Whey Protein Supplementation Preserves Postprandial Myofibrillar Protein Synthesis during Short-Term Energy Restriction in Overweight and Obese Adults1–3

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Abstract

Background: Higher dietary energy as protein during weight loss results in a greater loss of fat mass and retention of muscle mass; however, the impact of protein quality on the rates of myofibrillar protein synthesis (MPS) and lipolysis, processes that are important in the maintenance of muscle and loss of fat, respectively, are unknown.

Objective: We aimed to determine how the consumption of different sources of proteins (soy or whey) during a controlled short-term (14-d) hypoenergetic diet affected MPS and lipolysis.

Methods: Men (n = 19) and women (n = 21) (age 35–65 y; body mass index 28–50 kg/m2) completed a 14-d controlled hypoenergetic diet (∼750 kcal/d). Participants were randomly assigned, double blind, to receive twice-daily supplements of isolated whey (27 g/supplement) or soy (26g/supplement), providing a total protein intake of 1.3 ± 0.1 g/(kg · d), or isoenergetic carbohydrate (25 g maltodextrin/supplement) resulting in a total protein intake of 0.7 ± 0.1 g/(kg · d). Before and after the dietary intervention, primed continuous infusions of L-[ring-13C6] phenylalanine and [2H5]-glycerol were used to measure postabsorptive and postprandial rates of MPS and lipolysis.

Results: Preintervention, MPS was stimulated more (P < 0.05) with ingestion of whey than with soy or carbohydrate. Postintervention, postabsorptive MPS decreased similarly in all groups (all P < 0.05). Postprandial MPS was reduced by 9 ± 1% in the whey group, which was less (P < 0.05) than the reduction in soy and carbohydrate groups (28 ± 5% and 31 ± 5%, respectively; both P < 0.05) after the intervention. Lipolysis was suppressed during the postprandial period (P < 0.05), but more so with ingestion of carbohydrate (P < 0.05) than soy or whey.

Conclusion: We conclude that whey protein supplementation attenuated the decline in postprandial rates of MPS after weight loss, which may be of importance in the preservation of lean mass during longer-term weight loss interventions. This trial was registered at clinicaltrials.gov as NCT01530646.


Keywords: leucine, lean body mass, protein quality, hypocaloric, myofibrillar protein synthesis

Introduction

High quality weight loss is a term used to describe the loss of weight during hypocaloric feeding with the lowest possible ratio of lean body mass (LBM)7 to fat mass. It is well known that LBM is a major contributor to resting energy expenditure, mobility, and glucose disposal (1), whereas excess fat mass, particularly visceral adipose tissue, contributes to inflammation in obesity, which is correlated with increased risk of developing cardiovascular disease and type 2 diabetes (2, 3). We proposed that weight loss plans should aim to maximize retention of LBM and reduction of fat mass in order to achieve the greatest metabolic health benefits.

The maintenance of muscle mass, a large component of LBM, is determined by the balance between myofibrillar protein...
synthesis (MPS) and myofibrillar protein breakdown (MPB) (4). During an energy deficit, rates of MPS are blunted in the postabsorptive (5–7) and postprandial (6) states, which would clearly lead to loss of lean mass. One strategy to promote retention of muscle mass includes the consumption of dietary protein at intakes greater than the RDA of 0.8 g/(kg · d). Multiple studies and meta-analyses showed that consumption of protein at amounts greater than the RDA improves lean mass retention (8–10) and increases fat mass loss during energy restriction (8, 9). Interestingly, Pasiakos et al. (6) reported that postprandial rates of MPS were reduced during energy restriction in participants consuming the RDA for protein, but not when protein intakes were 2 or 3 times the RDA.

