Abstract

Skeletal muscle hypertrophy is a beneficial adaptation for many individuals. The metabolic basis for muscle hypertrophy is the balance between the rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), i.e. net muscle protein balance (NMPB = MPS – MPB). Resistance exercise potentiates the response of muscle to protein ingestion for up to 24 h following the exercise bout. Ingestion of many protein sources in temporal proximity (immediately before and at least within 24 h after) to resistance exercise increases MPS resulting in positive NMPB. Moreover, it seems that not all protein sources are equal in their capacity to stimulate MPS. Studies suggest that \( \sim 20\text{–}25\) g of a high-quality protein maximizes the response of MPS following resistance exercise, at least in young, resistance-trained males. However, more protein may be required to maximize the response of MPS with less than optimal protein sources and/or with older individuals. Ingestion of carbohydrate with protein does not seem to increase the response of MPS following exercise. The response of inactive muscle to protein ingestion is impaired. Ingestion of a high-quality protein within close temporal proximity of exercise is recommended to maximize the potential for muscle growth.

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Introduction

Skeletal muscle hypertrophy is a beneficial adaptation for many athletes, as well as other populations, including older adults, insulin resistant/diabetic individuals and others. Probably the most effective stimulus for muscle hypertrophy is resistance exercise. The hypertrophic response to resistance exercise is enhanced by nutrition, in particular provision of protein. Thus, study of the interaction of nutrition and exercise offers valuable information that may be used to enhance muscle hypertrophy and alter body composition during training.
Metabolic Basis for Muscle Hypertrophy

The metabolic basis for muscle hypertrophy is the balance between the rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), i.e., net muscle protein balance (NMPB = MPS – MPB). The processes of MPS and MPB occur concurrently and either or both may change in response to the nutrition and exercise circumstances at a given time. NMPB alternates between positive and negative periods throughout the day depending on proximity to a protein-containing meal. Periods of positive NMPB must be of larger duration and magnitude than negative periods over any given time for muscle growth to occur. In particular, changes in the balance of the muscle myofibrillar proteins are responsible for changes in muscle mass since they comprise the majority of muscle proteins. Studies over the past 15 years or so indicate that it is predominantly changes in the rate of MPS in response to exercise and nutritional perturbations that have a much greater impact on changes in NMPB than changes in MPB [1–3].

Methodological Considerations

The importance of protein consumption for muscle hypertrophy may be investigated by both chronic longitudinal resistance training studies and acute metabolic studies examining MPS and in some cases MPB. Chronic studies assess changes in muscle mass and strength over a given time period, often from 6 up to 16 weeks, but rarely for longer periods. The acute metabolic studies assess the response of muscle anabolism, often in the form of MPS, to exercise plus some sort of nutrient ingestion. For the acute studies to be meaningful, an assumption is that the metabolic response to exercise and nutrition over a period of only a few hours represents the potential for an intervention to influence muscle growth over the longer term.

Intuitively, it is easier to accept that long-term training studies measuring changes in muscle mass are better to discern the influence of diet or dietary components on muscle hypertrophy. However, the control, expense and difficulty of performing these studies make them less than ideal. Longitudinal studies are often small, and physiologically important changes may not be detected due to limitations in measurement methods and/or variation in the response of individual participants. These difficulties may easily obscure the interpretation of results from these studies. Nevertheless, ultimately longitudinal end point studies can make important contributions to the body of knowledge concerning protein and muscle growth.
An alternative method to longitudinal studies for assessment of the efficacy of nutritional interventions to stimulate muscle hypertrophy is to perform acute metabolic studies. Over the past 15–20 years, stable isotopic tracer methodology has been used to investigate the response of MPS, MPB and NMPB to various nutrition and exercise combinations. The assumption is that the acute, i.e. over a few hours around the exercise, response to an intervention is representative of the muscle hypertrophy that would occur over a longer period of time. There is now evidence that this assumption is valid. Recently, several studies have demonstrated that the muscle hypertrophy over several weeks of resistance exercise training mirrors the acute response of MPS and NMPB [4, 5]. Thus, the acute metabolic response may be considered predictive of the potential for muscle hypertrophy. It should be noted that the predictive capacity of the acute studies is qualitative, rather than quantitative. Nevertheless, we propose that acute studies measuring MPS in response to resistance exercise and nutrition provide important information that may be used to design programs that will lead to muscle growth in various populations. Accordingly, this review will focus primarily on results from these acute metabolic studies.

