Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion

Michelle M. Farnfield, Leigh Breen, Kate A. Carey, Andrew Garnham, and David Cameron-Smith

Abstract: Purpose: To investigate the impact of whey protein ingestion and resistance exercise training on the phosphorylation of mRNA translational signalling proteins in the skeletal muscle of young and old men. Methods: Sixteen healthy young (aged 18–25 years) and 15 healthy older men (aged 60–75 years) completed 12 weeks of resistance exercise and were randomly assigned to consume a whey protein (WPI) or placebo drink after each session. Muscle biopsies were collected before and 2 h after an acute exercise bout at the beginning and the end of training. Results: All subjects significantly increased strength after following strength training. Phosphorylation of mTOR was significantly greater in the WPI groups compared with placebo for both younger and older subjects. Phosphorylation of p70S6K, eIF4G, and 4EBP1 was greater for older subjects consuming WPI. Phosphorylation of rpS6, eIF4G, and 4EBP1 tended to increase in the younger subjects that had consumed WPI. Post-training, younger subjects demonstrated a similar pattern of mTOR phosphorylation as seen pre-training. In contrast, the initial heightened phosphorylation of mTOR, p70S6K, rpS6, and eIF4G in older muscle to combined resistance exercise and WPI ingestion became less pronounced after repeated training sessions. Conclusions: In the untrained state, resistance exercise coupled with WPI increases the phosphorylation of proteins involved in mRNA translation compared with exercise alone. Post-training, WPI- and exercise-induced protein phosphorylation was reduced in older men, but not in younger men. Thus, strategies to induce hypertrophy should utilize protein and resistance training concurrently. Further investigations should delineate interventions that will maintain sensitivity to anabolic stimuli in older populations.

Key words: resistance exercise training, amino acids, translation initiation, age.

Résumé : But de l’étude. Cette étude se propose d’analyser l’effet de la protéine lactosérique et de l’entraînement contre résistance sur la phosphorylation des protéines de signalisation de la traduction de l’ARNm dans le muscle squelettique d’hommes jeunes et moins jeunes. Méthodologie. Seize hommes âgés de 18 à 25 ans et quinze hommes âgés de 60 à 75 ans, tous en bonne santé, participent à un programme d’entraînement contre résistance d’une durée de 12 semaines ; les participants divisés aléatoirement en deux groupes boivent un verre contenant de la protéine lactosérique (WPI) ou un placebo après chaque séance d’exercice. On prélève des biopsies musculaires au début et à la fin du programme d’entraînement ainsi qu’avant et 2 h après une séance d’exercice. Résultats. À la fin du programme d’entraînement contre résistance, on observe chez tous les sujets une augmentation significative de la force musculaire. Comparativement au groupe placebo, on observe chez le groupe WPI une phosphorylation de la mTOR significativement plus grande, et ce, tant chez les jeunes que chez les moins jeunes. Chez les hommes plus âgés ayant consommé du WPI, on observe une plus grande phosphorylation de p70S6K, d’eIF4G et de 4EBP1. Chez les plus jeunes sujets ayant consommé du WPI, on observe une tendance à l’augmentation de la phosphorylation de rpS6, d’eIF4G et de 4EBP1. À la fin du programme d’entraînement, on observe que chez les jeunes sujets le schéma de phosphorylation de la mTOR est similaire à celui observé au début du programme. Par contre, l’augmentation de la phosphorylation de mTOR, p70S6K, rpS6 et d’eIF4G observée au début du programme d’entraînement chez les sujets les plus âgés consommant du WPI est moins marquée au fur et à mesure de la répétition des séances d’entraînement. Conclusion. Un programme d’entraînement contre résistance combiné à la consommation de WPI chez des sujets non entraînés augmente la phosphorylation des protéines impliquées dans la transcription de l’ARNm, comparativement à un programme d’entraînement sans consommation de WPI. À la fin du programme d’entraînement, on observe chez les sujets plus âgés ayant fait de l’exercice et consommé du WPI une diminution de la phosphorylation, mais pas chez les plus jeunes. En conséquence, dans le but de susciter l’hypertrophie, il faut combiner l’entraînement contre résistance et l’apport de protéines. Il faut faire d’autres études pour définir les interventions qui maintiendront la réceptivité des personnes âgées aux stimuli anabolisants.

Received 27 May 2011. Accepted 23 September 2011. Published at www.nrcresearchpress.com/apnm on 13 December 2011.

M.M. Farnfield, K.A. Carey, and A. Garnham. School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria 3125, Australia.