The source of protein (animal vs. plant) may also be an important factor affecting body composition changes during weight loss. For example, during energy restriction with exercise, the consumption of higher protein meals (30% of total energy intake) rich in dairy-source proteins promoted greater fat mass loss and lean mass retention (11). Admittedly, the contribution of other constituents of dairy foods responsible for these effects and the underlying physiologic mechanisms are unclear (12). Whey protein is one potential protein component that was speculated to contribute to the bioactivity of dairy (13). The branched-chain amino acid leucine is found in high proportions in whey and was shown to be a potent stimulator of muscle protein synthesis in humans (14, 15). In addition, data exist (at least in adipocytes and muscle cells) to suggest that leucine alone may have a synergistic role in muscle and adipocyte cells because it inhibits adipocyte lipogenesis and stimulates lipolysis (16, 17). Therefore, whey protein (containing leucine) may be a dairy constituent that is important for greater fat mass loss and lean mass retention. However, with regards to lipolysis, little data exist to expand this knowledge in humans. A meta-analysis conducted by Miller et al. (18) found a modest effect from whey protein supplementation on LBM retention and fat mass loss compared with carbohydrate during energy deficit; however, it was noted that currently there are not enough studies to compare whey protein with other protein sources.

The effect of protein source during weight loss requires further study; thus, the aim of this study was to examine the impact of protein in affecting protein turnover during energy deficit; however, it was noted that currently there are not enough studies to compare whey protein with other protein sources. These records were provide an estimate of their habitual dietary intake. These records were used to calculate the use of the Mifflin-St Jeor equation (20), with an estimated body mass and body mass index in kilograms and meters squared, respectively. The day after the infusion protocol, participants consumed pre- and postprandial (2 weekdays and 1 weekend day) to provide an estimate of their habitual dietary intake. These records were analyzed with the use of a commercially available software program (The Food Processor, ESHA). Participants were instructed not to consume any vitamin or mineral supplements, particularly calcium or vitamin D, for the duration of the study. Participants also were instructed not to consume alcohol for the duration of the study.

**Study design.** The timeline of the overall study is shown in Supplemental Figure 1A. In a double-blind investigation, 40 men and women were randomly assigned to a hypocaloric diet with twice daily supplementation with isolated whey protein, isolated soy protein, or an isoenergetic amount of carbohydrate. Groups were matched and stratified by age, sex, and BMI. Participants’ energy requirements were calculated with the use of the Mifflin-St Jeor equation (20), with an appropriate activity factor (calculated for each participant based on an activity log) by a registered dietician. Three days before the start of the experimental infusion trial, participants were provided with a 3-d weight maintenance diet designed to provide 100% of their estimated energy requirements and at a protein intake of 1 g/(kg · d). Participants were supplied with all the food required for the entire duration of the study. To enhance participant compliance, this mainly was in the form of pre-packaged meals (Copper County Foods).

After the weight maintenance diet, participants underwent their first infusion trial. Briefly, after an overnight fast, participants consumed a standardized breakfast at 0530 consisting of Ensure Plus (15% protein, 29% fat, 56% carbohydrate; Abbott) providing 6 kcal/kg body mass at home before arriving to the laboratory. Participants arrived at the laboratory at McMaster University at 0730. A 20-gauge catheter was inserted into the antecubital vein of one arm of each participant, and a baseline blood sample was drawn. A second catheter was inserted in the contralateral arm and primed continuous infusions of ring-[13C6]-phenylalanine [0.05 μmol/kg · min]; 2.0 μmol/kg prime] and [3-H]-glycerol [0.1 μmol/kg · min]; 1.5 μmol/kg prime] (Cambridge Isotope Laboratories) were initiated. After 3 h of tracer infusion, a muscle biopsy was obtained from the vastus lateralis, after which participants consumed their assigned study beverage composed of isoenergetic quantities of whey (27 g protein; Agropur Isochill 8000 Whey Protein Concentrate), soy protein isolate (26 g protein; SoyPro950M, International Trade Company), or an isoenergetic amount of carbohydrate (<1 g protein, 25 g maltodextrin; Globe Plus). Protein beverages were enriched to 4% with ring-[13C6]-phenylalanine according to their phenylalanine content. After a 3-h postprandial period, a second muscle biopsy (fed state) was obtained from the vastus lateralis (Supplemental Figure 1B). The day after the infusion protocol, participants consumed pre-packaged meals marking the onset of the 14-d weight loss program providing a 750 kcal/d deficit from subjects’ estimated energy requirements based on the Mifflin-St Jeor equation. In addition to the meals, study completion. Participants were informed of the experimental procedures to be used, the purpose of the study, and all potential risks before providing written consent. The study was approved by the Hamilton Health Sciences Research Ethics Board and was in accordance with standards set by the Canadian Tri-Council Policy (19) on the use of human participants in research.
participants also consumed study supplements (whey, soy, or carbohydrate), which were included within the energy allowance. Whey and soy supplements were isonitrogenous and both were isoenergetic with the carbohydrate control supplement. The supplements (27 g whey, 26 g soy, or 25 g carbohydrate) were consumed twice a day: midmorning (between breakfast and lunch) and midafternoon (between lunch and dinner). After the 14-d weight loss period, a second infusion was performed. This was identical to the first infusion with the exception of one extra biopsy before the infusion began to account for the new baseline isotope enrichment level.

