

Whey protein supplementation and muscle mass: current perspectives

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Abstract: Whey is one of the high-quality sources of protein with a higher proportion of indispensable amino acids compared to other sources. Its high leucine concentration makes whey an optimal protein source to maximize muscle protein synthesis (MPS) and to attenuate muscle protein breakdown at rest and following exercise. This review describes the main characteristics of the currently commercialized whey protein products and summarizes the available scientific evidence on the use of whey protein supplementation to maximize muscle mass gain in young adults without considering the impact on strength performance. Results of studies conducted on humans to date indicate that the integration of whey protein in the diet of resistance-trained individuals is effective in order to maximize muscle mass accretion. Nonetheless, the observed improvements are minimized when the total daily protein intake reaches a minimum of ≥ 1.6 g/kg. Under resting conditions, a single serving of ~ 0.24 g/kg body mass seems to be enough for stimulating a maximal postprandial response of MPS. Although this amount is effective to significantly promote an anabolic response after exercise, higher single doses of protein >0.40 g/kg after high volume workouts, involving large muscle mass, along with a minimum daily protein intake of >1.6 g/kg have been proposed as optimal to maximally stimulate MPS. Additionally, it seems that consuming whey protein as a part of a multi-ingredient admixture composed of carbohydrate, other protein sources and creatine monohydrate is more beneficial in order to maximize muscle mass gain in young resistance-trained individuals. These recommendations need to be confirmed by studies analyzing the MPS response to different workout configurations using a variety of intensities, training volumes (low, moderate or high) and the amount of the exercised muscle mass.

Keywords: indispensable amino acids, leucine, hypertrophy, nutrition, lean mass

Introduction

Whey protein has been proposed as an optimal protein source for supporting muscle mass accretion in humans.^{1,2} In comparison to other protein sources, whey protein has greater bioavailability, solubility and concentration of branched-chain amino acid (BCAA), particularly leucine.³ Findings from well-conducted meta-analyses of randomized controlled trials support the effect of combining whey protein supplementation with resistance training to optimize muscle mass accretion in trained² and non-trained^{4,5} individuals. Additionally, a more inclusive (49 studies with 1863 participants) meta-analysis by Morton et al⁶ supported the effect of protein supplementation to augment fat-free mass accretion by 27% (~ 0.3 kg) on average when combined with resistance training programs lasting for ~ 6 weeks. This figure is smaller than the 0.7 kg reported by Cermak et al⁴ or the 1.3 kg determined by Naclerio and Larumbe-Zabala.² Nonetheless, the improvements reported by

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Cermak et al⁴ and Morton et al⁶ resulted from a general analysis, merging trained and untrained, younger and older participants. When data from these two studies are analyzed using subgroups differentiated by the training status, the impact of protein supplementation on lean mass gain increased by +1.05 kg⁶ and +0.98 kg.⁴ These results are very similar to those reported by Naclerio and Larumbe-Zabala² who only used young resistance-trained individuals.

Despite the available evidence supporting the benefits of protein supplementation, mainly from whey, to maximize muscle mass gain, there are still some controversial results^{7,8} questioning the effectiveness of using protein for supporting muscle mass accretion in recreationally trained participants. It is likely that some uncontrolled factors, such as the type and quality of the protein source, the protocol of ingestion, including the dose per singular intake, timing of ingestion, eating patterns, including meal frequency and macronutrient distribution, the co-ingestion of other nutrients, and the energy content of the daily diet could have caused discrepancies between studies. In addition, the influence of the participants' training status and the training program configuration (volume, intensity and exercise selection) represent two of the most relevant variables to be considered to correctly interpret the observed results.⁹ In this short review, after describing the main characteristics of the currently commercialized whey protein products, the effectiveness of whey protein supplementation to maximize muscle mass gain in young adults, without considering the impact on strength performance will be analyzed.

An exhaustive literature review, considering solely human intervention studies, was performed until 31 December 2018 by using PubMed, Science Direct, Web of Science and Google Scholar. Combinations of the following keywords were used as search terms: "whey protein supplementation" AND "resistance exercise" OR "resistance training" OR "strength exercise" OR "strength training" OR "weight lifting" AND "muscular hypertrophy" OR "muscle mass" OR "fat free mass" OR "lean body mass" AND "humans". After an initial screening of title and abstracts, selected manuscripts were examined, including the reference lists of the retrieved articles. The inclusion criteria for this short narrative review were the following: 1) randomized controlled trial studies using trained and untrained young adults (aged ≥ 18 years up to 45 years); 2) the participants were classified as healthy with no medical contraindications; 3) the participants were assessed under resting or post-resistance exercise conditions in the fed or fasted

state; 4) trials should involve at least two groups or conditions (eg, treatment and control) to analyze the acute or long-term effects (>4 weeks) of whey protein supplementation, administered alone or as a part of multi-ingredient vs calorie equivalent contrast supplement (eg, carbohydrates). There were no restrictions on the number of participants, nor for sex, sports discipline or level of performance. Studies where participants were classified as patients, eg, unhealthy, including overweight or obese or any non-human intervention, were excluded.