L. Breen. Department of Kinesiology, McMaster University, Hamilton, ON L8S 4K1, Canada.

D. Cameron-Smith. School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria 3125, Australia; Liggins Institute, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.

Corresponding author: David Cameron-Smith (e-mail: d.cameron-smith@auckland.ac.nz).
Introduction

Age-related loss of muscle mass, independent of disease or other wasting environments, is termed “sarcopenia” (Janssen & Ross 2005). Changes in muscle mass reflect changes in protein turnover. Evidence has accumulated to suggest that it is not the profile of basal muscle protein metabolism in older people that causes an imbalance in protein turnover (Yarasheski 2002; Guillet et al. 2004), but rather deficits in the ability of older muscle to adequately respond to anabolic stimuli, such as dietary protein (Volpi et al. 2000; Paddon-Jones et al. 2004; Cuthbertson et al. 2005; Katsanos et al. 2005, 2006) and resistance exercise (Welle et al. 1995a; Kumar et al. 2009; Fry et al. 2011). A diminished response to anabolic stimuli in older muscle compared with younger muscle may be due to a dampened intracellular signalling transduction, as observed by others (Cuthbertson et al. 2005; Funai et al. 2006; Kumar et al. 2009).

Amino acids, particularly the branch-chain amino acid leucine, and resistance exercise stimulate muscle protein synthesis through activation of the mTOR signalling pathway (Anthony et al. 2000; Dickinson et al. 2011) that serves to regulate the mRNA translation initiation (Bodine et al. 2001). Activation of translation initiation involves the phosphorylation of the mammalian target of rapamycin (mTOR) and sequential activation of 70-kD S6 protein kinase (p70S6K) and the eukaryotic initiation factor 4E-binding protein (4E-BP1). p70S6K increases the capacity of the translational machinery through subsequent phosphorylation of ribosomal protein S6 that serves to selectively translate 5’-terminal polypyrimidine (TOP sequences) mRNA encoding ribosomal proteins and translation factors (Bolster et al. 2003). Phosphorylation of 4E-BP1 by mTOR enables dissociation from initiation factor eIF4E (Wang & Proud 2006). Once liberated, eIF4E is able to bind to eIF4G and form the eIF4F complex necessary for the uncoiling and binding of mRNA to the 43S ribosomal complex, thereby increasing the translational efficiency (Wang & Proud 2006).

Critical to the induction of a positive net protein balance when undertaking resistance exercise training, is the provision of adequate essential amino acids, with protein deficiency abolishing this effect (Biolo et al. 1997). In fact, increased amino acid availability synergistically enhances the stimulation of muscle protein synthesis that occurs in response to resistance exercise (Biolo et al. 1997; Moore et al. 2009b), whilst milk-based proteins promote muscle protein accretion to a greater extent than soy-based proteins (Wilkinson et al. 2007; Tang et al. 2009). Whey proteins have been touted as a more palatable, accessible, and practical nutritional supplement for the elderly, despite a more enhanced protein synthesis from an isocaloric EAA dose (Paddon-Jones et al. 2006). To our knowledge, no studies have systematically compared the of mRNA translational signalling response in the context of resistance exercise training combined with amino acid provision between young and old muscles. Therefore, the purpose of this study was to compare the impact of whey protein ingestion and resistance exercise training on the phosphorylation of mTOR and downstream signalling proteins in the skeletal muscle of young and “anabolically resistant” old men in the acute 2 h post-exercise phase.

Materials and methods

Subjects

Sixteen healthy young men (aged 18–25 years) and 15 healthy older men (aged 60–75 years), who had not participated in regular resistance exercise within a year prior to commencing the study, were recruited. A medical history questionnaire was used to identify and exclude subjects with a diagnosed condition or illness that would endanger subjects during strenuous exercise. Older participants were required to undergo a complete medical screening, including a 12-lead ECG exercise stress test to detect any underlying medical conditions. Subject characteristics are shown in Table 1. All subjects were informed of the nature and possible risk of the experimental procedures before their written informed consent was obtained. This study was approved by the Deakin University Human Research Ethics Committee.