**Blood analyses.** Plasma amino acid concentrations were determined through use of the Phenomenex EzFamast amino acid analysis kit with gas chromatography-mass spectrometry (GC Model 6890 Network, Agilent Technologies; MSD model 5973 Network, Agilent Technologies). Insulin concentrations were determined in plasma through use of solid-phase, 2-site chemiluminescence immunassays (Immulite; Intermedico) and glucose was measured through use of the glucose oxidase method.

Glycerol concentration and enrichment was measured through use of gas chromatography-mass spectrometry (GC Model 6890 Network, Agilent Technologies; MSD model 5973 Network, Agilent Technologies) to measure whole body lipolysis. Briefly, 50 μL plasma was deproteinized on ice in 500 μL 0.3 N Ba(OH)2 and 500 μL 0.3 N ZnSO4 for 20 min, and then centrifuged at 500 × g for 20 min at 4°C. The supernatant was collected and flowed through an ion exchange column consisting of 1 mL Dowex cationic resin (50WX8–200 resin; Sigma-Aldrich) and 1 mL Dowex anionic resin (1 × 8 chloride form; Sigma-Aldrich). The resin was washed 4 times with 1 mL of distilled deionized water and all flow-through was collected. Samples were then dried. The glycerol rate of appearance (Ra) was calculated through use of different equations in the fasted state (steady state) and after supplement ingestion (nonsteady state) as defined by the Steege equation (21). To calculate fasted glycerol Ra, the glycerol enrichment measured in the first blood sample of the infusion day (0 h) and after 2 h of infusion was used. To calculate fed glycerol Ra between 1 and 2 h after supplement ingestion, both the glycerol concentration and the enrichment 1 and 2 h after consuming the study supplement were used in the equation.

**Muscle analyses.** Approximately 50 mg wet muscle was homogenized on ice in buffer [10 μL/mg muscle of 25 mmol/L Tris 0.5% v:v Triton X-100 and protease/phosphatase inhibitor cocktail tablets (Complete Protease Inhibitor Mini-Tabs, Roche; PhosSTOP, Roche Applied Science)] and centrifuged at 15,000 × g for 10 min at 4°C to separate the supernatant (sarcoplasmic) and pellet (myofibrillar) fractions. The myofibrillar fraction was stored at −80°C for future processing.

To determine myofibrillar protein-bound enrichments, the myofibrillar fraction (pellet) was washed with distilled deionized water and then purified from collagen in sodium hydroxide (NaOH). The myofibrillar fraction was then hydrolyzed for 72 h in 0.1 M HCl and Dowex cationic resin (50WX8–200 resin; Sigma-Aldrich) at 110°C and mixed on a vortex every 24 h. The free amino acids were purified with the use of Dowex ion exchange chromatography, and the N-acetyl-n-propyl derivative was prepared and run on an isotope ratio MS to measure bound enrichment of ring-[13C6]-phenylalanine as described previously (16). The fractional synthetic rate (FSR) of myofibrillar protein was calculated with the use of the standard precursor-product equation and methods as described previously (22, 23). The postintervention infusion trial included a baseline muscle biopsy before the infusion began to account for changes in protein-bound enrichment from the preintervention infusion trial.

**Body composition.** Body composition was determined at the same time of day under the same nutritional conditions before and after the protocol. A DXA scan (QDR-4500A; Hologic software version 12.31) was performed with the use of a standardized protocol with participants positioned similarly on the scan table (24). Trunk fat was measured by sectioning lumbar 1 to lumbar 4 of the spine from the DXA scan. This was reported as an accurate surrogate measure of visceral adipose tissue (25).

