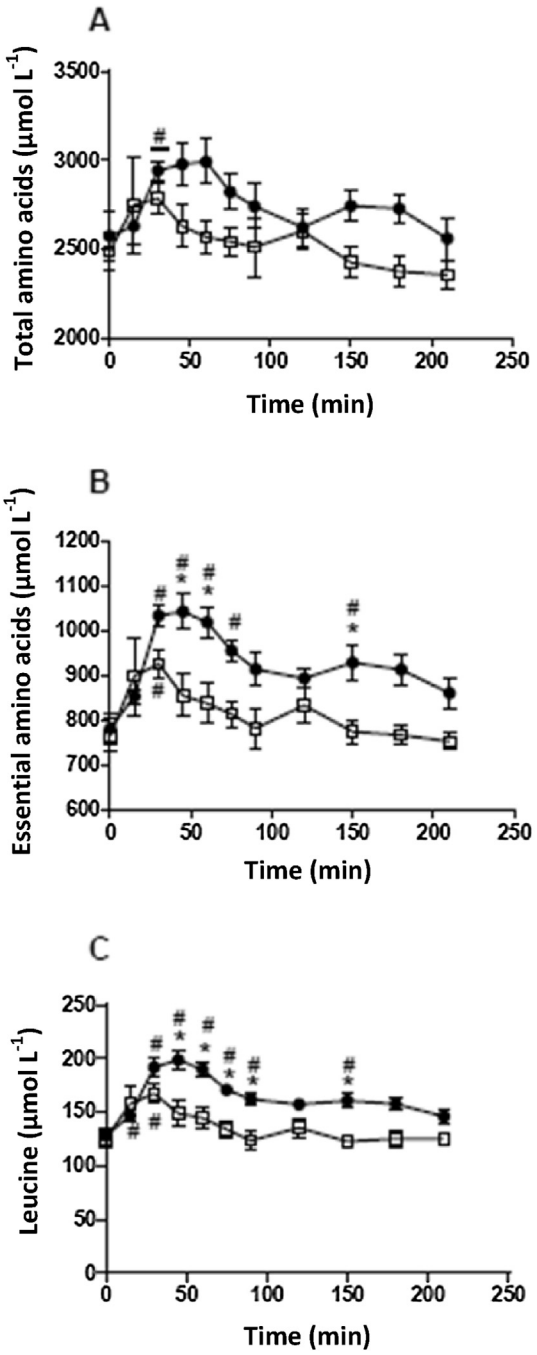

Fig. 2. Plasma total amino acid (A), essential amino acid (B) and leucine (C) concentrations in response to consumption of MP10 (■) and MP6 + CHO (□). Data are means ± SEM and represent 8 participants per group; * denotes difference between MP10 and MP6 + CHO at the same time point, $P < 0.05$; # denotes the difference from time 0 within the same group, $P < 0.05$. The horizontal line represents a main effect for time, irrespective of the beverage group.

MP6 + CHO groups, respectively, and the AUC was greater in the MP10 group ($P = 0.005$). Leucine was elevated above baseline to a greater extent following MP10 ingestion than following MP6 + CHO ingestion (time × beverage, $P < 0.001$; Fig. 2C). The leucine concentration was higher in the MP10 group between 45 and 90 min post beverage consumption. The leucine AUCs were 6440 ± 2678 AU and 2480 ± 835 AU in the MP10 and MP6 + CHO groups, respectively, and the AUC was greater in the MP10 group ($P = 0.001$).

3.4. Anabolic signalling

Akt phosphorylation at the serine (Ser) 473 residue was significantly increased above that at the fasted baseline at 90 min post ingestion of the study beverage in both groups (time, $P = 0.002$; Fig. 3A), but no change was seen at the threonine (Thr) 308 residue ($P = 0.273$; Fig. 3B), and no treatment difference was observed at either phosphorylation site. Phosphorylation of rps6 at the Ser 235/236 residues was significantly increased at both 90 and 210 min post beverage ingestion (time, $P = 0.003$; Fig. 3C), and no treatment difference was observed at either phosphorylation site. No change in rps6 phosphorylation at the Ser 240/244 residues was observed; however, at 90 min post beverage ingestion, there was a trend for greater phosphorylation in the MP10 group than in the MP6 + CHO group ($P = 0.070$; Fig. 3D).

