

# The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise

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## Introduction

This scientific review aims to concentrate all of the research on protein and its effect in muscle growth across a variety of conditions.

## Conclusions

Protein consumption increases protein synthesis and reduces breakdown, but its more profound effect is in synthesis.

20 grams of protein is sufficient to largely increase protein synthesis rates in cells, and consuming double only slightly increase synthesis rates further (10-20% increased boost).

Other details not mentioned here are in the body of the review.

## Amendments



# The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise

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## Abstract

Protein ingestion following resistance-type exercise stimulates muscle protein synthesis rates and consequently enhances the skeletal muscle adaptive response to prolonged training. Ingestion of ~20 g of quickly digestible protein isolate optimizes muscle protein synthesis rates during the first few hours of post-exercise recovery. However, the majority of daily protein intake is consumed as slower digestible, nutrient-rich, whole-food protein sources as part of mixed meals. Therefore, the muscle protein synthetic response to the ingestion of protein supplements and typical foods or mixed meals may differ substantially. In addition, the muscle protein synthetic response to feeding is not only determined by acute nutrient intake but is also likely modulated by habitual energy and nutrient intake and nondietary factors such as habitual physical activity, body composition, age, and/or sex. Therefore, nutritional recommendations to maximize the muscle protein synthetic response to exercise depend on the type of meal (e.g., protein supplements vs. mixed meals) and the time until the next feeding opportunity (e.g., feeding before overnight sleep) and, therefore, need to be personalized to the individual athlete.

## Key Points

Ingestion of 20 g of isolated, quickly digestible protein results in a near-maximal muscle protein synthetic response at rest and post-exercise, with a 10–20% further increase when the ingested amount is doubled to 40 g.

The ingestion of ≥40 g of slow digestible protein is recommended to maximize muscle protein synthesis rates when there is a prolonged period until the next feeding opportunity (≥6 h, e.g., overnight sleep).

Nutritional recommendations to optimize the muscle protein synthetic response to feeding should be personalized to the individual athlete (i.e., age, sex, and body composition, and type, intensity, and duration of exercise).

## 1 Introduction

While muscle mass is remarkably constant in healthy adults, it is a highly adaptive organ capable of changing in size and/or function. Even when muscle mass is constant, muscle tissue is constantly turning over, i.e., the rates at which muscle proteins are synthesized and broken down are in balance. This turnover allows muscle tissue to remodel, e.g., replacing damaged proteins with new proteins or changing the composition of muscle proteins to adapt to challenges such as exercise. An imbalance between protein synthesis and protein breakdown rates in skeletal muscle results in either a net gain (synthesis > breakdown) or net loss (breakdown > synthesis) in muscle mass.

A single session of exercise stimulates muscle protein synthesis (MPS) rates and, to a lesser extent, muscle protein breakdown rates [1, 2]. However, muscle protein net balance will remain negative in the absence of food intake [2]. Protein ingestion stimulates MPS and inhibits muscle protein breakdown rates, resulting in net muscle protein accretion during the acute stages of post-exercise recovery [3]. Therefore, post-exercise protein ingestion is widely applied as a strategy to augment post-exercise MPS rates and, as such, to facilitate the skeletal muscle adaptive response to prolonged exercise training.

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1. Muscle is constantly turning over (replacing the proteins within the cells), requiring the consumption of new protein from food to do so. If breakdown of old functional proteins within the muscle cells exceeds the replenishment of these proteins, the muscle cell shrinks.

One exercise session excites protein synthesis rates and muscle breakdown, but more so on the protein synthesis. Protein consumption excites protein synthesis (muscle functional protein production), and inhibits muscle breakdown.

Amount of protein, type, and timing of consumption all play as factors (among others, like what the protein is paired with).

Various factors have been identified that can modulate the MPS response to feeding, including the amount [4, 5], type [6, 7], and timing [8] of protein ingestion. However, much of this work has focused on isolated amino acids or quickly digestible protein isolates ingested in the absence of other nutrients [4–6, 8]. Such conditions may not be reflective of the postprandial MPS response to the ingestion of a mixed meal, in which protein is typically consumed in the form of slower digestible, whole-food protein sources [9]. In addition, the MPS response to feeding is not only determined by acute nutrient intake (i.e., meal composition) but is also likely modulated by habitual energy and nutrient intake and nondietary factors such as habitual physical activity, body composition, age, and/or sex [2, 10–13]. Therefore, nutritional recommendations to maximize the MPS response to feeding may depend on the type of meal (e.g., protein supplements vs. mixed meals) and time until the next feeding opportunity (e.g., feeding before overnight sleep) and should be personalized to the individual (e.g., accounting for physical activity level). While protein ingestion has been shown to stimulate the post-exercise MPS response following various modes of exercise [14–16], the majority of work has focused on resistance-type exercise. Therefore, the purpose of this review is to discuss our current understanding of dietary and nondietary factors modulating the MPS response to feeding and following resistance-type exercise.

## 2 Acute Dietary Factors Modulating the Muscle Protein Synthetic Response to Feeding

### 2.1 Amount of Protein

Few studies have investigated the dose–response relationship between protein ingestion and MPS rates during recovery

when protein were compared in a crossover design following whole-body resistance-type exercise [17]. The 40 g dose resulted in significant 20% higher MPS rates compared with the 20 g dose. These data may suggest that the amount of protein required to maximize MPS rates following whole-body resistance-type exercise is higher when compared with exercise during which less muscle is recruited. However, this hypothesis requires confirmation in a more direct comparison. Taken together, it appears that the ingestion of 20 g of isolated, quickly digestible protein results in a near-maximal MPS response at rest and post-exercise, with a 10–20% further increase when the ingested amount is doubled to 40 g (Fig. 1a).