**Statistical analyses.** Statistical analyses were performed with the use of SPSS version 18.0. A univariate (treatment) ANOVA was performed to compare all baseline anthropometric and dietary variables (all dietary variables in Table 2) between groups. A univariate ANCOVA was performed with energy deficit (difference from requirement) as the covariate on changes in body mass and fat mass. A repeated measures ANOVA (treatment × time) was performed for the analyses of plasma amino acid–related variables, glucose and insulin concentration time course and AUC, Western blot, and glyceral Ra. A repeated measures ANOVA (time) was performed on the myofibrillar FSR within groups and a repeated measures ANOVA (treatment × time) was performed on the change in myofibrillar FSR from postabsorptive to postprandial states pre- and postdiet. Significant differences in ANOVA were isolated with Tukey’s post hoc test. Significance was set at P < 0.05. Data are presented as means ± SEMs.

**Results**

**Participant characteristics.** Baseline participant characteristics are shown in Table 1. There were no differences between treatment groups (all P > 0.05) for any of the variables.

**Dietary manipulation.** The 14-d energy restriction diet is shown in Table 2. The whey and soy groups consumed significantly more protein (P < 0.01) than the carbohydrate group, and there were no differences between whey and soy groups. Despite instruction and provision of prepackaged diets, the calculated energy deficit (difference between foods consumed and estimated requirement) was significantly higher (P = 0.007) in the carbohydrate group than in the whey group.

**Body composition.** Body composition changes are shown in Table 3. All groups lost LBM, fat mass, total body mass, and trunk fat mass, with a main effect (P < 0.05) for time; however, there were no significant between-group differences. Because of the significant difference in the energy deficit between the whey and carbohydrate groups, additional statistical analyses were performed (ANCOVA) with the use of energy deficit (difference from estimated requirement) as a covariate. There were no significant differences between groups in fat mass (P = 0.83) or total body mass change (P = 0.76) indicating that the small but statistically significant differences in the energy deficit (Table 2) did not affect changes in fat mass or total body mass loss between the whey and carbohydrate groups.

**Plasma glucose and plasma insulin.** Changes in plasma glucose followed expected patterns after ingestion of the respective supplements (Figure 1A and B). AUC for the 3-h sampling period was significantly higher (main effect for treatment, P < 0.001) was significantly higher (main effect for treatment, P < 0.001).

|TABLE 2| Composition of 14-d weight loss diet including supplements consumed by the overweight or obese participants in the whey, soy, and carbohydrate groups1 |
|---|---|---|---|
|Whey| Soy| Carbohydrate|
|Protein intake, g/(kg · d)| 1.3 ± 0.1a| 1.3 ± 0.1a| 0.7 ± 0.1b|
|Fat intake, g/d| 48 ± 4a| 48 ± 4a| 48 ± 4a|
|Carbohydrate intake, g/d| 206 ± 18ab| 214 ± 20a| 226 ± 14a|
|Energy intake, kcal/d| 1750 ± 120a| 1760 ± 142a| 1640 ± 97b|
|Estimated energy deficit, kcal/d| −680 ± 37a| −750 ± 38ab| −860 ± 39b|
|Protein, en%| 29 ± 0.8a| 30 ± 1.4a| 19 ± 0.6b|
|Carbohydrate, en%| 47 ± 0.8a| 48 ± 0.8a| 56 ± 0.3b|
|Fat, en%| 25 ± 0.4a| 24 ± 0.7a| 27 ± 0.5b|