Supplements

Subjects were randomly assigned to receive either a whey protein (WPI) or placebo drink. The whey protein powder contained 97% protein powder, 2.9% artificial flavour, and 0.1% aspartame sweetener. The protein powder component was a β-lactoglobulin enriched whey protein isolate, NatraBoost BLG (90% protein of which 87% is β-lactoglobulin), and contained only low levels of other constituents i.e., 0.5% fat, 0.5% lactose, 5% moisture, 3.7% ash, and 1.15% milk minerals. The whey protein powder was made up with water to 200 mL and contained a total of 26.6 g amino acids per serving. The placebo powder contained only the same amount of artificial flavour and aspartame sweetener as the WPI drink, and both drinks looked identical. The WPI and placebo powders were manufactured by Murray Goulburn Nutritionalis and provided in kind to Deakin University. The dose of protein administered was based on previous studies investigating postprandial protein synthesis or a measurable anabolic effect with whey protein products (Brown et al. 2004; Tipton et al. 2004; Moore et al. 2009a). The amino acid content of the WPI was (in grams per 26.6 g serving): histidine 0.56, isoleucine 1.49, lysine 2.87, methionine 0.71, phenylalanine 0.93, threonine 1.3, leucine 3.66, valine 1.41, alanine 1.57, arginine 0.73, aspartate 2.94, glutamate 4.8, glycine 0.39, proline 1.3, serine 1.01, tyrosine 0.95.

Acute resistance exercise

At least 4 days prior to the testing day, each participant completed a familiarization session on the Cybex NORM dynamometer (Cybex International Inc. UK). For 24 h prior to,
and the days of the trial, participants abstained from alcohol, caffeine, tobacco, and additional exercise. Following an overnight fast (10–12 h), participants reported to the exercise physiology laboratory at Deakin for a resting muscle biopsy. The subjects then completed 3 sets of 8 repetitions of a maximal single-legged knee extension exercise on the Cybex NORM Dynamometer, with a 2 min rest between each set. Each repetition involved concentric and eccentric contraction of the knee extensors at a set speed of 60 °/s. Participants were instructed to contract as hard as possible and were verbally encouraged throughout each set. On completion of the exercise, participants immediately ingested their designated supplement and were required to remain resting in the laboratory before a muscle biopsy was collected at 2 h post-exercise from the exercised and non-exercised legs.

**Resistance exercise training**

The participants completed a 12 week bilateral resistance exercise training programme, designed with reference to the ACSM recommendations to achieve muscle growth (hypertrophy) and literature pertaining to muscle gain in the elderly (Esmarck et al. 2001). Participants performed 3 training sessions per week (36 training sessions in total) with at least 48 h rest between each session and trained at the same time of day to control for daily hormonal fluxes. All participants were individually supervised by a certified trainer (blinded to the supplementation) and in the presence of the principle investigator to verify compliance with the training protocol. The first three training sessions were conducted using light resistance to familiarize the subjects with the equipment, training protocol, and correct execution of the exercises. After the familiarization sessions, strength testing was performed to determine appropriate starting weights for all subjects. One-repetition maximum (RM) strength was estimated from subjects’ 5RM results for all exercises. Subjects’ 5RM was re-tested at week 6 and week 12, and the training load was adjusted accordingly to ensure that the training was progressive. Each training session was preceded by a 5 min warm-up on a stationary cycle, followed by a full set of exercises with light (warm-up) weights. The exercises consisted of leg presses, bench presses, seated rows, leg extensions, dumbbell shoulder presses, and sit-ups. Following the warm-up weights, subjects completed 2 sets of the same exercises, always performed in the prescribed order with 2 min rests between each set and 3–5 min rest between each exercise. Initially, the exercises were set to 50% of subjects 1RM for one week, followed by a progressive increase in the weights lifted each week until 80% of 1RM was attained at week 6. The exercise intensity was set at 80% of 1RM for the remaining 6 weeks. During this time, the load for all exercises was adjusted, if necessary, at every third or fourth training session to ensure the participant was working within the required rep range and prescribed intensity. At the end of the program, participants again returned to the laboratory in the fasted state (10–12 h) to complete the exercise trial, consisting of a single bout of resistance exercise and collection of muscle biopsies. The exercise performed and timing of the supplement and muscle biopsies was identical to the exercise trial completed by the subjects before the commencement of the exercise training (see above). Thus, participants received a supplement only after each training session. Therefore, muscle biopsies were collected in four different states: (i) untrained rested, (ii) untrained acutely exercised, (iii) trained rested, and (iv) trained acutely exercised.

**Daily food intake**

Participants were encouraged to maintain their habitual diet during the 12 weeks of resistance training. Self-reported food diaries were kept for 3 consecutive days (Sunday, Monday, and Tuesday) within the first two weeks and within the last two weeks of the training period. This was to determine daily food intake and to observe energy and protein consumption over the course of the study.

