Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men

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Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 107: 987–992, 2009. First published July 9, 2009; doi:10.1152/japplphysiol.00076.2009.—This study was designed to compare the acute response of mixed muscle protein synthesis (MPS) to rapidly (i.e., whey hydrolysate and soy) and slowly (i.e., micellar casein) digested proteins both at rest and after resistance exercise. Three groups of healthy young men (n = 6 per group) performed a bout of unilateral leg resistance exercise followed by the consumption of a drink containing an equivalent content of essential amino acids (10 g) as either whey hydrolysate, micellar casein, or soy protein isolate. Mixed MPS was determined by a primed constant infusion of L-[ring-13C]phenylalanine. Ingestion of whey protein resulted in a larger increase in blood essential amino acid, branched-chain amino acid, and leucine concentrations than either casein or soy (P < 0.05). Mixed MPS at rest (determined in the nonexercised leg) was higher with ingestion of faster proteins (whey = 0.091 ± 0.015, soy = 0.078 ± 0.014, casein = 0.047 ± 0.008%/h); MPS after consumption of whey was ~93% greater than casein (P < 0.01) and ~18% greater than soy (P = 0.067). A similar result was observed after exercise (whey > soy > casein); MPS following whey consumption was ~122% greater than casein (P < 0.01) and 31% greater than soy (P < 0.05). MPS was also greater with soy consumption at rest (64%) and following resistance exercise (69%) compared with casein (both P < 0.01). We conclude that the feeding-induced simulation of MPS in young men is greater after whey hydrolysate or soy protein consumption than casein both at rest and after resistance exercise; moreover, despite both being fast proteins, whey hydrolysate stimulated MPS to a greater degree than soy after resistance exercise. These differences may be related to how quickly the proteins are digested (i.e., fast vs. slow) or possibly to small differences in leucine content of each protein, hypertrophy; muscle mass; weightlifting

TWO OF THE MOST POTENT stimulators of skeletal muscle protein synthesis (MPS) are feeding and resistance exercise (29). The postprandial increase in circulating essential amino acids stimulates a marked rise in protein synthesis (7, 15, 35); this effect appears to be due to the amino acids themselves acting as the stimulus and not an effect due to the modest increases in insulin that result from amino acid ingestion (16). Resistance exercise potentiates the anabolic effect of feeding (32, 35). Several studies examining the consumption of whole proteins have found that the type of protein, and not simply its amino acid composition, can differentially modulate the anabolic response (3, 8, 9, 37). For example, it has been suggested that milk promotes better whole body nitrogen retention at rest (4, 14), and greater skeletal muscle protein accretion after resistance exercise, compared with soy protein (37). The difference in the metabolism of milk and soy proteins has been attributed to their digestion kinetics, wherein milk is digested slower than soy (4). Milk contains two protein fractions, whey and casein, which have been characterized based on their rate of digestion as “fast” and “slow” proteins, respectively (3). Soy, on the other hand, contains a single homogeneous protein fraction, which is digested in a manner more similar to whey than casein (4).

Whey protein is acid soluble and thus is digested quickly and results in a pronounced aminoacidemia. Data obtained at the whole body level show that whey induces a transient rise in whole body protein synthesis and leucine oxidation at rest (3, 8, 9). Conversely, casein has a modest effect on whole body protein synthesis but instead inhibits whole body protein breakdown (3, 8, 9). Thus, at least at the whole body level, protein digestion rate appears to be an independent factor regulating protein anabolism (8). While the data from whole body protein kinetics point to differential effects of different proteins on synthesis and breakdown (3, 8, 9), skeletal muscle only contributes ~25–30% to whole body protein synthesis (25), and its turnover rate is much lower (on the order of 20 ×) than that of more rapidly-turning-over gut (26, 27) and plasma proteins (6). Thus protein turnover measured at the whole body level may or may not be reflective, or even representative, of the anabolism of muscle proteins. At present, only a single study has measured the chemical net balance of amino acids across a limb following whey and casein ingestion (34), but these data do not give kinetic results and were equivocal depending on the choice of amino acid tracer studied. Thus to date no study has directly compared changes in skeletal MPS following the consumption of isolated proteins with differing rates of digestion in humans. The purpose of this study, therefore, was to measure the response of skeletal MPS following the ingestion of three distinct but high-quality proteins (from a dietary standpoint), whey, micellar casein, and soy, at rest and after resistance exercise. We chose to measure these responses following ingestion of similar quantities of these proteins but...
matched them on their total essential amino acid (EAA) content since only EAA are needed to stimulate MPS (36). We employed a unilateral model of exercise that permitted the comparison of the effect of protein ingestion on muscle anabolism both at rest and after resistance exercise within a given individual. Our hypothesis was that the consumption of whey hydrolysate, casein, and soy proteins would differentially stimulate MPS, based on the rate at which they are digested (whey > soy > casein), both at rest and after resistance exercise.

