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FACTORS THAT INFLUENCE THE AMOUNT OF PROTEIN NECESSARY TO MAXIMIZE THE ANABOLIC RESPONSE OF MUSCLE FOLLOWING RESISTANCE EXERCISE

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- The metabolic basis for changes in muscle mass is net muscle protein balance, i.e., the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). Changes in MPS are responsible for a much greater proportion of the change in net muscle protein balance than are changes in MPB.
- Many factors influence the response of MPS to protein ingestion following resistance exercise. However, the amount of protein consumed in a single serving following exercise is the most important factor that determines the magnitude of the MPS response.
- The increase in MPS with increasing amounts of ingested protein is not infinite and plateaus at some optimal amount of ingested protein.
- The optimal amount of protein to consume following exercise varies depending on a number of factors, including the characteristics of the exercise bout (i.e., leg-only vs. whole-body), the age of the individual, type of protein ingested and possibly the amount of muscle mass an individual possesses.
- The ingestion of 20-25 g of high-quality protein seems to be sufficient to maximally stimulate MPS in healthy young males following leg-only resistance exercise. However, it is clear that ingesting 20 g of protein does not maximally stimulate MPS under all circumstances.
- The amount of protein necessary to maximally stimulate MPS increases when exercising with greater amounts of muscle, increasing age and ingestion of proteins with inferior amino acid compositions.

INTRODUCTION

Muscle is an important tissue for those who participate daily in physical activity and exercise for health, enjoyment or training for competition – from elite athletes to recreational exercisers, older adults and hospital patients. The importance of muscle tissue most often is associated with locomotion and strength. However, muscle may also be the most crucial tissue for metabolic health (Wolfe, 2006). Thus, factors that enhance the maintenance and/or growth of muscle mass make important contributions to overall health. Two lifestyle factors with the most influence over muscle mass are nutrition and exercise/physical activity.

The metabolic regulation of muscle protein synthesis (MPS) and breakdown (MPB), i.e., net muscle protein balance (NBAL), determines changes in muscle mass. Muscle mass is gained over any given period of time when MPS exceeds MPB. NBAL naturally fluctuates between periods of positive NBAL (muscle gain) and negative NBAL (muscle loss) throughout the day in response to habitual diet and physical activity. Nutrient intake (primarily protein) and exercise regulate MPS, MPB and, therefore, NBAL. Specifically: resistance exercise increases fasting MPS, but NBAL remains negative in the absence of additional amino acids from a dietary protein source (Biolo et al., 1995); dietary protein stimulates MPS resulting in a transitory period of positive NBAL. The contribution of MPS to the overall response of NBAL to exercise and nutrition is far greater than the contribution of MPB (Biolo et al., 1995, 1997; Phillips et al., 1997). Thus, changes in MPS account for the bulk of the changes in muscle mass with training and nutritional support. Moreover, changes in MPS in response to exercise and nutrition play a wider role in the adaptive response to exercise via remodeling of muscle proteins. Exercise, and particularly resistance exercise, enhances the response of MPS to amino acids derived from dietary protein (Biolo et al., 1997; Pennings et al., 2011; Witard et al., 2014). Thus, protein-containing meals consumed within at least 24 h of an exercise bout result in protein deposition and ultimately phenotypic changes in muscle mass (Burd et al., 2011).

There are many factors that influence the response of MPS to protein ingestion. Timing of protein intake in relation to the exercise bout, other nutrients co-ingested with protein, characteristics of the protein (i.e., amino acid composition and digestive properties) and the amount of protein ingested all impact the response of MPS (Witard et al., 2016b). The pattern or distribution of the protein over a period of time also seems to influence the metabolic response (Areta et al., 2013). Of these factors, the amount of protein ingested at a single time point appears to have the largest influence on the MPS response (Schoenfeld et al., 2013). Thus, this Sports Science Exchange article will focus on the response of MPS to the amount of protein ingested in a single serving. The evidence regarding the amount of protein necessary to optimize the response of MPS in a single serving following exercise in various circumstances and human populations will be examined. The discussion will focus on the response of MPS following resistance exercise, since the bulk of the available information is limited to this type of exercise.