The primary outcome variables were lean body mass, fat-free mass, muscle protein synthesis response, muscle protein balance, muscle hypertrophy and muscular thickness. Finally, we also considered results and studies included in previous protein supplementation reviews.^{2,4-6,10}

The conducted search resulted in the assessment of 28 intervention studies for this narrative review. Of these, 15 were focused on the effect of whey protein supplementation to acutely enhance muscle protein synthesis (MPS) response and 13 examined the effect of whey protein to promote superior muscle mass accretion after a training period of >4 weeks.

Whey protein supplement types

There are several types of whey protein supplements on the market. The most common are 1) whey protein concentrate (WPC), 2) whey protein isolate (WPI) and 3) whey protein hydrolysate (WPH).

Protein concentrates are produced by the coagulation of milk with the enzyme rennet or acid, resulting in the separation of curds and whey. Further ultrafiltration and drying produces WPCs containing more than 25% up to $\sim 90\%$ of pure protein.¹¹ Additional processes such as selective elution, or ion exchange chromatography can be used to further produce a more pure and fractionated whey isolate product containing $\geq 90\%$ of pure protein with very low amounts of lactose and lipids.^{12,13} In both WPC and WPI, a mixture of native intact protein is available. Subsequent hydrolysis with enzymes or acids provides a way to breakdown the structure of protein contained in WPC and WPI,¹⁴ producing WPH mainly composed of di and tripeptides, with higher bioavailability and more rapid absorption time compared to intact protein products.¹⁵

Although current manufacturing techniques for producing WPC and WPI can preserve the native structure of whey protein, some fractions may change their concentration, increasing or decreasing the resulted proportion in the final whey product. In general, WPI could present a slightly reduced concentration of glycomacopeptides,

lactoferrin, lactoperoxidase and some bioactive peptides. Nonetheless, cross-flow microfiltration using low temperatures and not exposed to fluctuating pH changes, produces a WPI retaining a very similar proportion of nature whey protein sub-fractions with trace amounts of fat and lactose.¹³

The main advantage of ion exchanges hydrolysates products is their rapid uptake and availability of amino acids (AAs) and a possible strong insulinotropic effect that would elicit a fast and powerful stimulus on MPS during the postprandial period.¹⁶ This advantageous postprandial effect of WPH may contribute to optimizing the muscle anabolic response during exercise conditions,¹⁷ and provide potential cardio-protective effects associated with a reduced postprandial lipemia.¹⁸

Native whey protein is a relatively new whey product obtained by filtration of unprocessed raw milk.^{19,20} Native whey differs from the typical WPC and WPI by not containing glycomacropeptides and maintaining a higher leucine content with respect to the more common WPC or WPI.²⁰

In addition to the typical WPC or WPI that are very sensitive to the effect of ultra-high temperature (UHT), which may result in protein denaturation, aggregation and flocculation, a microparticulated form of whey protein concentrate (mWPC) is also available.²¹ Microparticulation is an advanced processing technology that is typically achieved by thermal aggregation and acid precipitation combined with high shear conditions.²² This treatment can improve heat stability aggregation and gelation in consumer products in which UHT processing is required.²¹ Although mWPC has increased stability and a lower pH,²³ no difference to improve MPS was observed 1 hr after consuming 20 g of mWPC or WPC, providing similar leucine concentrations in physically active healthy young men that were ingesting both supplements with water in resting conditions.

Whey protein quality: bioavailability and AA profile

Nutritionally essential or indispensable amino acids (IAAs) are defined as either those AAs whose carbon skeletons cannot be synthesized or those that are inadequately synthesized de novo by the body relative to needs, and which must be provided from the diet to meet optimal requirements.^{24,25} Non-essential or dispensable AAs are those which can be synthesized de novo in adequate

amounts by the body to meet optimal requirements.^{24,25} Dietary protein sources are considered complete when they provide all IAA. In general, many vegetable foods lack or contain very low amounts of one or more IAA, called a limiting AA, and therefore are termed incomplete protein foods.¹¹

Based on the digestible IAA score (DIAAS) methodology,²⁶ animal protein including whey, casein, egg or beef contains a higher amount and proportion of IAA compared with vegetable protein sources, such as soy, potato, cereals or wheat.²⁶ Moreover, certain plant-based proteins (eg, cereals) are limited in IAA such as lysine, threonine and tryptophan, whereas others (eg, legumes) are limited in cysteine and methionine. Therefore, whereas the DIAAS for milk, eggs and beef are well above 100%, plant-derived proteins generally score well below 80%. Nonetheless, some protein extracts obtained from soy,²⁷ rice²⁸ or pea²⁹ have demonstrated scores closer or above 100%. Figure 1 indicates that when the same amount of protein is consumed, whey isolate extracts provide more IAA, including leucine, compared with other animal (casein, egg, beef, insect or bovine colostrum) and plant (hemp, pea, brown rice) protein products.