**Amino acid analysis of supplements**

The placebo and whey protein drinks were analysed for amino acid composition at the Department of Primary Industries (Werritee, Australia), as described by Rayner (1985). Briefly, samples were hydrolysed in 6 mol/L HCl to determine total amino acids (excluding tryptophan, cystine, asparagine, and glutamine). The hydrolysates were reduced to dryness in vacuo to remove the HCl, and the residues reconstituted in sodium citrate buffer (pH 2.2), containing the internal standard norleucine. Samples were then assayed by high-performance liquid chromatography (Waters, USA), using an ion exchange column in the sodium form (Waters Part# WAT08002, Waters, USA) and quantified after post-column derivitization with ninhydrin. Empower Pro, Version 2, Software (Waters, USA) was used to process the chromatographic data.

**Muscle biopsy procedure**

The *vastus lateralis* muscle of the non-dominant, rested, and exercised leg was sampled under aseptic conditions after local anaesthesia (1% Xylocaine), using the percutaneous

### Table 1. Participant characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Young adult</th>
<th>Placebo</th>
<th>WPI</th>
<th>Old adult</th>
<th>Placebo</th>
<th>WPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td></td>
<td>(n = 9)</td>
<td>(n = 9)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.4±0.8</td>
<td>20.5±0.7</td>
<td>67.4±1.3</td>
<td>68.1±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183.9±1.6</td>
<td>178.5±2.7</td>
<td>174.3±2.1</td>
<td>173.1±2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>73.9±2.5</td>
<td>70.8±3.4</td>
<td>83.4±3.5</td>
<td>82.6±3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg·m²)</td>
<td>21.9±0.8</td>
<td>22.2±0.8</td>
<td>27.5±1.0</td>
<td>27.5±0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: * Significant difference, P < 0.05, between WPI and placebo within each age category.
needle biopsy technique modified to include suction (Evans et al. 1982) for all four biopsies. Excised muscle tissue from each biopsy was immediately frozen and stored in liquid nitrogen for subsequent analysis. To minimize the potential for interference, serial biopsy samples were collected at least 2 cm from previous biopsy sites.

**Western blot analysis**

Muscle samples (~30 mg) were homogenized in ice cold lysis buffer containing 20 mmol/L Tris-HCl, 5 mmol/L EDTA, 10 mmol/L Na-pyrophosphate, 100 mmol/L NaF, 2 mmol/L Na3VO4, 1% Igepal, 10 μg/mL Aprotinin, 10 ug/mL Leupeptin, 3 mmol/L Benzamidine, and 1 mmol/L PMSF. Homogenates were rotated on ice for 1 h and then centrifuged at 13 000 r/min (16 600 g) for 10 min. The resulting supernatant was analyzed for total protein content using the BCA protein assay (Pierce Biotechnology, Inc. Rockford, IL).

Fifty μg (rpS6, 4EBP1) or 75 μg (p70S6K, mTOR, eIF4G, Akt) of denatured total proteins from each sample, in Laemmeli buffer, were separated by electrophoresis on 5% (mTOR), 6% (eIF4G), 8% (Akt, p70S6K), 10% (rpS6) or 12% (4E-BP1) SDS-polyacrylamide gel and transferred to nitrocellulose membranes, or PVDF (for 4E-BP1), by electro-blotting. PVDF membranes were blocked by air drying and nitrocellulose membranes were blocked with 5% bovine-serum albumin in Tris-buffered saline for 1 h (rpS6, mTOR), 2 h (Akt, p70S6K), or overnight (eIF4G). All membranes were incubated overnight at 4 °C with polyclonal rabbit antibodies to total and phosphorylated (Ser473) Akt antibodies at 1:1000 dilution using Kodak ID 3.5 image analysis software. All phosphorylated proteins were adjusted for total protein.

**Dietary Analysis**

Analysis of the food diaries showed that the RDI for protein was met by both young men (0.84 g/kg BW/d) and older men (1.07 g/kg BW/d) during both early and late training. Despite added consumption of the WPI supplement by the WPI groups, no significant differences in protein intake in the early phase of training was observed between young placebo (1.6 ± 0.2 g/kg BW/d) and young WPI (1.9 ± 0.2 g/kg BW/d) or in the late phase of training between young placebo (1.6 ± 0.2 g/kg BW/d) and young WPI (1.8 ± 0.1 g/kg BW/d). However, in the early phase of training for older participants, protein intake by the WPI group (1.5 ± 0.1 g/kg BW/d) was significantly more than that consumed by the placebo group (1.1 ± 0.1 g/kg BW/d), P = 0.03. No significant differences in protein intake between these groups were observed during late phase training (old placebo, 1.3 ± 0.2 g/kg BW/d; old WPI, 1.6 ± 0.1 g/kg BW/d). Overall energy intake did not differ between supplement groups within each age category during both early and late phase training.