3.5. Plasma tracer enrichment

The plasma $^{13}C_6$ phenylalanine tracer to tracee ratio did not differ between the groups and did not differ during each period in which muscle FSR was calculated (−120 to 0 min, 0–90 min and 90–210 min). There was a slight increase in the tracer to tracee ratio from the −120 min time point to the 90 and 120 min time points (Fig. 4A, change from −120 min baseline, $P < 0.05$). The rate of phenylalanine appearance (R_a) was used as a marker of whole body protein breakdown, R_a decreased from fasted levels in the period 90–210 min following beverage consumption in both groups with no difference between groups (Fig. 4B, $P = 0.003$).

3.6. Muscle fractional synthetic rate

The myofibrillar FSR was increased above baseline levels at between 0 and 90 min following the ingestion of MP10 and returned to baseline at between 90 and 210 min following consumption (time × beverage, $P = 0.049$; Fig. 5). The consumption of MP6 + CHO did not increase the FSR above the baseline.

4. Discussion

This study aimed to identify the dose of dairy protein that is required to initiate a measureable protein synthetic response in the skeletal muscle of a major population group (middle-aged) that could potentially derive an anabolic benefit from regular ingestion of a high quality protein. This analysis is in contrast to the many studies that have been conducted previously to determine the quantity of dairy protein required to maximally stimulate MPS (Pennings et al., 2011; Witard et al., 2014; Yang et al., 2012). In this study, we demonstrated that 10 g, but not 6 g, of MP is sufficient to increase MPS above baseline in healthy middle-aged men. Moreover, we showed the lack of efficacy of 6 g of MP, even when combined with an additional 20 g of a carbohydrate matrix that stimulated a robust insulin response. Additionally, this study was conducted using a milk protein concentrate, thus providing data that a whole milk fraction delivering 10 g or greater of MP can have a beneficial effect on skeletal muscle anabolic responses.

The consumption of 10 g of MP resulted in a transient increase in MPS of approximately twofold above fasted levels for 90 min. The insulin response to this protein dose was minimal; however, it was large enough to stimulate the phosphorylation of Akt at Ser 473. This dose of protein was also sufficient to activate the downstream mTOR target rps6 at Ser 235/236. There was also a trend towards a greater activation of rps6 at Ser 240/244 ($P = 0.07$). The small magnitude of the observed signalling response was probably due to a combination of the small protein dose ingested and the choice of 90 min post dose as the first signalling time point. This choice was