**Importance of Protein**

Protein intake in conjunction with resistance exercise enhances the anabolic response of muscle. Elevated blood amino acid levels from infusion of amino acids or ingestion of a source of amino acids stimulates MPS resulting in positive NMPB [2, 6–8]. Moreover, it seems clear that it is the essential amino acids (EAAs) in the protein that are the key to muscle anabolism, i.e. provision of non-essential amino acids are unnecessary for stimulation of MPS [9]. However, it is unknown at this time whether someone could achieve an optimal MPS response and net balance consuming only EAAs. What we do know is that the response of muscle anabolism to exercise plus amino acids is greater than either alone [2]. Exercise potentiates the protein synthetic response in muscle allowing it to respond to provision of amino acids. Thus, the response of MPS, and specifically of the myofibrillar protein fraction, to protein ingestion is superior following exercise versus that seen at rest [10]. This response leads to muscle hypertrophy with repeated bouts of resistance exercise.

Recent work illustrates the importance of resistance exercise to potentiate the anabolic response of muscle to dietary protein. Witard et al. [11] demonstrated that MPS is elevated following exercise subsequent to a high protein meal consumed 3 h prior to the exercise. It is now clear that the amino acids from exogenous protein are being incorporated into the muscle protein following exercise.
Work from the laboratory of Prof. Luc van Loon using intrinsically labeled protein clearly demonstrates that incorporation of ingested amino acids, as well as endogenous amino acids, is increased by prior exercise [12]. Thus, the synergy of resistance exercise and ingested protein provide the optimal anabolic response of muscle that presumably leads to muscle growth.

**Type of Protein**

It is clear that ingestion of many protein sources in temporal proximity (immediately prior and at least within 24 h after) to resistance exercise increases MPS resulting in positive NMPB. Moreover, it seems that not all protein sources are equal in their capacity to stimulate MPS. Whereas studies have begun to elucidate the differences in the response of MPS during post-exercise recovery to various sources of protein, it is still difficult to unequivocally state that one source is ideal. Dairy proteins seem to offer some advantage for muscle anabolism over other protein sources. Wilkinson et al. [5] demonstrated that MPS was greater, resulting in greater positive NMPB with ingestion of fluid low fat milk compared to an iso-nitrogenous soy protein drink following resistance exercise. Moreover, when milk was ingested following each exercise bout during 12 weeks of resistance training, gains of muscle mass and strength were greater than when soy protein was ingested [4]. Subsequently, Tang et al. [7] demonstrated that the response of MPS to whey protein ingestion following resistance exercise was superior to that of either casein or soy protein. Similarly, myofibrillar MPS was greater with ingestion of whey protein isolate than micellar casein in older men following exercise [13] and at rest [13, 14]. These results suggest that dairy proteins – in particular whey protein – provide a superior anabolic response compared to other proteins.

The differences in the anabolic response to whey protein ingestion and the ingestion of other proteins likely is due to a combination of its high leucine and EAA content and the rapidity of digestion of the protein resulting in rapid hyperaminoacidemia. Data suggest that EAAs are the key to increasing MPS and NMPB with protein ingestion following resistance exercise [9]. In particular, the branched-chain amino acid leucine seems to be unique among the EAAs as a key regulator of translation initiation of MPS [3, 15]. Whey protein provides all of the EAAs, including leucine, in greater amounts than is present in human muscle protein, but soy and casein do not. In fact, recently, a ‘leucine trigger’ has been suggested to be a key factor for muscle anabolism. This thesis suggests that a threshold of leucine must be reached in the intramuscular pool before the maximal rate of MPS is stimulated. However, elevated leucine alone is insufficient to fully stimulate MPS [16]. Despite stimulation of translation initiation by
leucine, provision of the other amino acids, e.g., from an intact protein source, is necessary to supply the substrate for MPS. Thus, the amino acid composition of whey protein seems to be an important component leading to superior responses of MPS following exercise.