DOSE RESPONSE OF MUSCLE PROTEIN SYNTHESIS TO POST–EXERCISE PROTEIN INGESTION IN YOUNG TRAINED INDIVIDUALS

The acute response of MPS to increasing doses of protein following resistance exercise does not appear to be finite in healthy, trained males. Moore and colleagues (2009a) were the first to show a step-wise increase in MPS when resistance-trained males consumed increasing amounts of egg protein following leg-only resistance exercise. MPS increased progressively with increasing doses up to 20 g, but there was no further increase when 40 g were consumed. Moreover, amino acid oxidation increased after consuming the 40 g protein dose. Thus, the maximum anabolic response following exercise was deemed to result from ingestion of 20 g of high-quality protein and more than 20 g was unnecessary (Witard et al., 2016b). Ingestion of protein in amounts greater than 20 g simply resulted in increased non-anabolic fates of the ingested amino acids with no further increase in MPS.

The Moore et al. (2009a) study was the first to examine the dose response of MPS to ingested protein, but as with every study, a number of unanswered questions remained and our laboratory set out to address some of these. In the Moore et al. (2009a) study, the response of mixed MPS to protein ingested following exercise was measured after an overnight fast. It was possible that the response may be blunted after a prior meal ("muscle full effect"), thereby changing the dose-response dynamics (Atherton et al., 2010). We, therefore, examined the response to increasing amounts of protein ingestion in trained males following a breakfast 3 h prior to the exercise (Witard et al., 2014). Moreover, myofibrillar MPS was measured (as opposed to whole muscle MPS) to determine the response of the muscle protein fraction that contributes most to changes in muscle mass and strength. Myofibrillar MPS increased with the ingestion of 20 g of whey protein, but there was no further increase in the response to 40 g of protein following a leg-only resistance exercise bout. Thus, despite a number of methodological differences, these results supported the notion that MPS is maximal with the ingestion of ~20 g of protein following leg-only resistance exercise in trained, young weightlifters. Furthermore, consistent with the earlier results, amino acid oxidation (non-anabolic disposal) was increased dramatically with the ingestion of 40 g whey protein (Witard et al., 2014). So taken together with the results of Moore et al. (2009a), it was clear that the increase in myofibrillar MPS with increasing doses of protein, at least following leg-only resistance exercise (in a fed or fasted state), was not infinite. The excess amino acids not utilized for MPS with the ingestion of higher amounts of protein are shunted to non-anabolic pathways, such as oxidation. Therefore, the results of these studies have been used to make recommendations for post-exercise protein ingestion of no more than 20-25 g.

One factor deemed important for optimizing the amount of protein ingested following exercise is the total muscle mass of the individual consuming the protein (Breen & Phillips, 2011; Churchward-Venne et al., 2012b; Witard et al., 2016b). It is clear that MPS is increased in resting muscle following exercise in other muscles (Moore et al., 2009b; Witard et al., 2014) and that amino acid transport is increased into both resting and exercised muscles by high levels of amino acids in the blood (Biolo et al., 1997). This increase in transport results in greater incorporation of these amino acids (including those derived from ingested protein) into muscle protein (Pennings et al., 2011). Since increases in MPS ultimately are limited by availability of amino acids for incorporation into protein, a greater amount of muscle mass taking up amino acids from a finite amount of ingested protein may limit the response in any given muscle – contracted or not. Therefore, it seems intuitively logical that individuals with greater muscle mass may require greater amounts of ingested protein to maintain amino acid availability to all muscles and to maximally stimulate MPS. Thus, it is often asserted that MPS may not be maximal following the ingestion of 20 g of protein in individuals with large amounts of muscle mass (Churchward-Venne et al., 2012b; Witard et al., 2016b).

The question of the influence of muscle mass on the response of MPS to increasing doses of ingested protein was addressed by recruiting trained weightlifters and dividing them into two groups based on their measured lean body mass (LBM) (Macnaughton et al., 2016). Weightlifters with ≥ 70 kg of LBM were placed in the high LBM group and those with ≤ 65 kg LBM were placed in the low LBM group. MPS was measured following a bout of whole-body resistance exercise and the ingestion of either 20 g or 40 g of whey protein. We hypothesized that there would be no difference in the response of MPS to ingesting 20 g or 40 g of protein in the low LBM group, but the MPS response would be greater for 40 g vs. 20 g in the larger individuals. Interestingly, and to our surprise, the MPS response was not different between the two groups of individuals with different amounts of muscle. It should be emphasized that a whole-body resistance exercise bout was performed prior to protein ingestion in this study (Macnaughton et al., 2016) as opposed to leg-only in the previous studies (Moore et al., 2009a; Witard et al., 2014). Thus, it seemed that the amount of muscle actively engaged in the exercise prior to protein ingestion may be more important than the overall amount of muscle in a given individual. It is also critical to note that, to date, no study has directly compared the MPS response to different doses of protein in leg-only vs. whole-body resistance exercise. This comparison needs to be performed before it can be concluded with certainty that the difference between these studies is due to the amount of muscle mass involved in the exercise. It seems likely that a 40 g dose of protein would result in greater MPS in larger individuals following a leg-only resistance exercise bout, but not a whole-body workout.