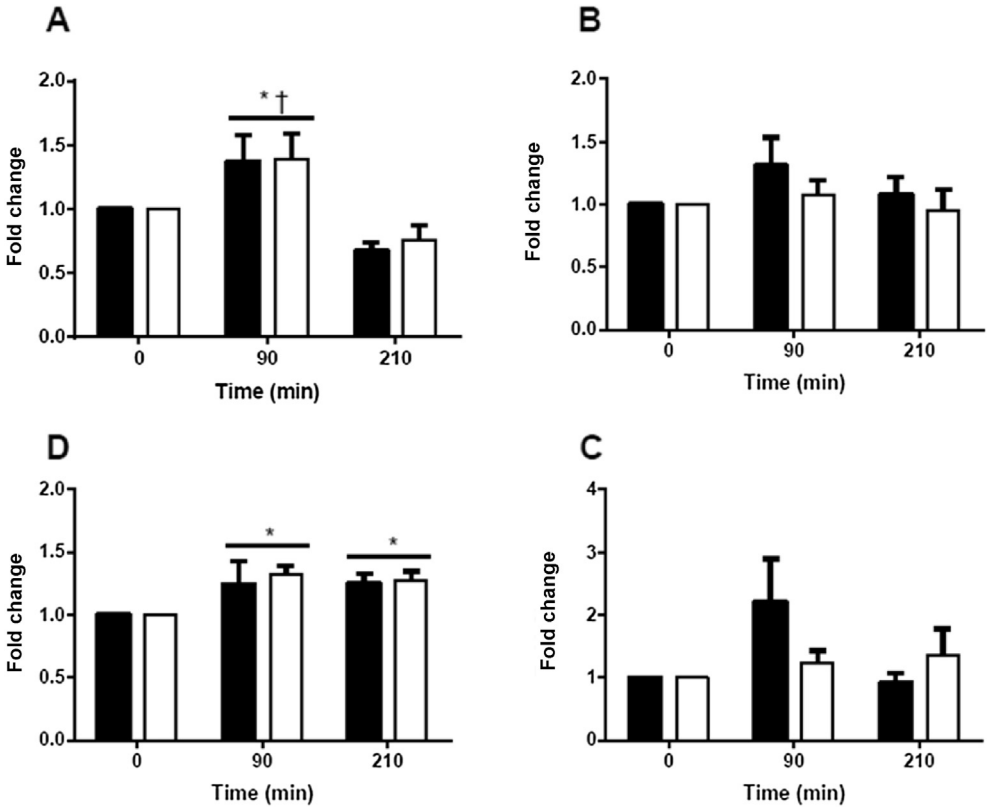

Fig. 3. Anabolic signalling: change in phosphorylated Akt (Ser 473) (A), Akt (Thr 308) (B), rps6 (Ser 240/244) (C) and rps6 (Ser 235/236) (D) in response to consumption of MP10 (■) and MP6 + CHO (□). All values are expressed as fold change from rest and are expressed relative to GAPDH. Data are means ± SEM and represent 8 participants per group; horizontal lines represent main effects; * denotes different from time 0, $P < 0.05$; † denotes different from time 210 min, $P < 0.05$.

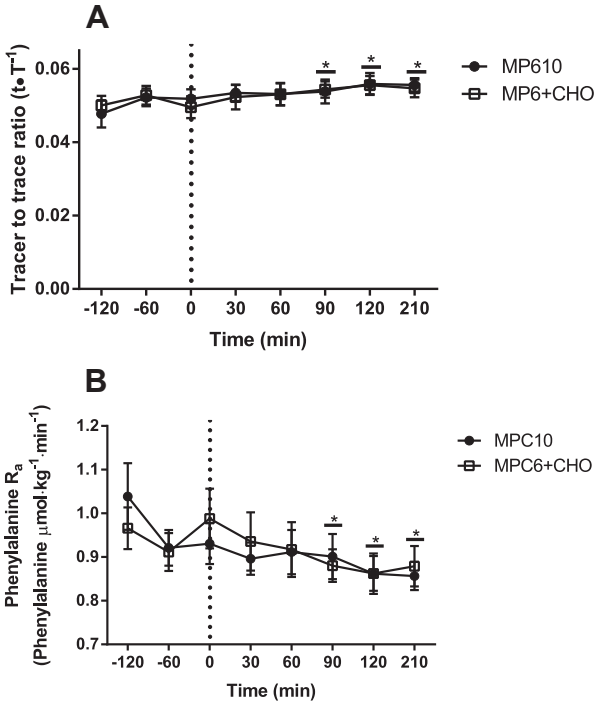

Fig. 4. Plasma $^{13}C_6$ phenylalanine kinetics: tracer to tracee ratio (A), phenylalanine R_a (B). The vertical dotted line represents the time when the beverages MP10 (■) and MP6 + CHO (□) were ingested. Data are means ± SEM and represent 8 participants per group; horizontal lines represent main effects; * denotes main effect for difference from baseline with no difference between groups, $P < 0.05$.