Digestive properties of the various proteins likely contribute to differences in the response of MPS following exercise. Rapid appearance of amino acids into the blood seems to be important for an optimal response of MPS [17]. Since whey protein is digested more quickly than micellar casein, hyperaminoacidemia develops more rapidly. Greater and more rapid aminoacidemia of EAAs likely contributes to the superior anabolic response noted for whey protein over casein. Since micellar casein coagulates and precipitates when it is exposed to acid, the curd (a complex of fat, if present in the milk, and protein) that is formed is digested more slowly. Thus, a more moderate but prolonged hyperaminoacidemia results from casein ingestion [13]. Taken together, the data suggest that the superiority of whey protein for stimulation of MPS following exercise results from a rapid increase in amino acid, in particular leucine, availability. This sentiment would also be true about meals that, due to their mixed macronutrient composition, will have slower digestion kinetics and thus will lead to a slower and protracted aminoacidemia. So the digestive kinetics resemble those of casein more than they do whey. As such, at this point, all we can say is that intact isolated proteins, if they are digested rapidly and have a high leucine content, work well. However, we know nothing about mixed meal consumption and the effect of protein in a matrix of fat and carbohydrate.

The superiority of the anabolic response to whey protein is not a universal finding. Previously, similar NMPB was observed with ingestion of whey protein and casein following resistance exercise [18]. These results are seemingly at odds with other data suggesting that MPS was greater with greater whey protein ingestion [7, 13]. Whereas there was no difference in NMPB between whey and casein protein, a direct measurement of MPS was not made [18]. Thus, it is possible that MPS was greater with the whey protein, and MPB was less with casein ingestion resulting in similar NMPB [18]. On the other hand, the form of the ingested casein may be the key. Micellar casein is digested more slowly than whey protein resulting in a less rapid increase in aminoacidemia. Caseinate was used to stimulate muscle anabolism in the previous study [18]. This notion is supported by recent studies from Copenhagen. These studies reported no differences in the response of MPS to ingestion of whey protein and caseinate [19]. Thus, the form of protein may impact the pattern of aminoacidemia leading to differences in the anabolic response. Pennings et al. [14] also showed recently that hydrolysis of casein prior to ingestion resulted in a more rapid aminoacidemia and greater MPS response than micellar casein, but that whey was superior to both micellar and hydrolyzed casein.
Muscle Protein Breakdown

Most studies measure only MPS in response to protein ingestion and exercise or their combination. We fully acknowledge that MPB also is stimulated with resistance exercise in the fasted state [1, 20]. However, when protein is provided (infusion of amino acids or ingestion), the rise in post-exercise MPB is ablated. It has been argued that this amino acid-induced 'shutting off' of MPB is due to the increased intracellular amino acid availability preventing an increase in MPB that was needed to ‘fuel’ the increase in MPS [1]. It appears, however, that this idea may not be correct and instead that the rise in insulin that often accompanies hyperaminoacidemia may be responsible for reducing MPB [21]. This argument is not to dismiss the importance of measuring MPB, which is regulated by at least four different systems, but it is known that the feeding and exercise-induced fluctuation in MPS is 3–4 times that of MPB, so in healthy persons the role of MPB in determining muscle protein balance is much less relevant versus MPS.

Amount of Protein

It is well established that protein ingestion following exercise stimulates MPS. However, the optimal amount of protein has yet to be firmly established. Moore et al. [22] examined the response of mixed MPS to ingestion of varying amounts of egg protein following resistance exercise. MPS increased stepwise in response to 0, 5, 10 and 20 g of protein. However, the response to 40 g was not significantly greater than that of 20 g. Moreover, leucine oxidation increased dramatically with ingestion of 40 g of protein. Thus, it was determined that ∼20 g of protein is sufficient to optimally stimulate MPS following exercise. Ingestion of more than 20 g simply results in oxidation of the excess amino acids [22]. Recently, we found that the response of myofibrillar MPS to whey protein was similar, i.e. the response to 40 g was not significantly greater than to 20 g [Witard et al., in prep.]. Moreover, the exercise was performed ∼3 h following a meal, rather than in the fasted state. This amount (20 g) also maximized MPS in non-exercised muscle. As with the earlier results [22], amino acid oxidation and urea production were dramatically increased when 40 g of whey protein were ingested [Witard et al., in prep.]. Thus, it seems that ∼20 g of a high-quality protein maximizes the response of MPS following resistance exercise, at least in young, resistance-trained males.