MATERIALS AND METHODS

Subjects. Three groups of six healthy young men (n = 18) who regularly engaged in whole body resistance training (2–3 days/wk) volunteered to take part in the study. There were no differences in age, height, or weight between groups (P > 0.56; 22.8 ± 3.9 yr; 179.7 ± 5.1 cm; 86.6 ± 13.9 kg; pooled mean ± SD for all subjects). Subjects were informed of the purpose of the study, experimental procedures to be used, and potential risks. Written consent was obtained from all subjects before commencing the study. This study was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board. All testing procedures conformed to those outlined in the Helsinki Declaration of 1963 on the use of human subjects in research.

Experimental protocol. The protocol was designed to examine the effect of consuming whey, casein, and soy protein on mixed muscle protein fractional synthetic rate (FSR) after an acute bout of resistance exercise. At least 1 wk before their first experimental trial, subjects participated in a familiarization session to become acquainted with the testing procedures and training equipment to be used. During the familiarization session, each subject’s 10-repetition maximum (RM) was determined for the seated leg press and knee extension exercises (Universal Gym Equipment, West Point, MS). Subjects performed both exercises unilaterally such that the contralateral leg served as a nonexercised control. For the 2 days before each experimental trial, subjects were asked to refrain from performing any resistance exercise with their legs. In addition, subjects consumed prepackaged diets on those 2 days designed to meet daily caloric (Harris-Benedict equation using an activity factor of 1.6 for all participants) and protein requirements for resistance-trained individuals (1.2–1.4 g/kg) (33).

Subjects arrived at the laboratory on the morning of each experimental trial after an overnight fast. After a baseline blood sample was drawn, subjects performed a bout of intense unilateral resistance exercise consisting of four sets each of leg press and knee extension exercises at a workload equivalent to previously determined 10- to 12-RM with 2 min of passive rest between sets. After the exercise bout, subjects had a 20-gauge catheter inserted into a dorsal hand vein, which was kept patent with a 0.9% saline drip, and a second blood sample was drawn. Subjects then consumed a drink (~100 kcal) containing whey (21.4 g), casein (21.9 g), or soy (22.2 g) protein dissolved in 250 ml water with sucralose (1 g, Splenda) for sweetening and vanilla extract (2 ml) to increase palatability (Table 1). In an effort to maximize protein synthesis with feeding, the amount of protein in each drink provided ~10 g of EAA (7.24). A small amount of tracer was added to each protein drink (8% of phenylalanine content) to minimize changes in blood enrichment after consuming the drink. Whey protein hydrolysate and micellar casein were obtained from American Casein (AMCO, Burlington, NJ) while isolated soy protein (Profam 891) was a generous gift from Archer Daniels Midland (ADM, Decatur, IL). A primed-continuous infusion of 1-[ ring-13C6]phenylalanine (0.05 μmol·kg⁻¹·min⁻¹, 2 μmol/kg prime; Cambridge Isotope Laboratories, Woburn, MA) was then administered through a 0.2-μm filter into an antecubital vein catheter after consumption of the drink to measure mixed muscle FSR. Articulated blood samples were obtained at 30, 60, 90, 120, and 180 min after consumption of the protein drink by warming the hand with a heating blanket (50°C). The infusion protocol is illustrated in Fig. 1.