With the DIAAS, protein quality is determined based on the ideal AA requirement pattern and the true ileal digestibility of each IAA as assessed in humans, growing pigs or growing rats.²⁶ In humans, the reference ideal protein source was established based on the AA requirement pattern for different age ranges: 0–0.5; 1–2, 3–10; 11–14; 15–18 and >18 years old.²⁶ Even though the ideal protein should cover all of the known requirements for the IAA in each of the age ranges, the overall requirements of 3–10-year-old children have been considered for adolescents and adults.^{26,30} Furthermore, it is important to highlight that as currently defined, the pattern of IAA requirements reflects the minimum level of required intake of each IAA that, although it may be slightly overestimated,³¹ should not be considered to represent an optimal or even a maximum level of IAA intake.³

The scoring pattern for protein quality is determined by calculating the ratio of each IAA established requirement to the protein need and expressed as mg of AA provided per g of the analyzed protein.²⁶ Thus, the criteria to determine both the IAA and protein requirements, influences the magnitude of each AA within the scoring pattern and consequently the extent to which the pattern would identify a food protein source as adequate or deficient in one or more IAA.³² Overall, a good quality protein source for adults has been defined as one that meets at least 100% of all

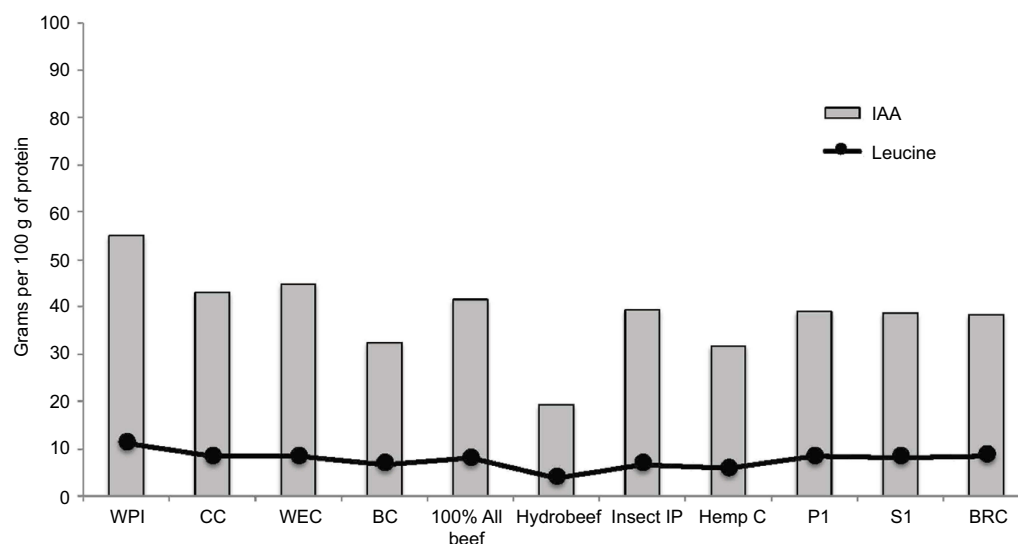


Figure 1 Amino acid profile of different protein sources describing the proportion of indispensable amino acids (IAA) and leucine in different animals and plant protein sources. Modified from: Phillips SM. The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab.* 2016;13:64. doi:10.1186/s12986-016-0124-8.³ Naclerio F, Seijo-Bujia M, Larumbe-Zabala E, Earnest CP. Carbohydrates Alone or Mixing with Beef or Whey Protein Promote Similar Training Outcomes in Resistance Training Males: A Double-Blind, Randomized Controlled Clinical Trial. *Int J Sport Nutr Exerc Metab.* 2017;27(5):408–420.⁷⁷ Churchward-Venne TA, Pinckaers PJM, van Loon JJA, van Loon LJC. Consideration of insects as a source of dietary protein for human consumption. *Nutr Rev.* 2017;75(12):1035–1045.⁷⁸
Abbreviations: WPI, whey protein isolate from the raw matter Optipep; CC, calcium caseinate; WEC, whole egg concentrate; BC, bovine colostrum; 100% All Beef, Hydrolysed Beef Protein from Crown Sport Nutrition (Spain) Hydrolysed Meat Protein from the raw matter Hydrobeef; Insect IP, insect isolate protein reported aminogram from Churchward-Venne et al; Hemp C, hemp protein concentrate; P1, pea isolate protein; S1, soy isolate protein; BRC, brown rice concentrate.

IAA requirements if 0.66 g/kg/day of this protein source is ingested.²⁶

Considering leucine as the key trigger IAA that turns on MPS³³ in situations like exercise-induced muscle mass gain, where MPS is the variable to maximize, it would be more important to focus on the leucine availability rather than the total IAA content.³ Thus, reaching a certain amount of leucine (leucine threshold) is an important aspect to consider in determining the potential effect of a given protein-rich food for further stimulating muscle mass accretion. For instance, in adults consuming 0.66 g/kg/d of protein which, as previously stated, is the lowest daily protein intake required to maintain body protein mass, a minimum amount of 0.039 g/kg/d of leucine

needs to be absorbed through the ileum.²⁶ The requirement of 0.039 mg/kg of leucine in adults represents the highest value of any IAA.³¹ In order to satisfy the minimum demand of leucine requirement, the ingested protein source should provide an average of 0.059 mg/g of digestible leucine.²⁶