The response to varying doses of protein is influenced by factors other than simply the amount of protein. Similar to the results from young males [Witard
et al., in prep.], Yang et al. [23] recently reported that 20 g of whey protein was sufficient to maximally stimulate myofibrillar MPS in non-exercised muscle. On the other hand, 40 g of whey protein was necessary to maximally stimulate myofibrillar MPS following exercise in older males [23]. Moreover, the type of protein impacts the dose response in older men. Soy protein ingestion (20 and 40 g) did not result in increased MPS in non-exercised muscle of older males [24]. However, with the anabolic potentiation of resistance exercise, myofibrillar MPS was increased by ingestion of 40 g, but not 20 g, of soy protein. When compared to whey protein, the response of MPS to soy protein was inferior. Thus, it seems that 40 g of protein is necessary for the optimal stimulation of MPS in older adults – a protein dose twice as great as that in young persons.

**Timing of Protein Ingestion**

It is clear that ingestion of protein in association with resistance exercise results in stimulation of MPS leading to positive NMPB and muscle growth; however, the precise timing of the ingestion in relation to the exercise may impact the response. Many feel that immediate (within as little as 45 min) post-exercise protein ingestion is crucial for the optimal response of muscle anabolism. This post-exercise period has been dubbed the ‘window of anabolic potential’, and whole books have been written supporting its importance. Clearly, early post-exercise ingestion of protein [7, 13, 17] or free amino acids [9, 25] results in increased MPS. Thus, immediate post-exercise ingestion of an amino acid source obviously is a sound method of enhancing muscle anabolism.

A training study from a Copenhagen laboratory may be the basis for the limited anabolic window purported by other authors. In that study, older men performed resistance exercise training for 12 weeks [26]. One group of volunteers consumed a protein-containing supplement immediately after exercise, whereas a second group waited 2 h to consume the supplement. Muscle mass in the group that waited 2 h to consume the protein did not increase, and the strength increase was much less than in the group that consumed protein immediately following exercise. Thus, it was concluded that waiting to consume protein not only inhibited, but also completely prevented, the anabolic response [26].

The notion that protein must be consumed immediately after exercise to have an anabolic impact is countered by data from studies on the acute anabolic response of muscle to feeding. MPS and NMPB were similar when EAA was ingested at 1 and 3 h following resistance exercise [27]. Moreover, the re-
response was actually greater when those nutrients were ingested prior to exercise compared to immediately after exercise [25]. At the very least, these data suggest that the ‘anabolic window’ is slightly broader than the first hour or two after exercise. Nevertheless, these time points may still be considered within close proximity of exercise. Moreover, the form in which an amino acid source is ingested seems to influence the anabolic response to the timing of ingestion. Whereas EAA ingestion prior to exercise results in a superior anabolic response to ingestion following exercise [25], the response to ingestion of intact whey protein prior to and following exercise was shown to be similar [8]. As with other factors influencing the anabolic response, these differences likely are due to the digestive properties of the source of amino acids.

More recent work suggests that this ‘window of opportunity’ may be even more extensive than just a few hours around the exercise bout. The response of MPS to resistance exercise is greatest within the first few hours following the exercise bout, but the response lasts for up to 48 h [1]. Thus, it is logical to suggest that the influence of the exercise on the ability of the muscle to respond to hyperaminoacidemia would still be enhanced. In fact, Burd et al. [28] recently demonstrated that the synergistic response of muscle to exercise and nutrition lasts for at least 24 h. Whey protein ingestion 24 h following exercise resulted in superior rates of MPS compared to ingestion at rest [28]. These results suggest that the ‘window of anabolic potential’ lasts for at least 24 h, and possibly as long as 48 h, following exercise (fig. 1). Thus, whereas the optimal response may occur when protein is ingested soon after exercise, a normal post-exercise feeding pattern will, in fact, support muscle anabolism.
Coingestion of Other Nutrients with Protein

The bulk of research regarding the nutritional influences on muscle anabolism has focused on protein. However, both carbohydrates and fats are typically consumed with protein in a mixed meal. Thus, investigation of the impact of the other macronutrients may give some insight into optimization of muscle anabolism. There is very little information on the role of fat ingestion in the anabolic response of muscle following exercise. One study suggests that the fat content of milk may play a role in this response. The increase in NMPB following ingestion of whole milk was greater compared to that to ingestion of an isonitrogenous amount of fat-free milk [6]. The mechanism for this response is unknown, but blood flow may play a role. Thus, it seems that coingestion of fat with protein following exercise may warrant further investigation.