The impact of varying amounts of ingested protein on post-exercise MPS rates in young adults has been limited to experimental settings investigating the impact of ingesting isolated, quickly digestible protein sources on MPS rates during 4–5 h of post-exercise recovery [4, 5, 17]. These conditions are ecologically valid for the acute post-exercise period in which the ingestion of quickly digestible protein supplements is common practice. However, such conditions do not reflect most meal situations in which protein is generally consumed as slower digestible, nutrient-rich, whole-food protein sources as part of a mixed meal. Therefore, it should be questioned whether these data can be directly translated to per-meal protein recommendations.

### 2.2 Type of Protein

Plant-based protein sources are typically considered less efficient at stimulating MPS rates than animal-based protein sources. The possible lower anabolic properties of plant-based protein sources may be attributed to the lower total essential amino acid content, limited content of specific amino acids, lower leucine content, lower digestibility, and/or higher splanchnic extraction of plant-based protein-

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2. There is a dose response relationship with protein and muscle protein synthesis - using egg protein/complete protein, there is an increase in muscle synthesis up to 20 grams of protein, then only a 10% further increase up to 40 grams.

△ This was confirmed with whey protein - same effect.

Other studies have shown a 20% increase from the 20 gram mark by consuming 40 grams.

3. Plant based proteins are considered less efficient for muscle protein synthesis. It is believed to be due to lower essential amino acids, lower digestibility, and higher splanchnic extraction (which means that less amino acids are deposited into the blood, but rather, those amino acids end up used by the intestines or excreted).

## to Feeding

### 2.1 Amount of Protein

Few studies have investigated the dose–response relationship between protein ingestion and MPS rates during recovery from resistance-type exercise in younger adults. Moore et al. [4] were the first to present a dose response in MPS rates following the ingestion of 0, 5, 10, 20, or 40 g of egg protein during recovery from lower-body exercise. They observed a dose-dependent increase in MPS rates up to the ingestion of 20 g of protein, with a nonsignificant ~10% further increase following the ingestion of 40 g (Fig. 1a). Witard et al. [5] followed up on this work by assessing the impact of ingesting increasing amounts of whey protein on MPS rates at rest and during post-exercise recovery using a unilateral leg exercise model. In addition, subjects ingested a standardized protein-rich breakfast 4 h before ingestion of the protein beverages. The ingestion of 20 g of whey protein was sufficient to maximize MPS rates at rest and during post-exercise recovery, with a nonsignificant ~10% further increase following the ingestion of 40 g of protein in the post-exercise condition. More recently, the ingestion of 20 g and 40 g of

efficient at stimulating MPS rates than animal-based protein sources. The possible lower anabolic properties of plant-based protein sources may be attributed to the lower total essential amino acid content, limited content of specific amino acids, lower leucine content, lower digestibility, and/or higher splanchnic extraction of plant-based protein-derived amino acids [18]. Indeed, most studies show that the ingestion of animal-based protein sources generally results in higher MPS rates at rest or following resistance-type exercise than plant-based protein sources in younger and older adults [6, 7, 19]. We have recently observed a significant increase in MPS in older adults following the ingestion of 60 g of wheat protein hydrolysate but not following the ingestion of 35 g of wheat protein hydrolysate [19]. These data suggest that consumption of a greater amount of plant-based protein is an effective strategy to compensate for its lower quality. However, whether the ingestion of large amounts of plant-based protein can maximize MPS rates in younger adults and/or following resistance-type exercise remains to be determined (Fig. 1b). It is important to note that investigations of the impact of plant-based protein sources on MPS rates has been limited to the ingestion of protein isolates. A typical mixed meal is likely to also

3. Plant based proteins are considered less efficient for muscle protein synthesis. It is believed to be due to lower essential amino acids, lower digestibility, and higher splanchnic extraction (which means that less amino acids are deposited into the blood, but rather, those amino acids end up used by the intestines or excreted).

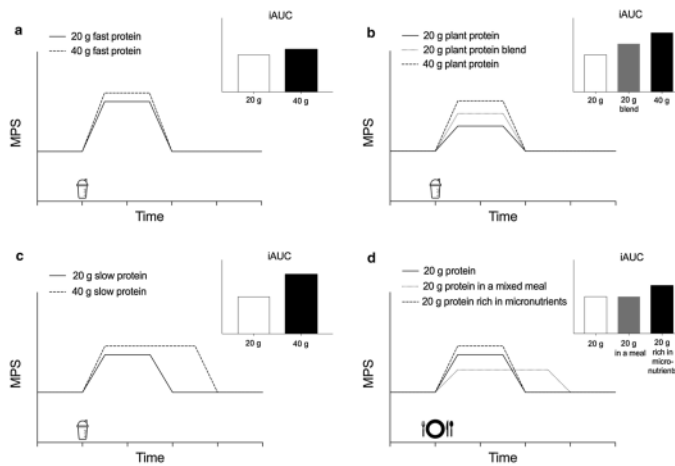
Some plant based proteins lead to no increase in muscle protein synthesis at levels around 35 grams consumed, but do show an effect at 60 grams.

However, using a variety of plant proteins can lead to increases in muscle protein synthesis beyond the same amount of a single plant protein.

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### Postprandial Muscle Protein Synthesis

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**Fig. 1** Conceptual representation of the muscle protein synthesis (MPS) response to various feeding protocols. **a** The ingestion of ~20 g quickly digestible protein results in a near-maximal MPS response. **b** The ingestion of 20 g plant-based protein typically results in a submaximal MPS response, but the consumption of a greater amount and/or mixing different sources may possibly augment the MPS response. **c** The ingestion of 20 g slowly digestible protein

results in a submaximal MPS response, but the ingestion of greater amounts may result in a more prolonged anabolic response. **d** Mixed meal ingestion may result in a more moderate but prolonged MPS response compared with ingestion of an isolated whole-food protein source. The micronutrient content of a whole-food protein source may further augment the MPS response. IAUC incremental area under the curve

include some animal-based protein and/or contain different plant-based protein sources that may provide a more balanced amino acid profile [20]. Therefore, the proposed lower MPS response to the ingestion of a single plant-based protein source can potentially be rescued by the ingestion of multiple (plant-based) protein sources (Fig. 1b).