A surprising result of the Macnaughton et al. (2016) study was that when participants in both groups were combined, the MPS response was greater following 40 g vs. 20 g of ingested protein. So, this study was the first to report that the MPS response to a 40 g dose was greater than the response to a 20 g dose in young, trained men. The obvious question is why were these findings different from previous studies? One possibility is simply statistical power. In the earlier studies, there was a mean difference of ~10% between the 40 g and 20 g doses that did not reach statistical significance. This more recent study assessed MPS in 30 participants compared to 12 per group (Witard et al., 2014) and six total participants in a crossover design (Moore et al., 2009a). So, it is conceivable that the difference between 40 g and 20 g reported in the previous studies was real, but the statistical power was low and thus the real, physiological difference between doses could not be detected. However, the difference between 40 g and 20 g (~20%) in our study was double that of the difference of the earlier studies. Therefore, a second and possibly more likely explanation for the different results between studies was the exercise bout employed (i.e., whole-body vs. leg-only). Of course, since the responses to whole-body resistance exercise and leg-only exercise have never been directly compared, we cannot rule out other possibilities to explain the discrepancies between the studies.

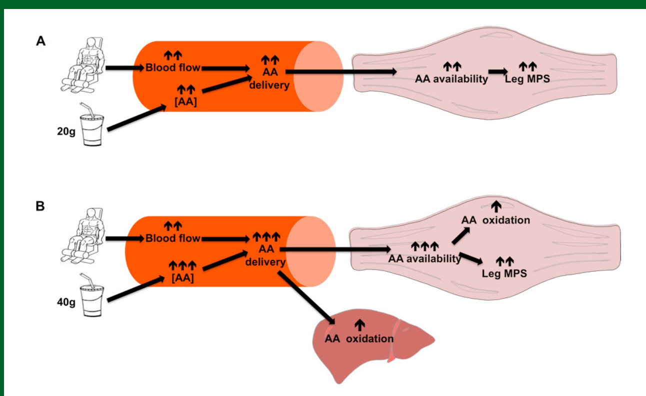

Figure 1. Proposed mechanisms for the differential responses of muscle protein synthesis (MPS) to 20 and 40 g of protein following leg-only resistance exercise.

A) Ingestion of 20 g protein following leg-only resistance exercise. Resistance exercise increases blood flow to the contracted muscle. Amino acids (AA) from ingested protein increase arterial AA concentrations. Delivery of amino acids (blood flow x [AA]) increases, resulting in increased availability of AA for MPS. MPS increases in the contracted muscle to near maximal rates.

B) Ingestion of 40 g protein following leg-only resistance exercise. Arterial [AA] increases more than with 20 g, so AA availability for MPS also is increased. However, MPS is maximal with less AA availability, so excess AA are shunted to non-anabolic pathways, in particular oxidation.

Arterial [AA] – amino acid concentration in the artery; AA delivery – delivery of amino acids to the muscle = arterial [AA] x blood flow to the muscle; AA availability – intracellular availability of amino acids to the muscle; AA oxidation – oxidation of excess amino acids.