**Strength data**

All groups significantly increased estimated 1RM strength after 12 weeks of resistance training in all three measured exercises (leg press, leg extension, bench press) (see Table 2). However, the magnitude of strength gain did not differ between WPI and placebo groups for each age category. Knee extensor exercise performed on the Cybex Dynamometer during biopsy trial days resulted in a significant increase in peak torque (Nm) for both concentric and eccentric strength before and after resistance training within each group. Furthermore, the young participants in the WPI group significantly increased their eccentric strength above that achieved by the young placebo group (P = 0.046, Table 2).

**Protein phosphorylation**

**Untrained state**

A single bout of maximal knee extension exercise, immediately preceded by either WPI or placebo ingestion, had no impact on the phosphorylation of Akt at Ser473 in both young and old skeletal muscle (Fig. 1). The fold-change in phosphorylation of mTOR at Ser2448 (Fig. 2) was significantly greater in the WPI groups for both young (3.3 fold-change; P > 0.01) and old (2.9 fold-change; P > 0.01) than that achieved by the corresponding placebo groups (young, 1.2 fold-change; old, 1.3 fold-change). Downstream of mTOR, phosphorylation of the binding protein 4EBP1 at
Thr37/46 showed a greater phosphorylation in the old WPI group (4.8 fold-change; $P > 0.01$) compared with old placebo (1.5 fold-change), the top band of the Western blot being the hyperphosphorylated form (Fig. 3). In the young, although significance was not achieved, supplementation with WPI resulted in an almost double activation of this protein compared with the placebo group. Phosphorylation of p70S6K at Thr389 (Fig. 4) was exceptionally responsive to combined WPI ingestion and exercise in old human skeletal muscle (28.4 fold-change; $P > 0.001$) with no change ob-
Fig. 3. Fold-change in phosphorylation of the eukaryotic initiation factor 4E binding protein-1 (4E-BP1) at Thr\(^{174/46}\) in young and old human skeletal after an acute resistance exercise bout with either a WPI or placebo supplement, in both untrained and trained muscle. (Top) Representative immunoblots of the 2 h time point obtained using the phospho-specific antibody. Values are expressed as mean ± SEM. \(*\): Significantly different, \(P < 0.05\), from respective placebo group within each age category.

![Image 366x577 to 560x595]

Preserved in the placebo group. A 2.2-fold increase in p70S6K phosphorylation was observed in the young WPI group compared with placebo, however this failed to reach significance \((P > 0.05)\). Further downstream of p70S6K is ribosomal protein S6. Consuming WPI immediately after an acute exercise bout dramatically increased rpS6 phosphorylation at Ser235/236 in both young \((P > 0.01)\) and old \((P > 0.01)\) human skeletal muscle (Fig. 5). However, because of the high within-subject variability, significance was not reached. Phosphorylation of eIF4G at Ser1108 (Fig. 6) was greater after combined WPI ingestion and exercise, but only significantly higher than exercise alone (placebo) in older human skeletal muscle \((P > 0.05)\).

**Trained state**

After completing 12 weeks of resistance exercise training and consuming either the placebo or WPI supplement, muscle biopsies were taken again before and after an acute exercise bout to assess activation of the regulators of translation initiation in a trained state. As with the untrained state, there were no significant differences between the WPI and placebo groups in the phosphorylation of Akt. Interestingly, enhanced phosphorylation of mTOR in the young WPI group was maintained, however no significant differences between the old WPI and old placebo groups were observed. In contrast, older muscle maintained its ability to phosphorylate 4E-BP1 to a greater extent when WPI was ingested. This effect was not seen in younger muscle. The large response in p70S6K phosphorylation after WPI ingestion in older muscle was not repeated in the trained state. The younger WPI group enhanced phosphorylation of p70S6K three times more than the placebo group, although no significance was observed. Trained and untrained younger muscle demonstrated markedly increased rpS6 phosphorylation, following exercise and WPI ingestion compared with exercise alone. In older muscle, the enhanced rpS6 phosphorylation following WPI ingestion seen pre-training was not observed in the trained state. Although significance was not reached, the elevated ability of WPI to phosphorylate mTOR signalling proteins remained consistent in the trained state. Similar patterns of eIF4G phosphorylation were observed pre- and post-training, although no significant differences were observed in the trained state.