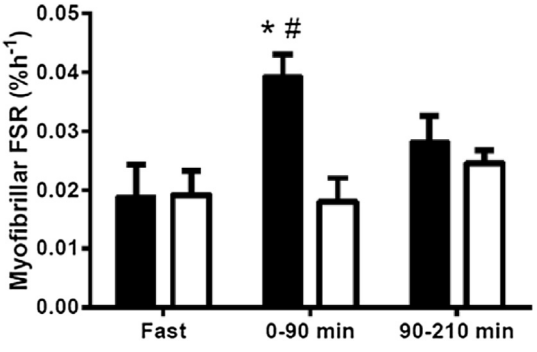

Fig. 5. Muscle protein synthesis. The rate of myofibrillar protein synthesis in the fasted state and in response to consumption of MP10 (■) and MP6 + CHO (□). Data are means ± SEM and represent 8 participants per group; * denotes different from MP6 + CHO at the same time point, $P < 0.05$; # denotes different from the fasted condition within the same beverage group, $P < 0.05$.

made to accommodate the measurement of MPS but was probably too late to capture the peak of the anabolic signalling response given the small protein does consumed (Glynn et al., 2010b).

The ingestion of a beverage containing 6 g of MP and 20 g of carbohydrate did not result in a detectable elevation in MPS for either 0–90 min or 90–210 min after the beverage was consumed. The combination of 6 g of MP with 20 g of carbohydrate resulted in an approximately 11-fold higher glucose AUC and an approximately twofold higher insulin AUC, compared with 10 g of MP over the full measurement period of 210 min. Despite the large divergence in insulin response, there were no differences in the degree of Akt phosphorylation at the Ser 473 and Thr 308 sites. This suggests that,

in agreement with previous work, insulin plays a permissive role for the stimulation of MPS, but a larger insulin response provides no additional benefit in terms of MPS or downstream signalling (Churchward-Venne et al., 2015; Greenhaff et al., 2008; Staples et al., 2011). Although carbohydrate can suppress MPB by stimulating insulin secretion, there is no evidence that carbohydrate can increase the stimulation of MPS that is induced by an optimal protein dose. In addition, it is not known if the addition of carbohydrate to a small protein dose could enhance MPS (Churchward-Venne et al., 2015; Figueiredo & Cameron-Smith, 2013; Kramer et al., 2015). The present study shows that, even at a low protein dose, additional insulin does not stimulate MPS.

Recent work (Kramer et al., 2015) showed that the addition of fat and carbohydrate to a serving of dairy protein slightly modified the plasma AA response to feeding but did not alter MPS, although the carbohydrate dose in this study was modest (9 g). Similarly, when 25 g of dairy protein was ingested in a milk matrix containing ~30 g of carbohydrate, the AA response to feeding was severely blunted but MPS was not altered (Churchward-Venne et al., 2015). It is unknown if a greater splanchnic retention is responsible for the observed differences. These findings agree with the current study, in which the AUCs for EAAs and leucine were more than twofold greater in the MP10 group than in the MP6 + CHO group, despite only a ~0.4-fold difference in ingested protein. Based on the above studies, it seems that the addition of carbohydrate was the probable cause of the lower AA appearance in the MP6 + CHO group. Despite this, the same studies suggest that it is highly unlikely that the addition of carbohydrate had any impact on MPS; nevertheless, because no MP6 group without added carbohydrates was included this possibility cannot be entirely ruled out. It is possible that the addition of carbohydrate in the MP6 + CHO group could have reduced protein breakdown to a greater extent than if 6 g of MP was consumed in isolation thus improving muscle protein balance. Based both on the similar observed reduction in phenylalanine R_a both the MP10 and MP6 + CHO groups and the lack of further suppression of protein breakdown when carbohydrate is added to an optimal dose of EAAs (Glynn et al., 2013) it is unlikely that the addition carbohydrates had any impact on net protein balance.