However, if the most likely explanation for the difference in results was the resistance exercise routines used, a physiological mechanism to explain these results is proposed in Figures 1 and 2. Essentially, it is based on the fact that the overall demand for amino acids will be greater with the involvement of more muscle during exercise, thus reducing the availability of amino acids to any given quantity of muscle. Nutritive blood flow to contracted muscle is increased following resistance exercise (Biolo et al., 1995). However, blood flow is reduced to both contracted and non-contracted muscle, when other muscles are also involved in the exercise (Volianitis & Secher, 2002), and increasing the active muscle will dilute the delivery of amino acids to each individual muscle group. With this reasoning, whole-body resistance exercise led to a broader dispersal of blood flow to muscle such that the supply of amino acids to each individual muscle was limited when 20 g was ingested. However, the ingestion of the 40 g protein dose provided enough amino acids to ensure sufficient delivery and availability of amino acids to further increase MPS following whole-body resistance exercise. The fact that the myofibrillar MPS rate we observed was reduced in our recent study (Macnaughton et al., 2016) compared to our previous study (Witard et al., 2014), supports the argument that the amino acids were limiting with whole-body exercise. The reduced response of MPS to the whole-body exercise may have obscured any differences present between weightlifters with different amounts of muscle mass. However, once again, until a comparison between whole-body to leg-only exercise has been made, we cannot conclude with absolute certainty that the response of MPS is different to these two types of resistance exercise. Nonetheless, at the very least, it seems clear that ingesting ~20 g of protein does not maximally stimulate MPS

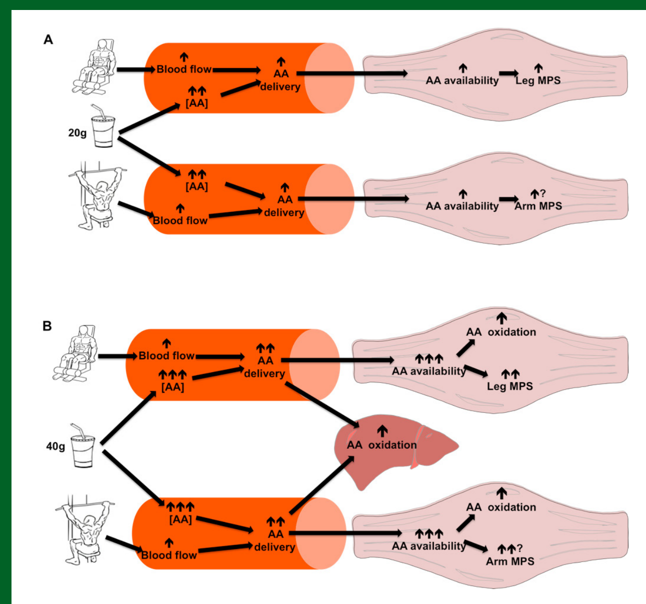

Figure 2. Proposed mechanisms for the differential responses of muscle protein synthesis (MPS) to 20 and 40 g of protein following whole-body resistance exercise.

A) Ingestion of 20 g protein following whole-body resistance exercise. Blood flow increases to both legs and upper body. Greater distribution of protein-derived amino acids (AA) results in less AA delivery and availability to each individual muscle. MPS in contracted leg muscles is less (Macnaughton et al., 2016) than with leg-only exercise (Witard et al., 2014).

B) Ingestion of 40 g protein following whole-body resistance exercise. Increased [AA] results in greater AA delivery, thus increasing AA availability to each muscle. MPS in contracted leg muscle (and presumably upper body muscles) is increased more than with 20 g.

Arterial [AA] – amino acid concentration in the artery; AA delivery – delivery of amino acids to the muscle = arterial [AA] x blood flow to the muscle; AA availability – intracellular availability of amino acids to the muscle; AA oxidation – oxidation of excess amino acids.

under all circumstances and recommendations for a single protein dose following resistance exercise appear to be too simple.

MPS RESPONSE TO INCREASING PROTEIN DOSES IN OLDER INDIVIDUALS

The loss of muscle with age (i.e., sarcopenia) is well-documented and is increasingly recognized as a critical problem in our ageing population. Clearly, all exercise and nutrition interventions that can help maintain, or even increase, muscle mass will be important for healthy ageing. Resistance exercise is a well-established means of increasing muscle mass in older adults as reviewed in the American College of Sports Medicine position stand (Chodzko-Zajko et al., 2009). Protein intake in association with resistance exercise increases the MPS response. However, the optimal dose of protein to maximize the response of MPS to resistance exercise leading to gains in muscle mass is not certain in older adults.

The metabolic changes that lead to muscle loss with age are not understood entirely. It now seems clear that the basal state (fasted and rested) rate of MPS is essentially the same in young and older adults (Volpi et al., 2001; Cuthbertson et al., 2005). However, a major contributing factor to sarcopenia with increasing age is the resistance of muscle to anabolic stimulation, or “anabolic resistance.” There is particular resistance to the anabolic stimulation of protein feeding (Witard et al., 2016a). Resistance exercise and protein nutrition are well-accepted interventions to counter age-related muscle loss. However, the optimal combination of protein nutrition and exercise to reduce muscle loss and/or optimize muscle gains has yet to be definitively determined in this population. It is important to understand the dose response relationship between MPS and protein intake in older adults to help formulate appropriate recommendations for exercise and nutrition to counter sarcopenia and dynapenia (decrease in strength).