**Discussion**

We report that 27 g of WPI consumed immediately after resistance exercise activates mRNA translational signalling in both young and old human skeletal muscle. Interestingly, the magnitude of enhanced activation of intermediates in the mTOR signalling pathway, from WPI ingestion compared with placebo, was greater in older muscle in the untrained state. However, it seems that the initial heightened response of older muscle to combined resistance exercise and WPI ingestion becomes less pronounced after repeated training sessions combined with WPI intake. This observation was apparent in the phosphorylation of mTOR, p70S6K, rpS6, and to a lesser degree in eIF4G, in the trained state. Following 12 weeks of strength training, all subjects demonstrated...
Fig. 5. Fold-change in phosphorylation of the ribosomal protein (rp) S6 at Ser235/236 in young and old human skeletal after an acute resistance exercise bout with either a WPI or placebo supplement, in both untrained and trained muscle. (Top) Representative immunoblots of the 2 h time point obtained using the phospho-specific antibody. Values are expressed as mean ± SEM. *: Significantly different, P < 0.05, from respective placebo group within each age category.

![Image of immunoblots](image)

significantly improved measures of muscular strength; however no differences were observed with differing age or supplement groups.

WPI ingestion following exercise enhances the activation of mTOR signalling in skeletal muscle

Amino acid ingestion following resistance exercise has been shown to enhance mTOR signalling (Karlsson et al. 2004; Dreyer et al. 2008; Holm et al. 2010; Moore et al. 2011) and muscle protein synthesis (Biolo et al. 1997; Moore et al. 2005; Hartman et al. 2007) compared with resistance exercise without nutrient ingestion. A dose dependency has also been shown in this relationship, and the dose of WPI we selected was close to that reported to optimally stimulate protein synthesis, at least for young persons (Moore et al. 2009a). In the present study, the provision of protein immediately post-exercise resulted in an increase in the phosphorylation of signalling proteins that are part of the mTOR signalling pathway. Specifically, WPI supplementation markedly increased the phosphorylation of mTOR, 4EBP-1, rpS6, and eIF4G compared with placebo. Increased mTOR phosphorylation was observed in the muscle of younger and older men in the untrained state, although only in the younger men following 12 weeks of strength training. WIPI-induced phosphorylation of mTOR was associated with a concomitant phosphorylation of p70S6K at Thr389. Following WPI ingestion, the response of p70S6K was almost doubled compared with placebo in the young subjects, whilst a more robust increase was observed in the muscle of older subjects. Marked, although variable, increases in rpS6 phosphorylation were observed in the muscle of subjects that consumed WPI, again consistent with previous results (Karlsson et al. 2004; Dreyer et al. 2008; Moore et al. 2011). Similarly, 4E-BP1 phosphorylation was increased with amino acid supplementation and exercise, compared with exercise alone, a response particularly evident in older muscle. In contrast, no differences were seen in the phosphorylation of Akt with either exercise or WPI supplementation. However, conflicting data concerning the responsiveness of Akt phosphorylation to exercise (Blomstrand et al. 2006; Dreyer et al. 2006; Camera et al. 2010) suggests that mTOR signalling activation by amino acids is independent of Akt and the upstream components of the IGF-1 pathway (Philp et al. 2011).

Activation of mTOR signalling is differentially regulated in young and old muscle following exercise

An interesting and novel finding of this study is the observation of differential phosphorylation of mTOR signalling proteins between younger and older men following an exercise training programme with or without WPI supplementation. Exercise and WPI increased mTOR phosphorylation to a similar degree in young and old before training, however WPI-induced mTOR phosphorylation following the training program was distinctly blunted in the old. Conversely, WPI-induced 4EBP-1 phosphorylation appeared to be more pronounced in older muscle before and after training compared with young muscle. Older muscle responded with a substantial increase (~25-fold) in phosphorylation of p70S6K 2 h
after WPI ingestion and acute exercise compared with a non-significant doubling in younger muscle supplemented with WPI. Similarly, greater phosphorylation of eIF4G was evident in older muscle supplemented with WPI before training compared with their younger counterparts.

Several reports have identified skeletal muscle signalling and protein synthesis inconsistencies between old and young individuals after an acute bout of resistance exercise (Drummond et al. 2008; Kumar et al. 2009; Fry et al. 2011). Kumar et al. (2009) showed that muscle protein synthesis and translational signalling, while fasted, was blunted in the elderly after a single bout of resistance exercise compared with the young. However, the blunted translational signalling response in this study (Kumar et al. 2009) was abolished by 2 h post-exercise, which may explain why we were unable to detect any difference in fasted, exercise-induced translational signalling between young and old. It should be noted that others have demonstrated blunted translational signalling in the elderly 3 h post-exercise, but only when a relatively large total exercise volume is completed (Fry et al. 2011). Previously, Drummond et al. (2008) demonstrated that the muscle protein synthetic response to combined resistance exercise and amino acid ingestion is simply delayed, as opposed to blunted, with aging. These authors further reported that mTOR signalling (mTOR, S6K1, 4E-BP1, eEF2) was similar between young and elderly, in contrast to the results shown here. Methodological differences including the source (crystalline essential amino acids vs. WPI) and timing of protein ingestion (immediately vs. 1 h post-exercise) likely contributed to these discrepancies. Thus, the precise mechanisms contributing to anabolic resistance in elderly muscles remain elusive; however, we are able to shed light on the differential modifications in mTOR-mediated signalling of young and old muscles that occur with prolonged training.