1 Values are means ± SEMs. Whey and soy groups, n = 14; carbohydrate group, n = 12. Means in a row without a common letter differ between groups, P < 0.05. en%, percentage of energy.
Myofibrillar protein synthesis. Rates of MPS in the fasted (postabsorptive) and fed (postprandial) states are shown in Figure 3A. Baseline postabsorptive rates of MPS were similar across groups. In response to supplement ingestion, MPS increased significantly in the whey (pre- and postintervention, $P < 0.001$) and soy (preintervention, $P = 0.002$ and postintervention, $P = 0.001$) groups before and after the diet. There was no significant effect from ingestion of the carbohydrate supplement on postprandial MPS before ($P = 0.67$) or after ($P = 0.55$) the diet. After the weight loss diet, there was a significant decrease in postabsorptive (all groups, $P < 0.001$) and postprandial (whey, $P < 0.001$; soy, $P = 0.001$; carbohydrate, $P = 0.022$) MPS in all groups. The decrease in postabsorptive MPS did not differ between the whey ($\pm 15\%$), soy ($\pm 25\%$), and carbohydrate ($\pm 20\%$) groups ($P > 0.05$); however, postprandial rates of MPS were reduced by $9\%$ in the whey group, which was significantly less than the reduction in the soy ($\pm 28\%$; $P = 0.021$) and carbohydrate ($\pm 31\%$; $P = 0.013$) groups after the 14-d weight loss intervention. Figure 3B shows the change in MPS from the postabsorptive to the postprandial state before and after the diet. In response to the supplement, FSR increased significantly more after whey ingestion than soy or carbohydrate ingestion ($P < 0.001$) both pre- and post diet.

Discussion

The novel finding from this study was that twice-daily consumption of whey protein resulted in an attenuation of the

### Table 3

<table>
<thead>
<tr>
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<th>Pre</th>
<th>Post</th>
<th>Δ</th>
<th>Pre</th>
<th>Post</th>
<th>Δ</th>
<th>Pre</th>
<th>Post</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body mass, kg</td>
<td>98.8 ± 4.4</td>
<td>104 ± 6.6</td>
<td>-2.1 ± 0.3</td>
<td>101 ± 6.7*</td>
<td>105 ± 4.9</td>
<td>-2 ± 0.3</td>
<td>103 ± 4.8*</td>
<td>105 ± 4.9</td>
<td>-2 ± 0.3</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>60.6 ± 3.3</td>
<td>63.5 ± 3.9</td>
<td>-0.6 ± 0.5</td>
<td>62.5 ± 4.1*</td>
<td>62.6 ± 4.0</td>
<td>-3 ± 0.4</td>
<td>62.2 ± 3.8*</td>
<td>62.6 ± 3.8</td>
<td>-0.4 ± 0.3</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>35.1 ± 2.4</td>
<td>37.6 ± 3.3</td>
<td>-1.4 ± 0.4</td>
<td>36.1 ± 3.3*</td>
<td>39.6 ± 3.0</td>
<td>-1.5 ± 0.2</td>
<td>38.2 ± 3.1*</td>
<td>39.6 ± 3.0</td>
<td>-0.2 ± 0.1</td>
</tr>
<tr>
<td>Trunk fat mass, kg</td>
<td>9.1 ± 0.8</td>
<td>9.6 ± 1.3</td>
<td>-0.5 ± 0.1</td>
<td>9.1 ± 1.2*</td>
<td>9.8 ± 1.1</td>
<td>0.1 ± 0.1</td>
<td>9.3 ± 1.1*</td>
<td>9.8 ± 1.1</td>
<td>-0.2 ± 0.1</td>
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1 Values are means ± SEMs. Whey and soy groups, $n = 14$; carbohydrate group, $n = 12$. *Post means within a row significantly different from Pre means ($P < 0.05$).
postprandial decline in MPS during a short-term dietary hypoenergetic diet vs. twice-daily supplementation with soy protein or carbohydrate. This is an important discovery because it indicates that proteins such as whey may be more effective at preserving MPS and potentially LBM in longer-term weight loss interventions. In addition, rates of lipolysis, although suppressed in all conditions, were suppressed to a greater extent after carbohydrate ingestion than with the ingestion of whey or soy protein. Although no group effects were observed for measures of body composition during this trial, this was almost assuredly because of the short-term nature of the intervention and a lack of sensitivity toward detecting changes with the use of DXA.