When the AA profile of the ideal protein source is used to calculate the DIAAS, it is possible to establish the AA reference ratio (AARR) – defined as the digestible content of a given IAA in the protein measured, compared to a hypothetical best protein that provides the necessary amount of all IAA.³ Based on a rat model, and the AA requirement pattern of a 0.5–3-year-old child, Rutherford et al³⁰ calculated the AARR of all the IAA in several animal and plant

protein sources. Only milk protein concentrates and whey isolate extracts reached values higher than 1 (100%) in all the IAA. Furthermore, the AARR of leucine was higher in milk (1.77) and whey (isolate, 2.57 and concentrate, 1.93) compared to two sources of soy protein isolate (1.13 and 1.29) pea protein isolate (1.37) and rice protein concentrate (1.11). In certain circumstances, such as regular exercise training, some protein sources may be more appropriate to satisfy physiological demands. For example, an increased consumption of whey protein containing foods providing higher levels of leucine, compared to those suggested by the DIAAS, may be useful to optimize training adaptation in athletes³⁴ or overcoming the normal resistance to the anabolic effect observed in the elderly.³⁵

Effects of whey protein to maximize muscle gains in young resistance-trained individuals

Several researchers have studied the effectiveness of combining whey protein supplementation with resistance training to maximize muscle mass accretion in young trained individuals.^{1,2,36} The advantages of using whey protein to maximize muscle mass gains in trained participants have been proposed after observing acute significant increases in the MPS response (recognized as a primary determinant of exercise-nutrition induced muscle hypertrophy)⁶ following a single exercise bout, or by analyzing whether the addition of whey protein vs a contrast isoenergetic supplement (eg, maltodextrin) elicits superior outcomes over an intervention period (eg, 6, 12, 24 weeks or 1 year). In this section, a summary of the most relevant available literature regarding the effect of whey protein supplementation to 1) acutely enhance MPS response or 2) to promote superior hypertrophy outcomes after a minimum intervention period of 4 weeks will be presented.

Studies analyzing the acute dose-response effect of combining whey protein with resistance exercise to stimulate MPS

In the post-absorptive state, an acute bout of resistance exercise stimulates MPS by more than 100% above basal levels. However, as there is a concomitant proportional larger activation of the muscle protein breakdown, the net protein balance remains negative.³⁷ Only when protein is ingested in conjunction with workouts a synergistic effect will be obtained to

create a meaningful increase in MPS leading to a positive net protein balance.³⁸ Therefore, the summative effects of many bouts of resistance exercise in combination with protein intake will promote muscle protein accretion over time.³⁹ The effective dose of ingested AAs/protein for stimulating a maximal resting postprandial response of MPS has been very well analyzed^{34,40} and eventually established in 0.24 g/kg body mass per serving in young adults⁴¹ achieving a minimum daily intake of ~0.8 g/kg of protein to potentially maintain muscle mass in resting conditions.⁴² Nonetheless, in young resistance training individuals aiming to gain muscle mass, higher single dose of protein ~0.40 g/kg⁴³ along with a minimum daily protein intake >1.6⁶ to ~2.0 g/kg⁴⁴ have been recently suggested as the optimal amount for the post-workout intake.⁴⁴ Within this context, during exercise recovery conditions the effective doses to maximally stimulate MPS still remain undefined and may be influenced by the type of ingested protein source.¹⁰ The presence of a relatively high proportion of IAA including leucine has been considered one of the most relevant factors affecting the concomitant postprandial MPS response during the post-exercise recovery period.³ Indeed, a dose-dependent relationship between the amount of IAA ingested from different protein sources and MPS has been observed.^{40,45,46} Whey protein has proven to be the best high-quality, rapidly digestible source of IAA to maximize MPS rates at rest and during the initial several hours of recovery following exercises.⁴⁷ Nonetheless, when other plant-based sources with lower proportion of IAA are ingested, larger amount of proteins with a concomitant increased nitrogen, energy intake, longer digestion time, oxidation and ureagenesis,^{42,48} may be needed to compensate for the less efficient anabolic stimulus.⁴⁹

Tang et al⁴⁶ reported significant post-exercise MPS increases after consuming 10 g of whey protein combined with 21 g of fructose, in young well resistance-trained men. Considering that whey protein provides ~50% of IAA⁵⁰ and 11–12% of leucine,⁵¹ in a 10 g dose of whey, ~5 g of IAA and ~1 to 1.2 g of leucine are provided. The participants analyzed by Tang et al had a body mass of ~80 kg and consequently they were ingesting ~0.12 g/kg of protein including ~0.062 g/kg of IAA and ~0.012 to 0.015 g/kg of leucine. Despite the fact that this amount of protein falls slightly below the accepted doses (0.18–0.30 g/kg) to optimally stimulate MPS in young individuals⁴³ it can be considered a minimal threshold amount to increase MPS in the early post-exercise conditions. In this regard, Churchward-Venne et al⁵² reported that a suboptimal dose (6.5 g) of whey protein enriched either with leucine (containing a total of 8.4 g of protein, 5.1 g of IAA and 3 g