Whereas ingestion of fat with protein has not received much attention, ingestion of carbohydrate alone and with protein has been studied. The response of muscle protein metabolism to carbohydrate is thought to be due to the hyperinsulinemia. Earlier work demonstrated that hyperinsulinemia following resistance exercise contributed little to the response of MPS, but did impact NMPB due to a reduction in protein breakdown [29]. Subsequent work has confirmed that ingestion of carbohydrate with sufficient amounts of protein does not further increase MPS following exercise [30, 31]. Thus, both fat and carbohydrate may play a role in muscle anabolism following exercise.

Inactivity

It has been known for some time that inactivity reduces MPS [32] and that even a mild contractile stimulus like electrical stimulation can ablate this decline [33]. What we do know is that the reduction in fasted-state MPS also extends to the fed state at both low and high doses of amino acids [34]. Thus, inactivity and unloading induce a state of ‘anabolic resistance’ of the muscle to amino acids similar to that seen in the elderly [35]. It is unknown as to the cause of the inactivity-induced anabolic resistance, but it could be due to one or more of the following: reduced amino acid delivery as a result of an impaired insulin-mediated vasodilation [36], signaling defects inherent to the muscle itself, and/or another inactivity-related unknown mechanism. Whatever the mechanism, the fact remains that in a state of inactivity humans lose their ability to build protein. Interventions to prevent this decline from a nutrient standpoint have met with mixed success. Paddon-Jones [37] reported that consumption of a mixture of 16.5 g of EAAAs (~35 g of high-quality protein) and 30 g of carbohydrate thrice
daily preserved MPS, muscle strength, and skeletal muscle mass during 28 days of bed rest. The data from Paddon-Jones contrast somewhat with the findings of Trappe et al. [38] who reported no benefit of daily supplementation with a leucine-enriched whey protein supplement in women on 60 days of bed rest. In fact, the whey-supplemented group lost more leg muscle mass than the control (i.e. non-supplemented) group. Thus, it may be that in the short term (i.e. 4 weeks or less) amino acid supplementation is effective in attenuating muscle mass loss due to inactivity, but in the long term supplements are ineffective.

Conclusions and Recommendations

It is clear that the training impulse is the most important aspect of muscle hypertrophy. Nevertheless, it is equally certain that nutrition, particularly protein nutrition, may have an important influence. Current knowledge allows us to make a few recommendations to optimize the anabolic response to protein ingestion. First, it is clear that the total amount of protein consumed is not the most important factor to consider. Many other aspects of protein feeding play a role, including the amount, timing and source of protein, as well as coingestion of other nutrients with the protein. Thus, even consuming the same total amount of protein, utilization of these other factors could change the anabolic response. Consumption of ~20–25 g of high-quality, i.e. with ample leucine resulting in rapid hyperaminoacidaemia, protein, e.g. whey protein, is sufficient to optimize the response, at least in healthy young males. Older individuals may need more protein for optimal muscle anabolism. Moreover, it is unclear if the total mass of muscle or the mass that is exercised will influence the response. Thus, a 50-kg gymnast may need less protein. The recommendation could perhaps be tailored to body mass. So, based on the body mass from earlier studies [22; Witard et al., in prep.], ~0.25–0.3 g protein/kg body mass could be recommended. Whereas, it is likely best to ingest protein soon after exercise, it seems clear that muscle does respond to protein ingestion for at least 24 h following exercise. Thus, all meals within that time will contribute to muscle hypertrophy. Ingestion of carbohydrate and perhaps even fat along with protein may contribute to muscle growth. Given the benefit of replenishing glycogen with carbohydrate ingestion, adding carbohydrate to protein is likely also prudent.

Disclosure Statement

The authors of this chapter have no financial arrangements to disclose.
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