After the 14-d hypoenergetic diet, postabsorptive rates of MPS decreased in all groups. This finding is consistent with previous studies demonstrating a reduction in postabsorptive rates of MPS after short-term weight loss (5, 7). Interestingly, we observed that supplementation with whey protein resulted in the greatest retention of the postprandial MPS response over soy and carbohydrate after the intervention. Similar findings were demonstrated by Pasiakos et al. (6), who showed that consumption of 2 and 3 times the RDA for protein resulted in an impaired rate of postprandial MPS after the diet. Pasiakos et al. (6) suggested that the amino acids in the RDA group may have been sequestered as a source of energy instead of being used for MPS; consequently, more protein would be required to optimally stimulate MPS. The difference between whey and soy protein supplementation could be the result of the greater leucine content in whey, which results in greater postprandial hyperleucinemia and stimulus for MPS (26). Indeed, our data showed a greater peak and net (AUC) exposure to leucine and essential amino acids with whey than with soy and carbohydrate. There is evidence that whey protein, as opposed to soy protein, results in amino acids being directed more toward peripheral (i.e., muscular) rather than splanchnic tissues (27). Our findings are congruent with this concept and support our earlier work demonstrating a greater MPS response after whey ingestion than with soy protein ingestion (26).

Despite the increased MPS response with whey ingestion compared with soy, no difference in whole body lipolysis was observed between the whey and soy groups. This finding was surprising especially during a hypoenergetic diet, because the energy consuming process of MPS (28) is likely to require energy exposure to leucine and essential amino acids with whey than with soy and carbohydrate. In support of this theory, high protein intakes were shown to stimulate greater fat oxidation during energy restriction than do high carbohydrate intakes (29), and lead to greater fat mass loss after 10 wk (30). In addition, we demonstrated that whey protein consumption

![FIGURE 2 Glycerol Ra in the postabsorptive (Fasted) and postprandial (Fed) periods early (1–2h) and later (2–3h) after supplement ingestion, before (A) and after (B) the dietary intervention. Within-group comparisons omitted for clarity. *Carbohydrate significantly different from whey and soy groups (P < 0.001). Values are means ± SEMs, n = 14 for whey and soy groups, and n = 12 for carbohydrate group. CHO, carbohydrate; Ra, rate of appearance.](image-url)

![TABLE 4 Aminocidemia-related variables for leucine and the sum of essential amino acids and total amino acids after whey, soy, or carbohydrate ingestion in obese or overweight subjects before and after the 14-d diet](table-url)
results in greater plasma hyperleucinemia, which makes it available to peripheral tissues such as muscle and fat. This is significant because leucine administration to a coculture of adipocyte and muscle cells resulted in decreased fatty acid synthase gene expression in adipocytes and increased FA oxidation in muscle cells, resulting in a reduction in energy storage in adipocyte cells and increased energy use (presumably for protein synthesis) in skeletal muscle cells. These results suggest that leucine affects energy partitioning between adipose tissue and lean mass (16). Therefore, mechanistically, whey protein has the potential to be a dietary component known to promote fat loss. Previous work also demonstrated that high protein from dairy promotes greater fat mass loss (11). Future work would be needed to determine the effect of whey protein supplementation during energy restriction provided greater stimulation of MPS and maintenance of postprandial MPS rates. To show changes in body composition, we would need to have extended our study. These results demonstrate the impact of protein quality on MPS during energy restriction, and may be of importance in the development of nutritional strategies to promote higher-quality weight loss, which involves the loss of a high ratio of fat to LBM.

**Acknowledgments**

We thank Tracy Rereich and Todd Prior for their technical assistance. SMP designed the study. AJH, GRM, TAC-V, CHM, LB, MvA, SKB, and SMP conducted the research. AJH and SMP analyzed the data. All authors assisted in editing the manuscript. AJH and SMP had primary responsibility for the final content. All authors read and approved the final manuscript.
References


In the above mentioned article, the second author’s surname is spelled incorrectly. In the author list on page 564, “Niyati Parkeh” should be “Niyati Parekh.” Footnote 2 should read as follows: “Author disclosures: M Vadiveloo, N Parekh, and J Mattei, no conflicts of interest.”

doi:10.3945/jn.115.214031


In the above mentioned article, the lean mass values in Table 1 should be 63.5 ± 3.9 kg (soy) and 62.6 ± 4.0 kg (carbohydrate). These changes do not alter the overall conclusions of the article.

doi:10.3945/jn.115.214361