of leucine), or a mixture of all IAA without leucine (containing a total of 12.5 g of protein, 9.3 g of IAA and 0.75 g of leucine), similarly stimulated MPS with respect to the ingestion of 25 g of WPI (providing a total of 11.5 g of IAA and 3 g of leucine) under resting conditions, and for the first 3 hrs after an acute bout of unilateral resistance exercise in young resistance training males. However, only ingesting 25 g of complete whey sustained exercise-induced rates of MPS for more than 3–5 hrs post-workout and may be more appropriate to obtain a more optimal post-exercise anabolic response. It is worth noting that the average body mass ± 2 standard deviation of the participants used by Churchward-Venne et al was 76.4 ± 4.0 kg. Therefore, the participants ingesting the suboptimal enriched admixtures were always consuming >0.10 g/kg of protein, providing >0.060 g/kg of IAA and ~ 0.010 g/kg of leucine. These figures could represent a minimum effective, albeit not optimal, single serving dose of protein, IAA and leucine, respectively, to significantly enhance MPS in young individuals at rest, or during the early (up to 3 hrs) post-workout period. In support of the previous rationale, and although using a different aged population (38–55 years old), Mitchell et al (2017)⁵³ observed significant increases in the mammalian target of rapamycin C1 (mTORC1) pathway by the ingestion of only 9.2 g of milk protein providing ~ 0.12 g/kg of protein, 0.052 g/kg of IAA and ~ 0.011 g/kg of leucine in non-resistance-trained men after performing four sets of unilateral leg extension and leg press.

Taken together, it seems that at rest and during the early post-exercise recovery period, a minimum amount (>0.10 g/kg) of high-quality protein (eg, whey), providing ~ 0.060 g/kg of IAA and ~ 0.010 g/kg of leucine are necessary to significantly increase MPS. Nonetheless, a higher amount of ~ 0.24 g/kg of high-quality protein, including >0.10 g/kg of IAA and >0.010 g/kg of leucine will be needed to maximally stimulate MPS at rest.

As previously highlighted, under exercise conditions requirements increase, and although a minimal effective dose can enhance MPS compared to baseline, further increases of MPS can be much beneficial for maximizing training outcomes. In this regard, the impact of the workout configuration, particularly the volume⁵⁴ and the amount of exercised muscle mass⁵⁵ has shown to be crucial in altering the dynamics of the MPS response to protein feeding during the post-exercise time.⁵⁶ Witard et al⁴⁰ reported optimal MPS responses measured over ~ 4 hrs after performing eight sets of ten repetitions of two unilateral lower body exercises and ingesting 20 g of WPI containing ~ 10 g of IAA and ~ 2.2 g of leucine. It is worth highlighting that a dose of 40 g providing ~ 20 g of IAA and 4.4 g of leucine resulted in negligible, non-significant stimulation

of MPS. When the relative dose (g of protein per kg of body mass) is analyzed, the participants allocated to the 20 g serving consumed ~ 0.24 g/kg of protein, 0.12 g of IAA and 0.026 g/kg of leucine, while those who consumed 40 g were receiving ~ 0.50 g/kg of protein, 0.25 g/kg of IAA and 0.055 g/kg of leucine. Since the study of Witard et al limited the amount of exercised muscles to the lower limbs, Macnaughton et al⁵⁵ compared the MPS response to the ingestion of 20 vs 40 g of the same whey product after performing a whole-body resistance exercise routine including upper and lower muscle groups. In addition, the influence of the participants' body size was also analyzed. Results demonstrated that although both doses, 20 and 40 g significantly increased MPS, the highest dose elicited a significant superior ($+20\%$) MPS response during 5 hrs after the completion of the workout. Furthermore, no difference in the post-exercise MPS response to protein ingestion between participants with a relatively small or large amount of fat-free mass (~ 59 vs 77 kg, respectively) was observed. When the relative intake is considered, for the 20 g dose, 0.20–0.26 g/kg of protein, 0.10–0.13 g/kg of IAA and 0.022–0.028 g of leucine was administered. Consequently, twice these amounts (0.40–0.52 g/kg of protein, 0.20–0.26 g/kg of IAA and 0.044–0.056 g of leucine) were ingested for the 40 g intake. It seems that the training volume and the amount of muscle exercised during the workout, rather than the body size possessed by the individual, impact on the determination of the effective optimal protein dose to maximally stimulate MPS during the post-training period.

In summary, the current evidence suggests that in young individuals, there is a minimum threshold amount of both IAA (~ 0.10 g/kg) and leucine (~ 0.010 g/kg) needed to stimulate MPS at rest and during the post-exercise recovery time. However, higher doses (~ 0.20 and >0.040 g/kg of IAA and leucine, respectively) are needed to optimally stimulate MPS after very demanding exercise sessions (Figure 2). Even though the requested amount of IAA could be satisfied with different protein sources, whey protein extract seems to be more efficient on a gram per gram basis.