Middle age is a poorly defined stage of adulthood, but is generally held to encompass the years between 40 and 60. This life stage represents a transition period between a maintenance of skeletal muscle mass and function and a gradual decline in muscle mass and function, which is seen with ageing (von Haehling et al., 2010). This transition is at least partially underpinned by the transition from an anabolic sensitivity to protein feeding in young adults to the anabolic resistance to protein feeding observed in older adults (Breen & Phillips, 2011). As there has been very little research into the anabolic sensitivity of middle-aged adults, the present study is unique in showing that the minimum threshold for an anabolic response to feeding is between 6 and 10 g of dairy protein. This threshold might be slightly lower in younger adults (Moore et al., 2009) or after exercise (Timmerman et al., 2012) and is likely to be higher in older adults (Cuthbertson et al., 2005) or in very inactive adults (Breen et al., 2013). It is important to study the anabolic response to protein feeding in a variety of populations because the findings can not necessarily be generalized across age and sex groups (Smith et al., 2012).

5. Conclusions

Dairy protein is a common ingredient that can be used to add high quality protein to formulated beverages and foods. Protein is often added to foods to promote muscle health by increasing MPS (Day & Swanson, 2013), which, over time, may promote the maintenance of muscle mass and function and support exercise-induced gains in muscle mass and function (Phillips, 2014). The present study shows that 10 g of MP is an effective protein dose to stimulate MPS in middle-aged men at rest, whereas 6 g, even when combined with additional carbohydrate, is not. This information gives food formulators the ability to design a wide range of products that will generate an anabolic response. Doses of dairy protein of less than 6 g should not be used in products that are intended to increase MPS in middle-aged men, although they may be beneficial in the context of a complete meal.

Acknowledgements

The authors would like to thank the Liggins Institute Clinical Research Unit staff; we would also like to thank Angela Rowan, Linda Schollum and Carmen Norris for their helpful input. Lastly, we would like to thank all the participants for their time commitment. CJM, RAM, RFD and DCS received financial support from the New Zealand Primary Growth Partnership (PGP) post-farm gate programme, funded by Fonterra Co-operative Group Ltd and the NZ Ministry for Primary Industries (MPI), to conduct this study. SDP is the Fonterra Chair in Human Nutrition, University of Auckland; ACF is a current employee of Fonterra Co-operative Group Ltd.

References

Biolo, G., Tipton, K. D., Klein, S., & Wolfe, R. R. (1997). An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *American Journal of Physiology, 273*, E122–E129.

Breen, L., & Phillips, S. M. (2011). Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing. *Nutrition and Metabolism, 8*. Article 68.

Breen, L., Stokes, K. A., Churchward-Venne, T. A., Moore, D. R., Baker, S. K., Smith, K., et al. (2013). Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *Journal of Clinical Endocrinology and Metabolism, 98*, 2604–2612.

Burd, N. A., Holwerda, A. M., Selby, K. C., West, D. W., Staples, A. W., Cain, N. E., et al. (2010a). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *Journal of Physiology, 588*, 3119–3130.

Burd, N. A., West, D. W., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., et al. (2010b). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PloS One, 5*, e12033.

Churchward-Venne, T. A., Snijders, T., Linkens, A. M., Hamer, H. M., van Kranenburg, J., & van Loon, L. J. (2015). Ingestion of casein in a milk matrix modulates dietary protein digestion and absorption kinetics but does not modulate postprandial muscle protein synthesis in older men. *Journal of Nutrition, 145*, 1438–1445.

Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., et al. (2005). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB Journal, 19*, 422–424.

D'Souza, R. F., Marworth, J. F., Figueiredo, V. C., Della Gatta, P. A., Petersen, A. C., Mitchell, C. J., et al. (2014). Dose-dependent increases in p70S6K phosphorylation and intramuscular branched-chain amino acids in older men following resistance exercise and protein intake. *Physiological Reports, 2*, e12112.

Day, L., & Swanson, B. G. (2013). Functionality of protein-fortified extrudates. *Comprehensive Reviews in Food Science and Food Safety, 12*, 546–564.