### 2.3 Protein Digestion and Absorption Rate

Dietary protein sources can differ substantially in their digestion and absorption kinetics. For example, whey is a quickly digestible protein that results in a rapid but transient postprandial increase in plasma amino acid concentrations [21]. In contrast, casein is a slower digestible protein that results in a more moderate but prolonged postprandial increase in plasma amino acid concentrations. The ingestion of whey protein typically stimulates MPS rates to a greater extent than casein protein when assessed over periods of up

to 6 h at rest or following resistance-type exercise [6, 21, 22]. This has been attributed to the more rapid protein digestion and amino acid absorption kinetics as well as the higher leucine content in whey compared with casein protein, resulting in a more rapid rise in postprandial leucine concentrations [21, 23–25]. Furthermore, the post-exercise MPS response to whey protein ingestion is attenuated when ingested in multiple smaller doses over time versus bolus ingestion [26]. These data suggest that protein digestion and absorption kinetics, and timing of intake, modulate the MPS response even when amino acid composition is matched. Therefore, whether the optimal amount of ingested protein as established for quickly digestible protein sources can be translated to slower digestible protein sources can be questioned.

The post-exercise MPS response to the ingestion of different amounts of slow digestible protein has not been assessed in healthy young men. This response may have a temporal pattern that differs from that of more rapidly digested

4. Whey protein is quickly absorbed and leads to a rapid rise in blood amino acids (protein constituent parts). Casein, on the other hand, is a slower digesting protein, leading to a more sustained rise in amino acids. Overall, whey still stimulates muscle protein synthesis more than casein, likely because of its higher leucine content. Whey can also be spread out, allowing for a sustained effect, as well.

It also seems that more of the slower digesting casein is necessary to generate a muscle protein synthetic response over hours (especially in sleep over 7-8 hours) - something like 40 grams is effective, but 30 grams is not.

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proteins. Increasing amounts of a more slowly digestible protein may result in a moderate but more prolonged MPS response consistent with its protein digestion and absorption pattern (Fig. 1c). Some support for this concept comes from our observations that the ingestion of 30 g of casein protein before sleep did not result in a detectable increase in overnight post-exercise MPS assessed over a prolonged 7.5-h overnight period [27], whereas we observed a ~22% increase in overnight MPS rates following the ingestion of 40 g of casein protein under nearly identical conditions [28]. While it should be noted that these studies assessed post-exercise MPS rates in different muscle protein fractions (i.e., mixed-muscle vs. myofibrillar protein), the ingestion of 30 g of protein should be more than sufficient to stimulate post-exercise mixed-muscle or myofibrillar protein synthesis rates over a 4–5 h period [4, 5]. Therefore, it appears that larger amounts of slow digestible protein are required to obtain a more robust stimulation of post-exercise MPS rates over more prolonged periods such as overnight sleep [29]. This may suggest that the optimal amount and type of ingested protein depends on the meal timing, with ~20 g of quickly digestible protein being preferred when there is a relatively short 3- to 5-h period until the next meal, but ingestion of ≥40 g of slower digestible protein may be favored when the period until the next feeding opportunity is prolonged. However, whether the digestion and absorption rate of a protein, and/or the co-ingestion of other nutrients, modulates the MPS response when relatively large amounts (≥40 g) of protein are ingested and MPS is assessed over a prolonged (>6 h) period remains to be determined.

#### 2.4 Mixed Meal Composition

Most work assessing the MPS response to feeding has focused on isolated protein intake. However, dietary protein is typically consumed as part of a mixed meal. The co-ingestion of foods that are not necessarily high in protein may still impact the total protein intake, the amino acid profile of the meal, protein digestion and absorption kinetics, hormonal response, and micronutrient intake. Such factors can potentially modulate the MPS response to feeding, but their individual contributions are difficult to predict [30].

Carbohydrate co-ingestion with protein delays protein absorption and digestion kinetics [31], although this does not seem to attenuate the MPS response to protein ingestion at rest or following resistance-type exercise [31, 32]. However, the impact of carbohydrate co-ingestion has been limited to the addition of rapidly digestible, high glycemic index carbohydrates to protein-containing beverages. In contrast, mixed meals are typically in solid form and provide more slowly digestible carbohydrates and also contain dietary fiber. Therefore, a greater delay in protein digestion and absorption kinetics can be expected following mixed

meal ingestion compared with the ingestion of a protein–carbohydrate supplement. In support, the postprandial rise in plasma amino acid levels appears to be substantially attenuated when minced meat is consumed in a mixed meal [33, 34]. It has been suggested that carbohydrate co-ingestion may augment MPS rates via its ability to elicit a postprandial rise in insulin concentration. However, insulin is permissive for MPS during hyperaminoacidemia and does not stimulate MPS rates under conditions reflecting food ingestion at rest [35, 36]. Consistent with this notion, carbohydrate co-ingestion with protein does not augment MPS rates following resistance-type exercise [32].

Very little work has addressed the potential impact of fat co-ingestion on the MPS response to protein ingestion. Post-exercise amino acid uptake by the leg (indicative of muscle protein accretion) has been shown to be higher following the ingestion of high-fat milk compared with skim milk [37]. More recently, we observed no delay in protein digestion and amino acid absorption and the subsequent MPS rates when milk fat was co-ingested with a beverage containing casein protein in older adults at rest [38]. This absence of a delay in protein digestion and absorption kinetics may be attributed to layering of fat on top of protein in the stomach that may only occur with a liquid meal [39]. However, co-ingestion of 17 g of fat as provided by the consumption of whole eggs did not attenuate post-exercise protein digestion and absorption kinetics compared with the ingestion of an isonitrogenous amount of egg whites [40]. Therefore, fat co-ingestion does not appear to substantially impact protein digestion and amino acid absorption kinetics. However, there is some indication that an oversupply of lipid may impair postprandial MPS. We have shown that lipid infusion reduces the MPS response to the ingestion of amino acids during hyperinsulinemic–euglycemic clamped conditions in healthy younger adults at rest [41]. In addition, attenuated post-exercise intramuscular anabolic signaling (i.e., 4E-BP1 phosphorylation) has been observed following the combined ingestion of protein and fat compared with the combined ingestion of protein and carbohydrate [42]. The impact of the fat content of a protein-containing meal on the MPS response remains ambiguous.