Studies analyzing the long-term effect of combining whey protein with resistance exercise to maximize muscle mass gain

Previous⁴ and more recent meta-analysis⁶ supports the notion that as the training level increases, the more relevant is the role of protein supplementation in maximizing the anabolic response to resistance training. Although a

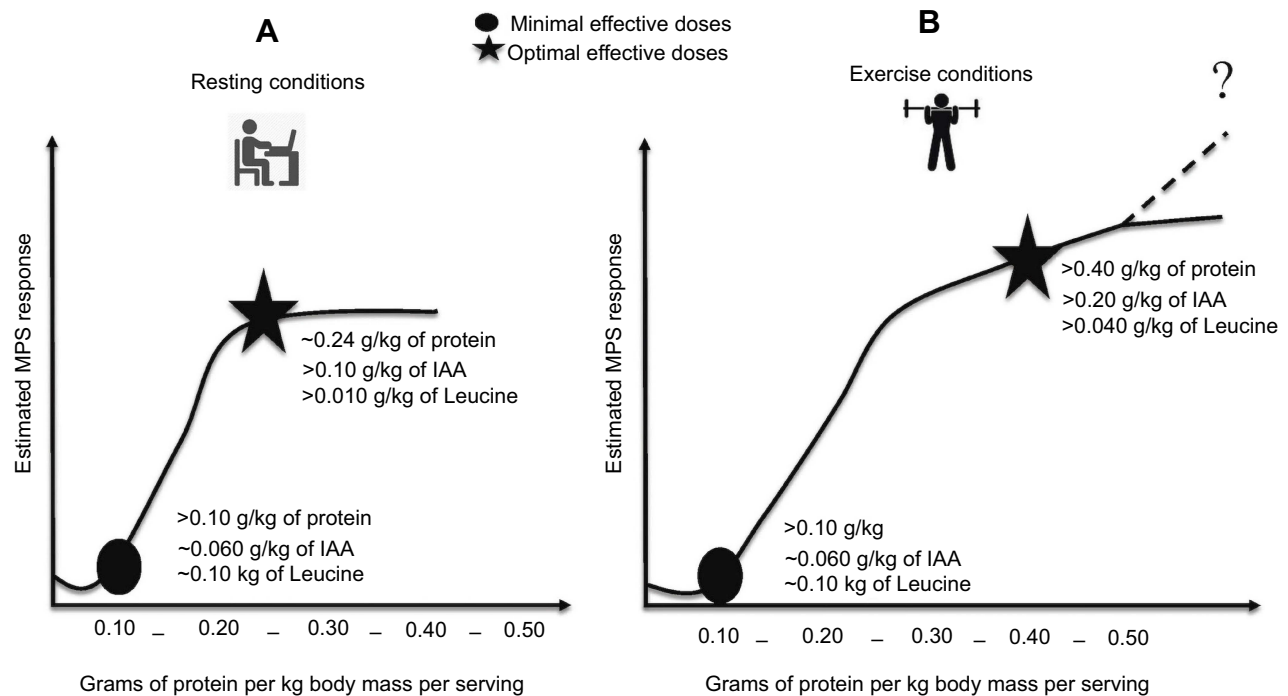


Figure 2 Theoretical model, showing the effect of a minimal and an optimal single dose (g/kg of body mass) of high-quality protein, IAA and leucine for stimulating muscle protein synthesis at rest (A) and after training (B).

minimum daily protein intake of 1.6 or as high as 2.2 g/kg appears to be the most influential factor in trained individuals focused on optimizing muscle mass accretion,³⁹ and only a small to modest, non-statistical significant, improvement can be obtained by any additional protein supplementation.^{6,39} From a practical point of view, a potentially slightly superior result obtained by a higher protein ingestion will still be important for trained individuals.² For instance, the meta-analysis of Naclerio and Larumbe-Zabala² reported that in well-trained athletes, an extra-increment of 2–4% in fat-free mass can be obtained from combining the ingestion of whey protein products with regular resistance training. Although these figures can be considered modest and are likely not to be statistically significant, for a typical trained 80 kg athlete an extra-gain of ~1.6 to 3.2 kg represents a valuable outcome, particularly if produced after relatively short intervention periods (6–12 weeks).

The recent meta-analysis from Morton et al⁶ suggests that protein supplementation augmented gains in muscle mass, with daily protein intakes of 1.6 g/kg body mass, being a plausible upper limit for eliciting lean mass accretion. Nonetheless, a potential confounding variable is the lack of an accurate control of the diet ingested by the participants. For instance, in meta-analysis of Naclerio and Larumbe-Zabala² that included randomized controlled

trials for young resistance-trained individuals, almost all the studies monitored the diet using 3–4 days diet records. Only the study of Joy et al²⁸ provided specific diet instructions in terms of macronutrient distribution rate, however, the daily amount of protein per kg of body mass was not reported. Similarly, in the recent meta-analysis of Morton et al,⁶ the only three studies^{57–59} that analyzed the impact of whey protein products on lean mass in young resistance-trained participants, also monitored their diet using a 3–4-day daily record. Although this approach has been extensively used, it is worth saying that it does not provide an ideal scenario to standardize and provide accurate information pertaining to participants' individual dietary intake.⁶⁰ As the amount of protein consumed from the supplement constituted only a fraction of the total dietary protein ingested during interventions, giving a pre-packed diet to participants during the study would offer a more accurate estimation of the impact of whey on the observed training outcomes when a suboptimal (eg, <1.6 kg/kg/d) or an optimal (>1.6 to 2 g/kg/d) diet protein intake is provided. For instance, two studies using trained individuals reported dissimilar outcomes regarding the impact of whey protein supplementation on muscle mass accretion in resistance-trained athletes. Cribb et al (2006) observed higher lean mass improvements in recreational bodybuilders who were ingesting >1.6 g/kg of protein (estimated by a 3-day diet record analysis) and further