Muscle needle biopsy. A percutaneous needle biopsy was taken, under local anesthetic, from the vastus lateralis muscle of both the exercised and nonexercised legs 180 min following the consumption of the protein drink. As subjects had not previously been infused with 1-[ ring-13C6]phenylalanine, baseline enrichment of the muscle was estimated from the enrichment of a mixed plasma protein pellet precipitated from the preinfusion blood samples (22, 23). The plasma protein pellet was processed and analyzed in the same manner as the muscle-bound protein pellet (see below).

Blood analyses. Blood samples were collected into evacuated containers containing lithium heparin and deproteinized in perchloric acid (PCA). Whole blood amino acid concentrations were determined on the PCA extract by high-performance liquid chromatography as previously described (37). The remaining whole blood was centrifuged at 1,200 rpm for 10 min at 4°C to separate the plasma. Plasma was removed and stored at −20°C until further analysis. Plasma insulin concentration was determined using standard radioimmunoassay kits (Diagnostic Products, Los Angeles, CA).

Ethanol was added to plasma to precipitate all plasma proteins. The sample was then centrifuged at 1,200 rpm for 10 min at 4°C to pellet the proteins, and the supernatant was decanted. Proteins were hydrolyzed using 6 N HCl (1,000 μl) for 24 h at 110°C. The protein hydrolysate was passed over a cation-exchange column (Dowex 50WX–200 resin; Sigma-Aldrich), then dried under dried N2 gas before analysis for isotopic enrichment, as described below.

Muscle analyses. Acetonitrile (10 μl/ml) was added to muscle samples (~20 mg) before being manually homogenized, vortexed, and then centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant containing the muscle intracellular free (MIF) amino acids was collected and the procedure repeated. The pooled supernatant was then dried under N2 gas for analysis of the MIF amino acid enrichments, as described below. The remaining muscle pellets were washed twice with distilled water, once with absolute ethanol, and then lyophilized to dryness. The dry muscle pellets were subsequently weighed and hydrolyzed with 6 N HCl (400 μl/ml) for 24 h at 110°C. The bound protein hydrolysate was passed over a cation-exchange column (Dowex 50WX–200 resin; Sigma-Aldrich), then dried under dried N2 gas before analysis, as described below.

Table 1. Total and essential amino acid content of protein drinks

<table>
<thead>
<tr>
<th>Protein Drink</th>
<th>Whey</th>
<th>Casein</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine, g</td>
<td>1.1</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Arginine, g</td>
<td>0.6</td>
<td>0.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Aspartic acid, g</td>
<td>2.2</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Cystine, g</td>
<td>0.4</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Glutamic acid, g</td>
<td>3.6</td>
<td>4.4</td>
<td>4.3</td>
</tr>
<tr>
<td>Glycine, g</td>
<td>0.4</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Histidine, g</td>
<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Isoleucine, g</td>
<td>1.4</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Leucine, g</td>
<td>2.3</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Lysine, g</td>
<td>1.9</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Methionine, g</td>
<td>0.5</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Phenylalanine, g</td>
<td>0.7</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Proline, g</td>
<td>1.4</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Serine, g</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Threonine, g</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Tryptophan, g</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Tyrosine, g</td>
<td>0.7</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Valine, g</td>
<td>1.0</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Total, g</td>
<td>21.4</td>
<td>21.9</td>
<td>22.2</td>
</tr>
<tr>
<td>EAA, g</td>
<td>10.0</td>
<td>10.1</td>
<td>10.1</td>
</tr>
</tbody>
</table>