Work from the laboratory of the late Professor Mike Rennie showed that stimulation of myofibrillar MPS was less for older adults than young with the ingestion of essential amino acids up to a dose of 20 g - equivalent to roughly 40 g of intact protein (Cuthbertson et al., 2005). We subsequently compiled data from several studies to garner information on the response to a single dose ingestion relative to total and lean body mass in young and older adults under resting conditions (Moore et al., 2015). The findings were consistent with the concept of anabolic resistance and the differences between young and older adults were marked when examined relative to lean body mass. The point where no further increase in MPS occurred with increasing protein ingestion was 0.60 g and 0.25 g protein/kg lean body mass for older and young adults, respectively. When considered in terms of total body mass, the response of MPS plateaued at ~0.40 g protein/kg total body mass in older men and 0.24 g protein/kg total body mass in young men. It is interesting to note that consuming 0.40 g protein in each of three meals in a day would result in a total ingestion of 1.2 g protein/kg for the day. This is the same amount that was associated with greater retention of lean body mass by older men (Houston et al., 2008). However, these results were measured under resting conditions. Since exercise, particularly resistance exercise, has a profound influence on the response of MPS to protein ingestion for up to 24 h following exercise (Burd et al., 2011), it is important to examine the single meal dose relationship of MPS following exercise.

The resistance of muscle to anabolic stimulation appears to impact the relationship between protein dose and the MPS response in older adults. Yang et al. (2012a) examined myofibrillar MPS at rest and following leg-only resistance exercise in older men with the ingestion of 0, 10, 20 and 40 g of whey protein. They reported that the ingestion of 20 g was maximally effective for MPS stimulation at rest in these older participants, as there was no further increase with ingestion of 40 g of whey protein. However, Pennings et al. (2011) reported that MPS was increased to a greater extent at rest by 35 g vs. 20 g of whey protein. There is no obvious explanation for the discrepancy between these two studies.

Nevertheless, resistance exercise enhanced the ability of the muscle to utilize ingested protein-derived amino acids for MPS in older men (Pennings et al., 2011). MPS was also greater at each dose of protein ingested in older adults in the Yang et al. study (2012a). On the other hand, unlike the earlier studies in young adults performing leg-only resistance exercise, the response of myofibrillar MPS to 40 g of protein was greater than the response to 20 g and there was no clear plateau in the MPS response (Yang et al., 2012a). So, it is unknown if ingestion of even higher doses of protein following exercise will further stimulate MPS in older men. It should be noted that the ingestion of > 40 g of protein in a single meal would likely not be well-tolerated by most older adults. Nevertheless, it seems clear that the dose response relationship between protein ingestion and MPS following resistance exercise is altered in older adults, at least with leg-only exercise.

SOURCE OF PROTEIN

Another important factor that influences the response of MPS to protein ingestion following exercise is the source of ingested protein. The amino acid composition and digestive properties of a protein are considered to be important factors that impact MPS (Witard et al., 2016b). The leucine content of the protein is considered to be the most important factor to maximize MPS. In fact, the “leucine threshold” hypothesis has been proposed to explain differences in the response of MPS to different proteins (Breen & Phillips, 2011). In this theory, the proteins with a greater proportion of leucine would be predicted to stimulate greater postprandial MPS. Another consideration for determining postprandial MPS is the digestive properties of the protein. There is evidence that, all else being equal, the protein that produces the fastest rise in the blood leucine concentration stimulates MPS to the greater extent (West et al., 2011). The faster rise in blood leucine explains why whey protein results in greater MPS following resistance exercise than micellar casein (Tang et al., 2009). These differential characteristics among different proteins will impact the relationship of the single meal dose that is ingested to the response of MPS following exercise.