supplemented their diet with the addition of 1.5 g/kg/d of WPH isolate vs the same amount of casein over a 10-week intervention period.⁶¹ Conversely, a more recent study⁵⁶ reported similar gains in lean mass over a 6-week resistance training intervention combined with the ingestion of 1) 25 g of whey 2) incremental whey protein ingestion protocol from 25 to 150 g/d from weeks 1 to 6; or (iii) 30 g of maltodextrin. All participants, regardless of the group, consumed >2.0 g/kg/d of dietary protein. Authors concluded that the ingestion of low (25 g/d) or high (25–150 g/d) whey protein daily doses may not provide substantial benefits in promoting hypertrophy when protein intake is >1.6 g/kg/d. Even though Haun et al⁵⁶ intended to control the diet based on participants' energy expenditure, the participants were instructed to self-report their dietary intake but some of them were unable to meet this requirement. Additionally, although, participants were instructed to refrain from ergogenic aids throughout the duration of the study, if consumed prior to the study, there were not restricted from using creatine monohydrate which has a significant impact on muscle mass accretion.⁶²

In summary, in resistance-trained individuals, integrating whey protein into their habitual diet represents a valid nutritional strategy for maximizing muscle mass gain as a result of middle (~6 weeks) to long duration (>12 weeks) resistance training intervention. These maximizing effects are minimized when the total daily protein intake achieves a minimum of ≥ 1.6 g/kg. Nonetheless, the current literature is still unable to accurately analyze the contribution of the supplement to the daily protein intake. This further confounds the measured outcomes and is potentially a source of variation between the observed effects. In this context it would be appropriate to analyze the convenience of using whey protein as a high-quality protein-rich food that can be integrated into the diet to help athletes to achieve the required daily amount of protein, thereby facilitating the ingestion of protein under special circumstances where the access to more traditional forms of foods (steak, chicken, eggs) becomes more difficult, eg, before, during or after training.

Co-ingestion with other macronutrients

Different multi-ingredient admixtures combining whey protein with carbohydrates,^{63,64} other protein sources such as casein,⁵⁷ bovine colostrum,⁶⁵ beef,⁶⁶ AAs,^{57,67} creatine monohydrate,⁴⁴ β -hydroxy- β methylbutyrate

(HMB)⁶⁸ or L-carnitine^{69,70} have been proposed for maximizing resistance training outcomes in athletes.

Combining carbohydrates and whey protein has shown to enhance cellular hydration, glycogen resynthesis and favor positive protein balances,⁷¹ compared to the ingestion of whey protein or AAs on their own. These beneficial effects are in part related to higher insulin anabolic-related stimuli caused by the addition of carbohydrates to whey protein.^{49,72} Since insulin initiates a suppression of muscle protein breakdown via the ubiquitous proteasome pathway,⁷³ the co-ingestion of carbohydrate acts as an optimizing, permissive nutrient for achieving a more favorable net muscle protein balance. Indeed, in the absence of sufficient blood AA availability, the carbohydrate-induced insulin concentration rise will likely target a suppression of muscle protein breakdown with no additional stimulation of MPS.⁷⁴

Multi-ingredients admixtures combining whey, with carbohydrates, casein, AAs and creatine monohydrate have shown superior enhancement effects on gaining lean mass compared to the ingestion of whey protein or carbohydrates alone.² Kerkick et al⁵⁷ reported significant increases in fat-free mass in 36 resistance-trained men after combining a 10-week strength training program with the ingestion of 40 g whey, plus 8 g casein and 2 g of carbohydrates, compared with both a carbohydrate-placebo and a similar multi-ingredient containing only 40 g of whey enriched with 3 g of BCAAs, 5 of glutamine and 2 g of carbohydrates. Authors concluded that the co-ingestion of whey and casein may be more effective to maximize muscle accretion compared to the ingestion of only one protein source.

Cribb et al⁷⁵ compared the effects of ingesting four different supplements: 1) whey protein only; 2) whey plus creatine monohydrate; 3) creatine plus carbohydrate and 4) carbohydrate only, on muscle mass in a group of recreational male bodybuilders over an 11-week intervention period. Supplementation with whey protein only, whey protein plus creatine monohydrate, and carbohydrates plus creatine monohydrate, resulted in greater hypertrophy responses compared with the ingestion of carbohydrates alone. Additionally, the consumption of creatine monohydrate mixed with whey protein or carbohydrate resulted in similar improvements that were still significantly greater compared with the ingestion of only carbohydrates or whey. More recently, Jakubowski et al⁶⁸ observed no differences between ingesting two daily 25 g doses of whey protein enriched with 1.5 g of HMB, or 1.5 of leucine during a 12-week periodized resistance training program on increasing muscle mass in trained men. On average, for all the participants regardless

of the group, 25 g of whey provided ~0.29 g/kg of protein 0.14 g/kg of IAA and >0.025 g/kg of leucine per intake. Moreover, all participants ingested an average of 1.8–1.9 g/kg/d of protein from the diet. It is possible that for individuals consuming a daily protein intake of ~1.6 g/kg, when the amount of IAA and leucine per intake reaches a threshold (~0.10 and 0.010 g/kg, respectively), no further stimulus on the MPS response will be produced by the addition of leucine and its metabolite HMB.⁷