EAA, essential amino acids.
Gas chromatography-mass spectrometry. Blood, plasma protein, and MIF enrichment were determined by making the heptafluorobutyl isobutyl (HFB) derivative of phenylalanine (28). Isotopic enrichments were measured by gas chromatography-mass spectrometry (GC-MS; Hewlett-Packard 5980/5989B, Palo Alto, CA) with ions selectively monitored at mass-to-charge (m/z) ratios of 316 and 322, and a skewed abundance distribution correction was applied (38). Baseline plasma protein and bound muscle protein enrichments were determined by measuring the N-acetyl-n-propyl ester (NAP) derivative of phenylalanine by gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS; Hewlett-Packard 6890, Palo Alto, CA; Thermo Finnigan Delta Plus XP, Waltham, MA). Derivatized amino acids were separated on a 30m DB-1701 column before combustion (temperature ramp: 110°C for 2 min; 20°C/min ramp to 210°C; 5°C/min ramp to 280°C; hold for 5 min).

**Calculations.** Mixed muscle protein FSR was calculated from the determination of the rate of tracer incorporation into muscle protein and using the MIF phenylalanine enrichment as a precursor, according to the equation:

\[
\text{FSR} \, (\%/h) = \frac{(E_m - E_0)}{[E_f(t_1 - t_0)]} \times 100
\]

where \(E_0\) is the enrichment of the protein-bound isotope tracer from isolated plasma proteins with the assumption that tracer-naive subjects would have an \(m+6\) phenylalanine enrichment of virtually zero (i.e., equivalent in muscle and blood). The enrichment obtained from the pool of all plasma proteins therefore represents a basal measure of isotopic enrichment for \(m+6\) from which the enriched measurement can be taken. \(E_m\) is the enrichment of the protein-bound isotope tracer from the second biopsy, \(E_f\) is the mean MIF tracer enrichment during the time period for determination of protein incorporation, and \((t_1 - t_0)\) is the incorporation time. It is possible that the assumption of zero for a baseline enrichment would serve to overestimate the true FSR; however, we believe this overestimation would be the same between conditions.

**Statistical analyses.** Subject anthropometric data and leucine area under the curve (AUC) data were analyzed using t-tests with Bonferroni correction. All other data were analyzed using a two-factor repeated-measures ANOVA. When significance was indicated, a Tukey honestly significant differences (HSD) post hoc procedure was used to identify pairwise differences. All statistical analyses were performed using SigmaStat 3.10.0 (www.systat.com, Systat Software, Point Richmond, CA), and significance was accepted at \(P < 0.05\). All data are presented as means ± SD.

**RESULTS**

**Plasma insulin concentration.** Plasma insulin at baseline was similar between all three groups (Fig. 2). There was a small rise in plasma insulin at 60 min following whey and soy consumption (both \(P < 0.05\)). Plasma insulin was unchanged after the ingestion of casein protein (\(P = 0.43\)).

**Blood amino acid concentrations.** Changes in the concentration of EAA, branched-chain amino acids (BCAA; data not shown), and leucine in the blood followed the same general pattern. All proteins stimulated a rise in EAA (whey = soy > casein; Fig. 3A) and leucine (whey > soy > casein; Fig. 3B) concentration by 30 min postingestion; however, whey protein resulted in a more pronounced aminoacidemia than either casein or soy (\(P < 0.05\)). At 60 min postconsumption, the concentration of EAA and leucine was also higher following whey consumption than either casein or soy (whey > soy > casein; all \(P < 0.05\)). The AUC for blood leucine after whey ingestion was ~73% greater than soy and ~200% greater than casein (Fig. 3B, inset).

**Plasma and muscle intracellular free phenylalanine enrichment.** Plasma and muscle intracellular free phenylalanine enrichments are shown in Fig. 4, A and B, respectively. Linear regression analysis (not shown) indicated that the slopes of the plasma enrichments over time were not significantly different from zero (\(P > 0.05\)), suggesting that plasma enrichments had reached a plateau and subjects were at isotopic steady state over the incorporation period.