Recent work suggests that the MPS response to feeding may be modulated by the consumption of micronutrients (Fig. 1d). In support, the ingestion of whole eggs was more effective in stimulating post-exercise MPS rates than was the ingestion of an isonitrogenous amount of egg whites [40]. The differential response could not be attributed to differences in protein digestion and amino acid absorption or caloric intake between the treatments [31, 32, 38]. A possible explanation is the considerably higher content of fat and/or micronutrients in whole eggs. As previously discussed, fat co-ingestion may augment the post-exercise MPS response to protein ingestion [37]. Furthermore,

- Mixed meals (with carbohydrates or fats) change the digestion of protein. Carbohydrates slow the digestion and absorption of protein. Fat consumption does not seem to impact protein digestion and absorption like carbohydrates do, except potentially in high amounts of fat consumption.

It is also thought that micronutrients increase muscle protein synthesis response to protein. Usually concentrations found in food helps, but over consumption, like from a concentrated vitamin, may be detrimental to muscle protein synthesis.

several micronutrients that are contained primarily in the yolk, such as vitamin A, vitamin D, vitamin E, zinc, selenium, and cholesterol, are potential candidates to augment the anabolic response to feeding [30, 43]. In addition, the co-ingestion of an amylopectin/chromium complex has recently been shown to augment the post-exercise MPS response to a suboptimal amount of protein [44]. However, it should be noted that some nutrients may potentially impair MPS when ingested in high amounts that are typically not found in whole foods. For example, the ingestion of the lipid second messenger phosphatidic acid has recently been shown to impair the post-exercise MPS response in older adults [45]. Furthermore, high-dose antioxidant supplementation (i.e., vitamin C and E) may blunt the adaptive response to exercise [46]. Therefore, high doses of micronutrient supplements with strong antioxidant properties is not recommended for athletes. Taken together, emerging evidence suggests that certain micronutrients in a meal may be able to modulate postprandial MPS rates. The MPS response to mixed meal ingestion may, therefore, differ from the ingestion of protein isolates due to changes in protein and amino acid absorption kinetics and/or specific micronutrient(s) content(s) (Fig. 1d).

To date, only one study has assessed postprandial MPS rates following mixed meal ingestion. Subjects consumed a ~1300 kcal lean beef mixed meal containing either 40 g or 70 g of protein [34]. Postprandial MPS rates did not differ between the ingestion of the moderate- or the high-protein meal at rest or during post-exercise recovery. Interestingly, plasma essential amino acid concentrations were highest at the end of the 4-h postprandial period. This may suggest that large protein-rich mixed meals result in a protein digestion and absorption pattern that reflects a more slowly digestible protein. Clearly, more research is warranted to assess the MPS response to the ingestion of mixed meals and its modulation by meal composition.

### 2.5 Alcohol

Despite warnings from health agencies, alcohol consumption remains culturally engrained worldwide [47]. Interestingly, several studies have reported that athletes are more likely to consume excessive amounts of alcohol, especially as part of binge-drinking practices in team sports [48, 49]. Parr et al. [15] demonstrated that alcohol ingestion impaired the MPS response to post-exercise protein ingestion. A total of 1.5 g alcohol per kg bodyweight ( $12 \pm 2$  standard drinks) was consumed to reflect alcohol intake levels reported in binge drinking practices of team athletes. These data provide clear proof of principle that ingestion of excessive amounts of alcohol can impair post-exercise recovery. Furthermore, Parr et al. [15] observed that alcohol co-ingestion downregulated intramuscular anabolic signaling (i.e., phosphorylation of mammalian target of rapamycin), which is consistent with

the majority of data in rodent models [50]. How this alcohol-induced attenuation of anabolic signaling is regulated is unclear, but direct effects via REDD (regulated in development and DNA damage)-1 and indirect effects via the modulation of the activity of circulating anabolic hormones such as insulin-like growth factor 1 have been proposed [50]. Further work is required to provide more mechanistic insight and to determine whether there is a dose-response relationship between alcohol intake and MPS rates and to determine the impact of more moderate alcohol consumption, e.g., drinking one to two glasses of wine with dinner.

## 3 Habitual Food Intake and Postprandial Muscle Protein Synthesis

### 3.1 Habitual Energy and Protein Intake

The MPS response to feeding is not only modulated by acute nutrient intake but may also be affected by habitual food intake (Fig. 2). Athletes may intentionally eat a caloric surplus to gain lean body mass (i.e., bulking), but the impact of caloric overfeeding on MPS rates remains to be determined. It is more common for athletes to purposely restrict caloric intake to reduce body fat [51]. While it is feasible to increase lean body mass during a marked energy deficit when a high protein consumption is combined with a high volume of resistance and anaerobic exercise [52], energy restriction generally leads to muscle mass loss [53, 54]. Consistent with this notion, integrated MPS rates are reduced during energy restriction but can be rescued by resistance-type exercise and may be potentiated by a higher protein diet [55]. While acute caloric intake does not seem to modulate the MPS response to feeding [31, 38], both acute postabsorptive and postprandial MPS rates at rest have been shown to be attenuated following a short-term (3–14 day) energy intake restriction [10, 56]. Greater reductions in the MPS response to feeding can be expected during more severe energy deficits and in individuals with lower body fat levels, as these conditions have been shown to increase lean body mass loss during energy intake restriction [57, 58]. The blunted MPS response to protein ingestion during energy restriction may be rescued by the consumption of a higher protein diet. Subjects consuming 1.6 or 2.4 g protein/kg/day during a 40% energy deficit for 21 days had a preserved MPS response to protein ingestion, whereas this anabolic response was attenuated in subjects consuming the recommended daily allowance (RDA) for protein of 0.8 g/kg/day [59]. In contrast, the opposite pattern was observed in these subjects following 10 days of weight maintenance. A significant increase in MPS was observed in subjects ingesting 0.8 and 1.6 g of protein/kg/day, but no anabolic response was observed in subjects consuming 2.4 g of protein/kg/

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6. Alcohol reduces muscle synthesis signaling, as assessed in binge drinking (not sure about single drink, but likely has a minor effect).