The potential biological significance of altered mTOR signalling with age remains unclear. We show that older muscle supplemented with WPI tended to exhibit greater activation of mTOR signalling proteins, but without direct measures of muscle protein synthesis, it is unclear whether this heightened activation would have resulted in greater muscle protein synthetic rates. Based on the recent data of Atherton et al. (2010) showing reasonable concordance between the increase in rates of muscle protein synthesis and translational signalling with whey protein ingestion, we can reasonably speculate that increased protein synthesis rates might be expected within the older muscle supplemented with WPI, compared with placebo. However, based on the work of Drummond et al. (2008), any muscle protein synthetic response subsequent to changes in translational signalling may have been delayed compared with the young. It is equally plausible that in older muscle, greater activation of translational signalling pathways is necessary to achieve a comparable level of protein synthesis to that seen in the young. In support of this thesis, no differences were seen in 1RM strength between the age groups or with WPI supplementation. Thus, the greater mTOR signalling response in the elderly did not translate to an improved muscular adaptation, compared with the young. However, the measures of strength employed by this study may not have been sensitive enough to detect small but potentially significant increases in muscle strength. In support of this supposition, we were able to detect a greater increase in eccentric knee extensor torque, as measured by dynamometer, in the young WPI group compared with the young placebo group. Finally, the time-course of this study may not have been sufficient to detect meaningful differences in strength outcomes. Studies are therefore required to further explore the physiological significance of altered mTOR signalling with WPI supplementation and age.

Repeated training sessions impair WPI-induced mTOR activation in the skeletal muscle of older individuals

To our knowledge, this is the first study to investigate the acute response of the regulators of mRNA translation initiation after chronic training and protein supplementation in young and older individuals. WPI taken immediately after acute resistance exercise activated mTOR signalling to a similar degree before and after training in young muscle. Conversely, the initial heightened response of older muscle to combined resistance exercise and WPI ingestion became less pronounced after repeated training sessions. This was particularly observed in phosphorylation of mTOR, p70S6K, rpS6, and to a lesser degree in eIF4G. Thus, whilst it appears that the activation of key proteins involved in translation initiation with amino acid supplementation is preserved in young muscle following training, deficiencies are evident in the activation of members in this signalling cascade in the muscle of older men. These data are in accordance with muscle protein synthetic responses that have been shown to be dampened after prolonged resistance training in young adults (Phillips et al. 1999; Kim et al. 2005; Tang et al. 2008).

The ability of skeletal muscle to hypertrophy in response to resistance exercise is not lost with advancing age (Trappe et al. 2000; Häkkinen et al. 2001), however the magnitude of change in myofiber growth has been shown to be blunted in older adults (Welle et al. 1996; Kosek et al. 2006; Petrella et al. 2006). Similarly, the increase in muscle protein synthesis with resistance exercise training in the old is lower than the young (Welle et al. 1995b). The reduced activation of translation initiation signalling proteins demonstrated following 12 weeks of strength training observed in the current study may explain, at least partially, this impaired hypertrophic capacity within older skeletal muscle.

In conclusion, the results of this study show that in the untrained state, resistance exercise coupled with whey protein ingestion increases the phosphorylation of proteins involved in mRNA translation compared with exercise alone and to a similar extent in young and old skeletal muscle. Whilst this enhanced activation occurs independently of training status in younger men, in older men, the WPI and exercise induced increase in phosphorylation was attenuated by 12 weeks of strength training. Strategies to improve muscle growth in the elderly should not dismiss the use of protein, particularly whole proteins high in leucine, in conjunction with a resistance exercise program, although further investigations are required to delineate interventions that will maintain sensitivity to anabolic stimuli.