There is relatively little information available on the response of MPS to various doses of protein other than whey protein. Given the importance of the leucine composition to the response of MPS, it is believed that proteins that contain less leucine, such as plant proteins, (van Vliet et al., 2015), will produce a smaller anabolic response. Yang et al. (2012b) investigated the response of MPS to ingestion of soy protein following resistance exercise in older adults. At rest, the MPS response following ingestion of 20 or 40 g of soy protein was not greater than ingestion of no protein at all. However, following resistance exercise, MPS was greater with ingestion of 40 g, but not 20 g, of soy protein (Yang et al., 2012b). Moreover, the MPS response to soy protein ingestion was less than with ingestion of whey protein at all doses of protein at rest and following exercise. Thus, these data are consistent with the leucine threshold concept suggesting that ingestion of greater amounts of so-called inferior protein sources (those with lower leucine composition), is necessary to maximally stimulate MPS. Unfortunately, this is the only study to date to systematically investigate the response of MPS to increasing doses of plant proteins in humans and there are no available data on younger individuals. Nevertheless, since plant proteins typically contain less leucine than animal proteins (van Vliet et al., 2015), it would be expected that a greater dose of protein would be required to produce MPS rates similar to those achieved with the ingestion of whey protein. Thus, it would be interesting to determine dose response curves for various plant proteins, particularly sources with higher leucine compositions, like legumes or quinoa (van Vliet et al., 2015).

The importance of the leucine composition in protein sources for the stimulation of MPS has led to the suggestion that the addition of leucine to an “inferior” protein may increase the MPS response (Witard et al., 2016a). There have been a limited number of attempts to investigate the response of MPS to various blends of proteins and free amino acids designed to manipulate the proportion of leucine (Atherton et al., 2017; Churchward-Venne et al., 2012a, 2014; Reidy et al., 2013). However, no systematic investigation of the dose response relationship of ingested protein blends with post-exercise MPS has been performed. Nevertheless, this information will be important to understand these variables so that appropriate recommendations for protein intake following exercise may be made.

Since the digestive properties of a protein influence the anabolic response to ingestion of that protein (Breen & Phillips, 2011; Witard et al., 2016a, 2016b), the form in which the protein is ingested may also have an important impact on the dose response. Most studies examining the response of MPS to protein ingestion utilize protein supplements. However, there are a few studies that have examined these responses to the ingestion of protein in food form. Symons et al. (2009) compared the response to different amounts of beef ingestion at rest and reported that MPS was increased, but there was no difference following ingestion of beef containing 30 vs. 90 g of protein in young and older adults (Symons et al., 2009). Subsequently, Robinson et al. (2013) reported that MPS was not increased by the ingestion of beef containing 12 or 24 g of protein at rest or following resistance exercise. However, the ingestion of 36 g of beef protein resulted in a significant rise in MPS in both situations (Robinson et al., 2013). To date, these studies provide the only information available on the response of MPS to various doses of food protein sources.

SUMMARY AND PRACTICAL APPLICATIONS

In summary, the optimal amount of protein to consume following exercise should not be considered as a one-size-fits-all proposition. We are only just beginning to delineate the many factors that may influence the optimal amount of protein to ingest to stimulate the maximal response of MPS. Recommendations from initial studies were to ingest 20-25 g of high-quality protein following exercise in young adults. However, we now know that this amount may not be sufficient to stimulate the maximal response of MPS in all circumstances and with all types of protein. There is still a great deal of information needed to make definitive recommendations regarding the optimal dose of protein for maximal stimulation of MPS in all situations and for all people.

- The best recommendation for the amount of protein to ingest to maximally stimulate MPS following leg-only (or other isolated body parts) exercise in young, healthy males is 20-25 g or ~0.25 g/kg of a high-quality protein, such as whey or egg protein. Greater amounts do not further stimulate MPS, but do stimulate amino acid oxidation.
- Young males participating in whole-body resistance exercise should consume up to 40 g of high-quality protein to maximally stimulate MPS.
- Older adults should consume more than 20 g, and up to at least 40 g of high-quality protein following leg-only exercise. However, given that whole-body exercise further increases the demand for protein-derived amino acids, even more protein may be required to maximally stimulate MPS.
- More protein is necessary if the amino acid composition of the protein is less than ideal, i.e., with lower amounts of leucine and perhaps other essential amino acids, as with most plant proteins.
- There is currently insufficient information on the response of MPS to ingesting proteins in forms other than isolated protein supplements to make solid recommendations, as the optimal amount of protein in a solid matrix or consumed in a meal with other nutrients is unknown. At present, the best practical course is to simply extrapolate from the results of studies with isolated proteins and aim for the amount of protein necessary to maximally stimulate MPS when consumed in supplemental form.

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