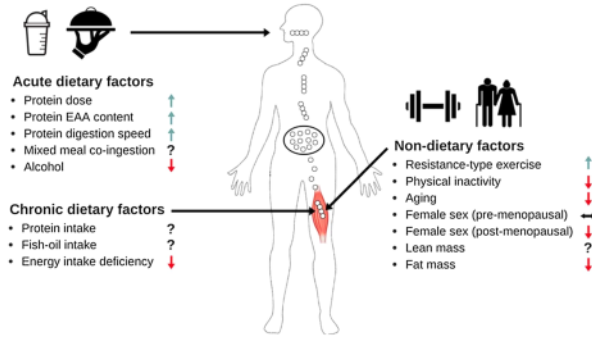
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7. Overfeeding (consuming more calories than you need) is thought to promote muscle protein synthesis, but the studies on that have yet to be conducted in earnest. Calorie deficit, especially severe, can lead to muscle loss from reduced muscle protein synthesis, which is combated by resistance training and a high protein diet.

People consuming 1.6 and 2.4 grams per kilogram of bodyweight a day in a severe calorie deficit (40%) were able to maintain their muscle protein synthesis ability. Interestingly, when they were back on a maintenance diet (no calorie restriction), these same people consumed 0.8 grams per kilogram and 1.6 grams per kilogram experienced increases in muscle protein synthesis, but did not get more muscle protein synthesis from 2.4 grams per kilogram.





**Fig. 2** Schematic representation of factors modulating the muscle protein synthetic (MPS) response to feeding. The MPS response to feeding is augmented by prior exercise and a higher amount, essential amino acid (EAA) content, and digestion and absorption rate of the ingested protein. The MPS response to feeding is attenuated by alcohol co-ingestion, during prolonged energy deficit, during muscle inactivity, with aging (especially in females), and by individuals with excessive fat mass. ↑ indicates the MPS response to feeding is augmented, ↓ indicates the MPS response to feeding is attenuated, ↔ indicates the MPS response to feeding is not affected, ? indicates the impact on MPS response to feeding is unclear

7 day [59]. We observed no impact of 14-day habituation to a higher (1.5 g/kg/day) or lower (0.7 g/kg/day) protein diet on postprandial MPS rates or the MPS response to the ingestion of 25 g of protein in weight-stable older adults. Furthermore, postabsorptive MPS rates do not appear to change even after a prolonged 12-week high (2.4 g/kg/day) or low (0.4 g/kg/day) protein diet in weight-stable healthy young adults [60]. While it is clear that prolonged energy restriction attenuates postabsorptive and postprandial MPS rates, the impact of habitual protein intake during energy restriction and energy balance on the MPS response to protein ingestion remains poorly understood.

### 3.2 Habitual Omega-3 Polyunsaturated Fatty Acid Intake

8 Omega-3 polyunsaturated fatty acid supplementation for 8 weeks has been shown to augment the MPS response to a hyperinsulinemic-hyperaminoacidic clamp in both younger and older adults [11, 61]. As postabsorptive MPS rates were not significantly increased following the supplementation period, omega-3 supplementation appears to specifically enhance the muscle anabolic sensitivity to hyperaminoacidemia. Insulin and amino acids were clamped to levels typically seen after a meal but were low enough to avoid a possible ceiling effect. In contrast, omega-3 supplementation

for 8 weeks did not increase the MPS response to the ingestion of 30 g of whey protein at rest or following resistance-type exercise [62]. As 30 g of protein should be more than sufficient to maximize the MPS response to protein ingestion in healthy young subjects, it could be speculated that fish oil supplementation may only augment the MPS response to suboptimal amounts of protein ingestion. While omega-3 fatty acid supplementation could provide an effective nutritional strategy to augment the MPS response to protein ingestion, it is unclear what the target omega-3 concentrations should be and thus what dose and duration of supplementation period should be recommended.

## 4 Nondietary Factors Modulating the Muscle Protein Synthetic Response to Feeding

### 4.1 Exercise and Physical Activity

Physical activity and exercise are potent stimulators of postabsorptive as well as postprandial MPS rates [2, 63–65] (Fig. 2). A single bout of exercise stimulates the use of dietary protein-derived amino acids as precursors for MPS, as dietary protein-derived amino acids are more directed towards activated muscle during their

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8. Omega-3 fats increase muscle protein synthesis when amino acids are present from protein, but has no effect if the amino acids saturate (reach their ceiling in their ability to maximize muscle protein synthesis). So, it may be helpful if less than an ideal amount of protein is consumed (say, 15 grams instead of 25 grams).

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recovery from exercise [66]. The MPS response to protein ingestion is maximally stimulated by the ingestion of 20 g of whey protein in rested conditions and following lower-body resistance-type exercise [4, 5], albeit with higher MPS rates during the post-exercise condition [4, 5]. However, exercise may increase not only the maximal rate of postprandial MPS but also the duration of the anabolic response. It has been suggested that the MPS response to protein ingestion is transient even in the presence of prolonged hyperaminoacidemia, a phenomenon often termed the “muscle full” effect [67]. However, the exercise-induced increase in anabolic sensitivity may supplant the muscle full effect and allow for a more prolonged MPS response to feeding. In support, two studies using a unilateral leg exercise model observed a prolonged MPS response to feeding in the exercised leg [68, 69]. MPS rates were elevated in the first 3 h following protein ingestion but had returned to basal levels in the subsequent 2 h in the rested leg, whereas MPS rates remained elevated during the entire 5-h period following protein ingestion in the exercised leg [68, 69]. Thus, exercise appears to increase both the rate and the duration of postprandial MPS rates.

