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

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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

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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