**Mixed MPS.** At rest, both whey and soy FSR were significantly greater than casein (\(P < 0.01\); Fig. 5). Whey FSR tended to be greater than soy at rest but was not significantly different (\(P = 0.067\)). After resistance exercise, FSR was greater compared with rest in all groups (\(P < 0.05\); Fig. 5). FSR following whey consumption was significantly greater than both soy and casein after resistance exercise (\(P < 0.05\)).

**DISCUSSION**

This is the first study to report directly measured rates of mixed MPS in response to ingesting isolated proteins that are known to be digested at different rates in humans. We found that the consumption of whey protein hydrolysate stimulated MPS to a greater degree than casein both at rest and after resistance exercise. While FSR in the whey group tended to be greater than soy in the rested muscle, this did not reach statistical significance. After resistance exercise whey hydrolysate stimulated a significantly larger rise in MPS than soy. In

![Image](http://jap.physiology.org at Occidental College Library on November 30, 2012)
congruence with our previous work showing a greater stimulation of MPS with milk vs. soy protein ingestion (37), soy appears to be less effective at stimulating MPS than whey protein despite inducing a similar rise in circulating EAA.

Based on previous literature identifying protein digestibility as an independent factor regulating whole body protein anabolism (8), we hypothesized that the pattern of appearance of amino acids in the systemic circulation following consumption of whey, casein, or soy would also result in a differential stimulation of protein synthesis at the muscle level. For example, several studies have noted that “fast” proteins stimulate a large rise in protein synthesis whereas “slow” proteins primarily inhibit protein breakdown, but these results come from data at the whole body level (3, 8, 9) of which muscle comprises only 25% (25) and turns over at a much slower rate than, for example, gut proteins (26, 27). In addition, milk proteins appear to support greater “peripheral” (i.e., muscle) vs. splanchnic protein synthesis than do soy proteins (4, 14). Our data extend these previous studies that measured only whole body protein turnover by demonstrating that the consumption of whey hydrolysate and soy isolate (i.e., “fast” proteins) result in considerably higher rates of muscle protein synthesis than casein (i.e., “slow” protein), both at rest (whey ≈ soy > casein) and after resistance exercise (whey > soy > casein). In our view, these differences are unlikely to be explained by our use of a whey protein hydrolysate, rather than isolate. This is because previous data have noted no difference in the pattern of aminoacidemia following ingestion of 36 g of whole whey protein or its hydrolysate (5). While the pattern of peripheral aminoacidemia yields no insight into the actual kinetics of protein absorption, this fact is of little consequences since the concentration of amino acids in the peripheral (i.e., non-
splanchnic) circulation would be those that are available for protein synthesis by peripheral tissues such as muscle (assuming of course equivalent flow). Interestingly, when examining whole body leucine kinetics, prior studies actually found that casein consumption promoted a higher whole body leucine balance than whey (3, 8, 9). While these findings may seem contradictory to what we observed here, the inhibitory effect of casein on protein breakdown, almost certainly in the splanchnic region (26, 27), was the largest contributor to the greater whole body leucine balance observed. In addition, the increase in whole body protein synthesis stimulated by whey was observed to be quite transient (3, 8, 9). Admittedly, we chose a 180-min time point in the present study to capture the acute MPS response, whereas the whole body data represented an aggregate 7-h response (3, 8, 9). We do not think, however, that extending our response beyond 3 h would have markedly affected our MPS results since amino acid concentrations were back down to baseline levels by 240 min.

Previously, whey and casein proteins were shown to improve net muscle amino acid balance (measured as a-v balance) to a similar extent, despite a marked difference in the pattern of aminoacidemia, presumably reflecting the rate of digestion of each protein (34). In contrast, we observed marked differences, using direct incorporation measurements, in rates of postexercise skeletal MPS after whey and casein feeding in the present study. It is known that resistance exercise increases muscle protein breakdown, albeit to a lesser extent than synthesis (1, 28). Thus, the results of Tipton et al. (34) could have arisen due to a marked suppression of muscle protein breakdown with casein and a stimulation of synthesis with whey. Ingestion of amino acids attenuates the post-resistance exercise-induced increase in muscle protein breakdown (2, 35); thus in the present study it is hard to envision that a marked suppression of muscle proteolysis occurred with casein ingestion that would not have occurred with whey or soy. We did not measure proteolysis, however, and thus can only speculate as to the effect of protein digestibility on muscle protein breakdown due to current methodological limitations that preclude the direct measurement of muscle protein degradation after physiological (i.e., bolus) protein ingestion.