MPS rates are also sensitive to habitual physical activity. Several studies have demonstrated that muscle disuse reduces postabsorptive and postprandial MPS rates following prolonged bed rest or immobilization [70–73]. Glover et al. [70] observed lower MPS rates during low- and high-dose amino acid infusion following 14 d of immobilization. As the higher amino acid infusion protocol provided ample amino acids to maximize the MPS response under normal conditions, it appears that hyperaminoacidemia cannot compensate for a disuse-induced decline in muscle anabolic sensitivity. Less extreme reductions in physical activity may also reduce anabolic sensitivity in muscle. For example, a reduction in daily step count has also been shown to reduce the MPS response to feeding in older adults [72]. To date, the impact of step reduction on MPS rates in healthy younger adults has not been assessed. It could be speculated that a reduction in daily step count has less impact on anabolic sensitivity in athletes undergoing intensive exercise training, as low-load resistance-type exercise has been shown to attenuate the decline in anabolic sensitivity during step reduction in older adults [71]. However, the MPS response to feeding is likely reduced during prolonged bed rest or immobilization and may contribute to injury-related muscle atrophy in athletes [74]. An injured athlete is likely to have a lower exercised-induced energy expenditure and is likely to reduce caloric intake to avoid gaining body fat. However, a reduction in energy intake may result in a lower absolute protein intake. While the limited work available does not support the idea that a higher protein intake can compensate for a

disuse-induced reduction in MPS, habitual protein should at least be maintained [74, 75].

#### 4.2 Ageing

Ageing is accompanied by a progressive decline in muscle mass, termed sarcopenia. Little to no differences in postabsorptive MPS rates are observed between younger and older adults [76, 77]. However, the MPS response to feeding is attenuated in older compared with younger adults, a phenomenon termed anabolic resistance [77]. Interestingly, it appears the age-related anabolic resistance to protein ingestion can be at least partly compensated for by increasing the amount of ingested protein. Whereas the ingestion of 20 g of high-quality protein appears sufficient to maximize the MPS response at rest or during post-exercise recovery in younger adults [4, 5], no clear plateau in postprandial MPS rates following graded doses up to ~40 g of ingested whey, beef, or soy protein has been observed in older adults at rest or during post-exercise recovery [78]. Therefore, possibly even greater amounts of ingested protein are required to maximize the MPS response to feeding in older adults. Alternatively, it is not clear whether 40 g of dietary protein is required to maximize the anabolic response in older adults or whether a more moderate dose such as 30 g is sufficient. Some support for the latter comes from a retrospective biphasic linear regression and breakpoint analysis that observed a dose–response relation between protein ingestion and postprandial MPS rates up to ~0.4 g/kg bodyweight in older adults at rest [79]. The average weight of the older adults in this analysis was 79.3 kg, which would suggest that the average older adult of ~80 kg would require ~30 g of protein in a meal to maximize the MPS response at rest. Therefore, older athletes should aim to ingest at least 30 g of high-quality protein per meal to improve exercise recovery and adaptations.

#### 4.3 Sex

Men have more muscle mass and less body fat than age- and bodyweight-matched females [80]. However, in healthy young adults, no such sexual dimorphism is apparent in basal MPS rates [11, 81–83]. MPS response to a hyperinsulinemic–hyperaminoacidemic clamp [11, 83], MPS response to resistance-type exercise [82], or postprandial MPS rates following resistance-type exercise [81]. A possible explanation for this apparent discrepancy is that the greater amount of muscle mass in males than in females primarily originates from an augmented growth spurt in males during puberty, which seems attributable to the surge in testosterone secretion [13, 84]. After puberty, muscle mass remains largely constant up to middle-age adulthood in both healthy males and healthy females, which is consistent with similar muscle

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9. Exercise increases the peak of muscle protein synthesis (MPS), but also the duration of MPS.

Inactivity from an active state leads to reduced muscle protein synthesis rates which cannot be compensated for by consuming more amino acids/protein. Still, the loss of muscle may be worsened by not consuming enough protein, so one should at least maintain high protein consumption to blunt the reduced MPS effect, even if it doesn't rescue it.

10. Aging leads a reduction in muscle mass, but not from a reduction in protein consumed induced muscle protein synthesis, which stays the same across age. However, the initiation of muscle protein synthesis takes more protein than in younger individuals - this is known as “anabolic resistance”. It may be necessary to consume 1.5 times to double, if not more, of protein if you are older (say, 60 years or older). So, a younger person (20s, 30s) would consume 20 grams of protein to achieve maximal MPS, and an older individual might need 30 or 40 grams of the same protein.

11. Men have more muscle, but that is not due to higher muscle protein synthesis rates at rest, post-protein consumption, or post-exercise. It is believed to be due to a spike in testosterone in puberty that allows more muscle mass (not sure I believe this, considering muscle cells are determined in-utero and testosterone is consistently much higher in men, so why would this one spike lead to more muscle mass? I think there may be some developmental differences.

Women and men maintain their muscle mass for decades (into middle age), and with age can begin experiencing loss, with women having a lower muscle protein synthetic ability from protein consumption which might be attenuated by simply consuming a bit more protein.

protein turnover rates between the sexes. Therefore, nutritional strategies to maximize the muscle anabolic response do not differ between young male and female adults per se.

In contrast to younger adults, a sexual dimorphism in MPS rates may exist in adults at a more advanced age. Older women have higher postabsorptive MPS rates but a blunted MPS response to feeding compared with older men at rest [13, 83, 85]. As greater amounts of protein in a meal can at least partly compensate for age-related anabolic resistance [78], it is tempting to speculate that older women require the ingestion of higher amounts of protein to maximize the anabolic response to feeding when compared with older men. In addition, whether age-related sexual dimorphism in the MPS response to protein feeding is present in active older adults engaging in regular exercise training remains to be determined.