Drummond, M. J., Dreyer, H. C., Fry, C. S., Glynn, E. L., & Rasmussen, B. B. (2009). Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *Journal of Applied Physiology, 106*, 1374–1384.

Figueiredo, V. C., & Cameron-Smith, D. (2013). Is carbohydrate needed to further stimulate muscle protein synthesis/hypertrophy following resistance exercise? *Journal of the International Society of Sports Nutrition, 10*. Article 42.

Glynn, E. L., Fry, C. S., Drummond, M. J., Dreyer, H. C., Dhanani, S., Volpi, E., et al. (2010a). Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 299*, R533–R540.

Glynn, E. L., Fry, C. S., Drummond, M. J., Timmerman, K. L., Dhanani, S., Volpi, E., et al. (2010b). Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *Journal of Nutrition, 140*, 1970–1976.

Glynn, E. L., Fry, C. S., Timmerman, K. L., Drummond, M. J., Volpi, E., & Rasmussen, B. B. (2013). Addition of carbohydrate or alanine to an essential amino acid mixture does not enhance human skeletal muscle protein anabolism. *Journal of Nutrition, 143*, 307–314.

Greenhaff, P. L., Karagounis, L. G., Peirce, N., Simpson, E. J., Hazell, M., Layfield, R., et al. (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *American Journal of Physiology: Endocrinology and Metabolism, 295*, E595–E604.

Hill, N. R., Levy, J. C., & Matthews, D. R. (2013). Expansion of the homeostasis model assessment of β-cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: iHOMA2. *Diabetes Care, 36*, 2324–2330.

Kramer, F. I., Verdijk, L. B., Hamer, H. M., Verlaan, S., Luiking, Y., Kouw, I. W., et al. (2015). Impact of the macronutrient composition of a nutritional supplement on muscle protein synthesis rates in older men: A randomized, double blind, controlled trial. *Journal of Clinical Endocrinology and Metabolism, 100*, 4124–4132.

Lagrange, V., Whitsett, D., & Burris, C. (2015). Global market for dairy proteins. *Journal of Food Science, 80*(Suppl 1), A16–A22.

Mamerow, M. M., Mettler, J. A., English, K. L., Casperson, S. L., Arentson-Lantz, E., Sheffield-Moore, M., et al. (2014). Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *Journal of Nutrition, 144*, 876–880.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*, 412–419.

Mikkelsen, U. R., Dideriksen, K., Andersen, M. B., Boesen, A., Malmgaard-Clausen, N. M., Sorensen, I. J., et al. (2015). Preserved skeletal muscle protein anabolic response to acute exercise and protein intake in well-treated rheumatoid arthritis patients. *Arthritis Research and Therapy, 17*. Article 271.

Milan, A. M., D'Souza, R. F., Pundir, S., Pileggi, C. A., Barnett, M. P. G., Markworth, J. F., et al. (2015). Older adults have delayed amino acid absorption after a high protein mixed breakfast meal. *Journal of Nutrition, Health and Aging, 19*, 839–845.

Mitchell, C. J., Churchward-Venne, T. A., Parise, G., Bellamy, L., Baker, S. K., Smith, K., et al. (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. *PloS One, 9*, e89431.

Mitchell, C. J., Della Gatta, P. A., Petersen, A. C., Cameron-Smith, D., & Markworth, J. F. (2015a). Soy protein ingestion results in less prolonged p70S6 kinase phosphorylation compared to whey protein after resistance exercise in older men. *Journal of the International Society of Sports Nutrition, 12*. Article 6.

Mitchell, C. J., McGregor, R. A., D'Souza, R. F., Thorstensen, E. B., Markworth, J. F., Fanning, A. C., et al. (2015b). Consumption of milk protein or whey protein results in a similar Increase in muscle protein synthesis in middle aged men. *Nutrients, 7*, 8685–8699.

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition, 89*, 161–168.