The differences in the stimulation of MPS after ingestion of whey hydrolysate and soy protein and casein at rest and after resistance exercise are somewhat surprising given their similar protein digestibility-corrected amino acid scores (PDCAAS) (13). Indeed, the PDCAAS of these proteins would suggest that they are high-quality complete sources of amino acids, which in theory should be able to equally support protein synthesis. However, the concept of PDCAAS and their relevance to physiological outcomes has drawn some criticism (31); our results suggest that in the context of skeletal muscle accretion following resistance exercise this is particularly the case. In the present study there were marked differences in the patterns of aminoacidemia, which likely reflect the rate of protein digestion not only between fast and slow proteins, but also within the fast proteins themselves (i.e., whey and soy). The rise in EAA (Fig. 3A), BCAA (data not shown), and leucine (Fig. 3B) was of greater amplitude and considerably more rapid following whey consumption compared with soy. These differences in the rate of EAA appearance in the circulation may be especially important to the differential stimulation of MPS we observed after whey or soy ingestion at rest and following resistance exercise. Recent work has demonstrated that supplementing soy protein with BCAA (leucine, isoleucine, and valine) is required to rescue its anabolic effect in elderly and clinical populations (11). Leucine has also been shown to enhance the activation of mTOR-related signaling proteins at rest and after exercise (17, 19, 21). Thus the greater total BCAA (~7%) and leucine content (~28%) in particular may have contributed to the larger increase in protein synthesis after whey ingestion compared with soy. We speculate that a critical "trigger" threshold of EAA, perhaps leucine in particular (10), has to be reached in the blood before MPS is maximally stimulated and that this threshold was not reached with soy ingestion. Such a thesis is supported by our recent work showing a saturable response in MPS following resistance exercise (24).

The differences in skeletal MPS that we observed may have implications for populations with compromised nutrient sensitivity (e.g., the elderly) (7). Indeed, it has been found that the protein digestibility paradigm observed in young individuals is actually "reversed" in the old with respect to whole body protein metabolism (9), and that "fast" protein ingestion is associated with a greater whole body leucine balance (7). If the leucine "trigger" concept is correct, then we would speculate that these results (7) may reflect the inability of casein to increase blood EAA, BCAA, or leucine concentration high enough to turn on MPS in older persons who appear to have a reduced sensitivity to amino acids or an "anabolic resistance" (7). For example, ingestion of larger doses of leucine has been shown to enhance feeding-induced increases in MPS in aged individuals (18, 30). Considering protein ingestion after exercise appears critical to enhance skeletal muscle hypertrophy with resistance training in the elderly (12), we propose that elderly individuals would likely obtain the greatest benefit with respect to stimulating MPS and likely muscle protein accretion by consuming a "fast" leucine-rich dietary protein such as whey both at rest and after resistance exercise (18, 20, 30). Future studies should directly measure skeletal MPS in populations such as the elderly after consuming different whole proteins to confirm this thesis.

In summary, we report that the consumption of whey protein hydrolysate stimulates skeletal MPS to a greater extent than either casein or soy. Our results suggest that the type of protein consumed is a modulating factor in determining postprandial resting and postexercise muscle anabolism in young healthy men, both at rest and after resistance exercise. Moreover, this effect may be related to the leucine content of the protein consumed and how quickly it is digested. Thus, it appears that when providing an optimal dose of protein (~10 g EAA) (7, 24), a rapid increase in EAA (perhaps leucine specifically) is important for supporting maximal rates of skeletal MPS.

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