#### 4.4 Body Size

It seems intuitive that individuals with a greater lean body mass require larger amounts of protein to be consumed than do individuals with less lean body mass. Consistent with this line of thinking, protein intake requirements are often expressed relative to body size, and most typically to body weight, as this is more practical to assess in individuals. However, only one study has directly examined the impact of body size on the MPS response to feeding. Macnaughton et al. [17] observed no difference in the post-exercise MPS response to protein ingestion between subjects with a relatively small or large amount of fat free mass (~59 vs. 77 kg, respectively). These data suggest that lean body mass is not a strong modulator of protein requirements in the initial several hours of post-exercise recovery. A possible explanation is that only a relatively small amount of essential amino acids is required as precursors for MPS, even in larger individuals. A factor that is more likely to limit the MPS response to feeding is the postprandial rise in plasma leucine concentration [68]. However, Macnaughton et al. [17] observed only a trivial difference in peak plasma leucine levels following protein ingestion between smaller and larger individuals. Therefore, the amount of ingested protein required to maximize the muscle anabolic response following resistance-type exercise may be less affected by body size than has been assumed.

It is not uncommon for athletes in various sports such as rugby or bodybuilding to intentionally overfeed and gain considerable amounts of body fat in an attempt to optimize lean body mass gains ("bulking up"). However, several studies have reported attenuated postabsorptive and/or postprandial MPS rates in overweight or obese subjects at rest [12, 86–88], but these observations have not been consistent [12, 86–89]. What may cause a reduced MPS response to feeding in subjects with excess body fat is unclear, but it

seems unlikely that the amount of body fat an individual possesses has a substantial impact on the amount of precursors required for MPS or the size of the plasma amino acid pool and consequently the peak plasma leucine concentrations following feeding. Therefore, it is more likely that excess body fat directly reduces anabolic sensitivity to protein ingestion in muscle. In support, lipid infusion has been shown to reduce postprandial MPS rates in healthy young adults at rest [41]. These data suggest that excess lipid availability per se reduces anabolic sensitivity within skeletal muscle, independent of body composition [41]. Therefore, athletes who are intentionally overfeeding to gain muscle mass may consider limiting excessive dietary fat intake and body fat accumulation. However, the proposed impact of excessive body fat mass on MPS rates is likely confounded by habitual physical activity levels and is perhaps less of a concern for athletes engaged in regular intense exercise training.

### 5 Is the Anabolic Response to Feeding Limited to Muscle Protein Synthesis?

#### 5.1 Muscle Protein Breakdown

It could be argued that the anabolic response to feeding is not limited to the MPS response. Muscle protein net balance is determined by the difference between MPS and muscle protein breakdown rates. However, changes in MPS rates in response to exercise and nutrition seem to be much greater than changes observed in muscle protein breakdown rates [2, 90]. Therefore, changes in net muscle protein balance appear to be largely determined by changes in MPS rates. While feeding reduces muscle protein breakdown rate via an increase in circulating plasma insulin concentrations, only a moderate rise in insulin concentration is required for maximal inhibition of muscle breakdown rates. Greenhaff et al. [35] assessed the impact of a constant amino acid infusion together with progressive increments in insulin on muscle protein breakdown rates and found that increasing insulin concentrations to 30 mU/L lowered muscle protein breakdown rates by ~50% compared with basal levels, with no further suppression at higher insulin concentrations. However, it appears even lower insulin concentrations may be sufficient to suppress post-exercise muscle protein breakdown rates. The ingestion of 20–25 g of protein increases insulin concentrations to ~15–20 mU/L, with no further suppression in post-exercise muscle protein breakdown rates when insulin levels are increased to ~65 mU/L via carbohydrate co-ingestion [32]. Therefore, food intake will substantially reduce muscle protein breakdown rates, with the macronutrient content of the food being of little impact.

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12. Lipid/fat nutrient/molecule given intravenously reduces the muscle protein synthesis capabilities. So, possibly, consuming high dietary fat may blunt muscle protein synthesis, but this has not been evaluated in exercising individuals.

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13. Muscle protein breakdown does not change as readily as muscle protein synthesis. Food consumption reduces muscle protein breakdown through its stimulation of insulin - however, on a small rise in insulin is necessary to get the maximal inhibition on muscle protein breakdown. Maximal protein breakdown is around 50% reduced from basal levels. Protein alone stimulates enough insulin release to reduce muscle protein breakdown maximally, with no added benefit of co-ingestion of carbohydrates.

Muscle protein breakdown has an important role in muscle remodeling and removal of non-functional proteins within the musculature. Knocking out (eliminating via genetic engineering) protein breakdown molecules leads to lowered muscle quality.



Although under normal, healthy conditions, protein breakdown does not seem to play an important quantitative role in net muscle accretion, it does have an important function in muscle tissue reconditioning. Proteolysis is required for the clearance of damaged and/or aberrant proteins, thereby allowing optimal tissue function and remodeling. In support, knocking out critical genes in the protein breakdown proteasome and autophagy pathways reduces muscle quality, muscle function, and muscle mass in animal models [91–94]. These data suggest that at least some amount of muscle protein breakdown is required for proper muscle conditioning. Therefore, inhibition of muscle protein breakdown beyond the normal postprandial reduction may not represent a desirable target in healthy populations.