Acknowledgements

The authors acknowledge the excellent medical assistance provided by Dr Andrew Garnham, School of Exercise and Nutrition Sciences, Deakin University; Murray Goulburn
Nutritional for providing the supplements; Dr Craig Tre
nery and Vilnis Ezerneiks from Department for Primary Indus
tries, Werribee, Australia for the amino acid analysis of the 
supplements; Gaye Rutherford for analysis of the food 
diaries; personal trainers Sam Wright, Christie Bence, and 
Genevieve Francis and Dr Stuart Phillips of McMaster Uni
versity for his insightful edits of the manuscript. This re
search was supported by a grant awarded to Dr. D.
Cameron-Smith from Dairy Australia (DU1111; Dairy Pro
tein Supplementation to Increase Muscle Strength).

References
Anthony, J.C., Anthony, T.G., Kimball, S.R., Vary, T.C., and 
Jefferson, L.S. 2000. Orally administered leucine stimulates 
protein synthesis in skeletal muscle of postsorptive rats in 
association with increased elf4F formation. J. Nutr. 130(2): 139–
145. PMID:10720160.

Aherton, P.J., Etheridge, T., Watt, P.W., Wilkinson, D., Selby, A., 
Rankin, D., et al. 2010. Muscle full effect after oral protein: time-
dependent concordance and discordance between human muscle 

abundant supply of amino acids enhances the metabolic effect 
E129. PMID:9252488.

Branchained amino acids activate key enzymes in protein 
273S. PMID:16365096.

Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., 
Bauerlein, R., et al. 2001. Akt/mTOR pathway is a crucial 
regulator of skeletal muscle hypertrophy and can prevent muscle 
ncb1101-1014. PMID:11715023.

control mechanisms modulate skeletal muscle gene expression 
1097/00003677-200307000-00002. PMID:12882475.

Soy versus whey protein bars: effects on exercise training impact 
1186/1475-2891-3-2-22. PMID:15588291.

2010. Early time course of Akt phosphorylation after endurance 

Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., 
contraction-induced human skeletal muscle mTORC1 signaling 
2044-5040-1-11. PMID:21798089.

Farnfield et al. 29

Evans, W.J., Phinney, S.D., and Young, V.R. 1982. Suction applied to 

Fry, C.S., Drummond, M.J., Glynn, E.L., Dickinson, J.M., Gunder-
contraction-induced human skeletal muscle mTORC1 signaling 
1852. doi:10.1249/E92–E400. doi:10.1152/ 
ejphysiol.00582.2007. PMID:18056791.

Drummond, M.J., Dreyer, H.C., Pennings, B., Fry, C.S., Dhanani, S., 
to resistance exercise and essential amino acids is delayed with 
aging. J. Appl. Physiol. 104(5): 1452–1461. doi:10.1152/ 

Esmarck, B., Andersen, J.L., Olsen, S., Richter, E.A., Mizuno, M., 
and Kjaer, M. 2001. Timing of postexercise protein intake is 
important for muscle hypertrophy with resistance training in 
7793.2001.00301.x. PMID:11507179.

Evans, W.J., Phinney, S.D., and Young, V.R. 1982. Suction applied to 

Fry, C.S., Drummond, M.J., Glynn, E.L., Dickinson, J.M., Gunder-
contraction-induced human skeletal muscle mTORC1 signaling 
2044-5040-1-11. PMID:21798089.

Age-associated decrease in contraction-induced activation of 
downstream targets of Akt/mTor signaling in skeletal muscle. Am. J. 

Guillet, C., Prod’homme, M., Balage, M., Gachon, P., Giraudet, C., 
protein synthesis is associated with S6K1 dysregulation in elderly 

Changes in electromyographic activity, muscle fibre and force 
production characteristics during heavy resistance/power strength 

Hartman, J.W., Tang, J.E., Wilkinson, S.B., Tarnopolsky, M.A., 
Consumption of fat-free fluid milk after resistance exercise 
promotes greater lean mass accretion than does consumption of 
soy or carbohydrate in young, novice, male weightlifters. Am. J. 

Holm, L., van Hall, G., Rose, A.J., Miller, B.F., Doessing, S., Richter, 
collagen and myofibrillar protein synthesis rates differently in 
298(2): E257–E269. doi:10.1152/ajpendo.00609.2009. PMID: 
19903866.

muscle mass and composition with metabolism and disease. J. 

R., and Blomstrand, E. 2004. Branched-chain amino acids increase 
p70S6k phosphorylation in human skeletal muscle after resistance 

Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., 
and Wolfe, R.R. 2005. Aging is associated with diminished 
accretion of muscle proteins after the ingestion of a small bolus of 
PMID:16280440.

Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., 
and Wolfe, R.R. 2006. A high proportion of leucine is required for 

Published by NRC Research Press