Murphy, C. H., Churchward-Venne, T. A., Mitchell, C. J., Kolar, N. M., Kassis, A., Karagounis, L. G., et al. (2015). Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are restored with resistance training and balanced daily protein ingestion in older men. *American Journal of Physiology: Endocrinology and Metabolism, 308*, E734–E743.

Pennings, B., Boirie, Y., Senden, J. M., Gijsen, A. P., Kuipers, H., & van Loon, L. J. (2011). Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *American Journal of Clinical Nutrition, 93*, 997–1005.

Pennings, B., Groen, B., de Lange, A., Gijsen, A. P., Zorenc, A. H., Senden, J. M., et al. (2012). Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *American Journal of Physiology: Endocrinology and Metabolism, 302*, E992–E999.

Phillips, S. M. (2014). A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Medicine, 44*(Suppl 1), 71–77.

Phillips, S. M., Tang, J. E., & Moore, D. R. (2009). The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons. *Journal of the American College of Nutrition, 28*, 343–354.

Roza, A. M., & Shizgal, H. M. (1984). The Harris Benedict equation reevaluated: Resting energy requirements and the body cell mass. *American Journal of Clinical Nutrition, 40*, 168–182.

Schönfeldt, H. C., & Gibson Hall, N. (2012). Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition, 108*(Suppl 2), S69–S76.

Smith, G. I., Reeds, D. N., Hall, A. M., Chambers, K. T., Finck, B. N., & Mittendorfer, B. (2012). Sexually dimorphic effect of aging on skeletal muscle protein synthesis. *Biology of Sex Differences, 3*. Article 11.

Smith, G. I., Villareal, D. T., Lambert, C. P., Reeds, D. N., Mohammed, B. S., & Mittendorfer, B. (2010). Timing of the initial muscle biopsy does not affect the measured muscle protein fractional synthesis rate during basal, postabsorptive conditions. *Journal of Applied Physiology, 108*, 363–368.

Staples, A. W., Burd, N. A., West, D. W., Currie, K. D., Atherton, P. J., Moore, D. R., et al. (2011). Carbohydrate does not augment exercise-induced protein accretion versus protein alone. *Medicine and Science in Sports and Exercise, 43*, 1154–1161.

Swaminathan, S., Vaz, M., & Kurpad, A. V. (2012). Protein intakes in India. *British Journal of Nutrition, 108*(Suppl 2), S50–S58.

Tang, J. E., & Phillips, S. M. (2009). Maximizing muscle protein anabolism: The role of protein quality. *Current Opinion in Clinical Nutrition and Metabolic Care, 12*, 66–71.

Tarnopolsky, M. A., Pearce, E., Smith, K., & Lach, B. (2011). Suction-modified Bergstrom muscle biopsy technique: Experience with 13,500 procedures. *Muscle and Nerve, 43*, 717–725.

Timmerman, K. L., Dhanani, S., Glynn, E. L., Fry, C. S., Drummond, M. J., Jennings, K., et al. (2012). A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults. *American Journal of Clinical Nutrition, 95*, 1403–1412.

Timmerman, K. L., Lee, J. L., Dreyer, H. C., Dhanani, S., Glynn, E. L., Fry, C. S., et al. (2010). Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. *Journal of Clinical Endocrinology and Metabolism, 95*, 3848–3857.

von Haehling, S., Morley, J. E., & Anker, S. D. (2010). An overview of sarcopenia: Facts and numbers on prevalence and clinical impact. *Journal of Cachexia Sarcopenia Muscle, 1*, 129–133.

Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., & Tipton, K. D. (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *American Journal of Clinical Nutrition, 99*, 86–95.

Wolfe, R. R. (2012). The role of dietary protein in optimizing muscle mass, function and health outcomes in older individuals. *British Journal of Nutrition, 108*(Suppl 2), S88–S93.

Yang, Y., Breen, L., Burd, N. A., Hector, A. J., Churchward-Venne, T. A., Josse, A. R., et al. (2012). Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *British Journal of Nutrition, 108*, 1780–1788.