## 5.2 Whole-Body Protein Balance

Assessment of MPS and/or breakdown rates requires sampling of skeletal muscle tissue. To avoid skeletal muscle tissue sampling, protein synthesis and protein breakdown rates are often assessed on a whole-body level, which only requires stable isotope tracer infusion and arterial or arterialized blood sampling [95]. As muscle tissue represents a large proportion (~40%) of total body protein content, it is often assumed that whole-body protein metabolism is a good proxy for muscle tissue protein turnover. However, muscle tissue has a relatively slow turnover when compared with other tissues, such as liver, kidney, lung, intestine [96], and even brain [97]. Therefore, muscle mass is estimated to contribute only ~25–30% to whole-body protein turnover [98]. As a consequence, whole-body and MPS rates in response to nutrition [27, 34, 40], exercise [65, 99], and disease [100, 101] do not necessarily align. Although ample data are available on the impact of various stimuli on muscle tissue protein synthesis and/or breakdown rates, the responsiveness of other tissues to similar factors is less well studied. Consequently, we should be cautious when applying whole-body amino acid kinetics to gain insight into protein metabolism at a tissue-specific level.

Practical inferences based on the assessment of whole-body protein metabolism are further complicated by methodological issues. A common method to assess whole-body protein metabolism is based on the amino acid flux in and out of the circulation. This method assumes that the rate at which amino acids are disappearing from the circulation (i.e., tissue uptake) minus amino acid oxidation rates reflects whole-body protein synthesis. This would require the tissue free amino acid pools to remain constant. However, tissue free amino acid pools are likely to change considerably in response to conditions such as feeding, invalidating whole-body protein synthesis rate calculations. Even more complex is the determination of whole-body protein breakdown rates in a postprandial setting. Whole-body protein breakdown

rates are determined by the rate at which amino acids are appearing in the circulation from tissues minus the rate at which dietary protein-derived amino acids appear in the circulation. Therefore, calculation of whole-body protein breakdown rates depends on accurate assessment of the amount of exogenous protein appearing in the circulation. Some researchers estimate the latter based on previously published values [34, 102, 103]. However, such values are specific to the experimental conditions under which they are obtained (i.e., the amount and type of protein, the pattern of protein ingestion, and the duration of the postprandial period assessed), and may not be applicable to other conditions. Therefore, assessment of postprandial whole-body protein breakdown rates is only reliable when the amount of exogenous protein appearing in the circulation is assessed appropriately [104].

The indicator amino acid technique is a noninvasive method to assess whole-body protein balance [105]. The ingestion of ~50 g of protein maximizes whole-body protein balance in healthy adult males following intermittent-type exercise as assessed by the indicator amino acid technique [106]. In contrast, the ingestion of 20–40 g of high-quality protein is sufficient to maximize the postprandial MPS response in healthy young adults at rest or following resistance-type exercise [4, 5, 17]. Therefore, athletes determined to maximize anabolism in both muscle and non-muscle tissues may consider the ingestion of ~50 g of protein per meal. However, clearly more research is warranted to determine which non-muscle tissues are responsive to protein feeding and whether this has any functional relevance for athletes.

## 5.3 Do Myofibrillar Protein Synthesis Rates Reflect Changes in Muscle Mass?

Whether changes in MPS rates following certain stimuli correlate with subsequent changes in muscle mass during more prolonged exposure to such stimuli has been questioned. Mitchell et al. [107] observed no correlations between acute post-exercise myofibrillar protein synthesis rates and muscle hypertrophy observed following more prolonged resistance-type exercise training. Myofibrillar protein synthesis rates were assessed in the first 6 h following the first exercise bout performed as part of the prolonged exercise training program. The lack of significant (positive) correlations is not surprising as basal and postprandial myofibrillar protein synthesis rates have been shown to be elevated for up to 72 h after a single bout of exercise [63]. A follow-up study assessed myofibrillar protein synthesis rates during a more prolonged 48-h post-exercise period after the initial exercise bout, at 3 weeks, and at 10 weeks of a prolonged resistance-type exercise training program [108]. While no correlations were observed between post-exercise myofibrillar protein

synthesis rates following the initial exercise bout and muscle hypertrophy following prolonged exercise training, strong positive correlations were observed between myofibrillar protein synthesis rates assessed over a 48-h period at 3 weeks and at 10 weeks of training and the increase in muscle mass. The initial exercise bout resulted in considerable muscle damage, but exercise-induced muscle damage was attenuated at 3 weeks of training and almost completely absent at 10 weeks. Therefore, it appears that the myofibrillar protein synthetic response to a single bout of unaccustomed exercise may be at least partly a response to muscle damage and directed at tissue repair rather than muscle hypertrophy. After the first couple of days or weeks of training, post-exercise myofibrillar protein synthesis rates may be more reflective of the net changes in muscle mass, i.e., muscle hypertrophy. Consistent with this notion, myofibrillar protein synthesis rates assessed during several weeks of a prolonged resistance-type training program have been shown to correlate with muscle hypertrophy [109]. Given these findings, it seems evident that changes in myofibrillar protein synthesis rates during recovery from successive exercise sessions can be predictive of net increase in muscle mass.

## 6 Conclusions

The ingestion of 20 g of high-quality, rapidly digestible protein results in a near-maximal stimulation of MPS rates at rest and during the initial several hours of recovery following lower-body resistance-type exercise. Ingestion of animal-derived proteins tends to result in a greater increase in MPS rates than ingestion of plant-derived proteins. However, ingestion of larger amounts and/or mixing of different plant-derived proteins may possibly compensate for the lower anabolic properties. The ingestion of relatively large amounts ( $\geq 40$  g) of slowly digestible protein may result in a prolonged MPS response and may be recommendable when there is a prolonged period until the next feeding opportunity ( $\geq 6$  h, e.g., overnight sleep). Recent evidence suggests that whole-food protein sources may contain micronutrients that can further augment the MPS response. The anabolic response to protein ingestion is attenuated during prolonged energy intake restriction, during muscle disuse, and in older adults (especially older females). The ingestion of greater amounts of protein can at least partly rescue the blunted MPS response during prolonged energy restriction and ageing but not during muscle disuse. In conclusion, nutritional recommendations to maximize the MPS response to feeding depend on both the type of meal and time until the next feeding opportunity and should be personalized to the individual athlete.

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## Compliance with Ethical Standards

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