Taken together, the available evidence supports the benefits of adding carbohydrates, creatine monohydrate or other protein sources to whey for maximizing muscle mass gain in resistance-trained individuals. Nonetheless, the additional benefits of adding AA or derivatives (eg, HMB) remain unclear and will be limited by the total daily protein intake and the relative amount (g/kg of body mass) of IAA and leucine ingested in each singular intake.

Conclusions

- A good quality protein source for adults has been defined as one that meets at least 100% of all IAA requirements if 0.66 g/kg/day of this protein source is ingested.
- To reach the minimum daily nutritional requirement of leucine (0.039 g/kg), the ingested protein sources should provide an average of 0.059 mg/g of digestible leucine.
- In certain circumstances, such as regular exercise training, whey protein sources (eg, WPI, WPC) possessing higher (>1) leucine AARR may be more appropriate to satisfy the physiological demands of exercise, favoring training adaptation and outcomes.
- The currently available evidence supports the use of whey protein to optimize muscle mass gain in resistance training individuals.
- At rest, young individuals may require a minimum threshold amount of >0.10/kg of protein, providing ~0.060 g/kg of IAA and ~0.010 g/kg of leucine per serving to stimulate MPS. Higher doses of ~0.24 g/kg of protein per intake, including >0.10 g/kg of IAA and >0.010 g/kg of leucine may be needed to maximize MPS response.
- Under exercise conditions, higher singular doses of protein may help to optimally stimulate MPS response. For instance, after very demanding exercise sessions (involving higher workout volumes and a large amount of exercising muscle mass) the ingestion of >0.40 g/kg of high-quality protein providing ~0.20 g/kg of IAA and >0.040 g/kg of leucine could be considered. In this context, compared to other

protein sources (pea, rice, soy or beef), the use of whey protein extracts (WPI, WPC or WPH) seems to be more efficient, on a gram per gram basis.

- In resistance-trained individuals the integration of different form of whey protein products in the diet may be considered as a valid nutritional strategy to satisfy the physiological demands and maximize training adaptation, eg, gaining muscle mass. These enhancement effects are minimized when the total daily protein intake achieves a minimum of ≥ 1.6 g/kg.
- Extra beneficial effects of whey protein-containing supplement on lean mass accretion are most evident when consumed as part of a multi-ingredient, containing carbohydrates, creatine monohydrate and other protein sources such as casein.

Practical application and futures perspectives

The integration of protein supplements as a part of regular diet represents a valid procedure to satisfy nutritional demands and avoid limitations in performance outcomes caused by a sub-optimal nutritional supply within the athletic context.

Whey protein extracts can be used to optimize exercise-induced benefits. Compared with the habitual protein-rich foods (eggs, cheeses, meat, milk, etc.), whey protein supplements possess a higher digestibility, leading to a rapid rise in the postprandial aminoacidemia. Even though muscles remain sensitized to protein ingestion for at least 24 hrs following exercises,⁷⁶ from a practical viewpoint some athletes may struggle, particularly those with high body masses, to consume enough protein to meet their required daily needs (>1.6 g/kg). Therefore, the pragmatic recommendation is for an athlete to eat as soon as possible after a workout, ingesting >0.24 to ~0.4 g/kg of high-quality protein sources, eg, WPI, WPC or WPH. In this respect, not eating does not offer any benefit regarding exercise adaptation and may also interfere with the subsequent training sessions.⁴⁴

Based on the current reviewed literature, the following considerations are proposed for futures studies or intervention protocols.

- The optimal dose of protein to be ingested in the post-workout meal needs to be determined based on each specific workout configuration.

- Whole-body resistance exercise routines involving larger muscle mass may require higher protein doses to maximize the anabolic effects during the post-exercise time.
- Studies using higher volumes along with high-intensity training sessions, typically designed for increasing performance or gaining muscle mass in athletes from different disciplines (American football, rugby, bodybuilding, wrestling, judo, etc.) characterized by a high component of strength, may need special attention in determining the optimal composition of the post-workout meal.
- The use of whey protein products should not be analyzed as an isolated strategy for increasing muscle mass or enhance performance in athletes. From a practical perspective, it should be considered as a valid dietary option to optimize nutrition and facilitate exercise-induced adaptations and outcomes.
- Future studies should consider the proportional contribution of whey protein products to the total daily protein intake and reveal its contribution to each individual meal with particular attention to the pre, during and post-workout food ingestion.

Disclosure

Fernando Naclerio and Marcos Seijo declare that they have no conflicts of interest relevant to the content of this review.

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