Nutrition and Dietary Supplements

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Whey protein supplementation and muscle mass: current perspectives

This article was published in the following Dove Press journal:
Nutrition and Dietary Supplements

Fernando Naclerio
Marcos Seijo

School of Human Sciences, Department of Sport Science and Physical Education, University of Greenwich, London, UK

Video abstract

Point your SmartPhone at the code above. If you have a QR code reader the video abstract will appear. Or use: https://youtu.be/dp7SPKgBSKk

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 accession. 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 trained[2] and non-trained[4,5] individuals. Additionally, a more inclusive (49 studies with 1863 participants) meta-analysis by Morton et al[6] 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 al[4] or the 1.3 kg determined by Naclerio and Larumbe-Zabala.[2] Nonetheless, the improvements reported by

Correspondence: Fernando Naclerio
University of Greenwich, Avery Hill Campus, Sparrows Farm, Sparrows Lane, Eltham, London SE9 2BT, UK
Tel +44 020 831 8441
Fax +44 020831 9805
Email f.j.naclerio@gre.ac.uk

http://doi.org/10.2147/NDS.S166195

Cermak et al[4] and Morton et al[6] 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 kg[6] and +0.98 kg.[4] These results are very similar to those reported by Naclerio and Larumbe-Zabala[2] 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 ~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 ≥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 glycomacropeptides, 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 IAAcompared 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] rice[28] or pea[29] 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

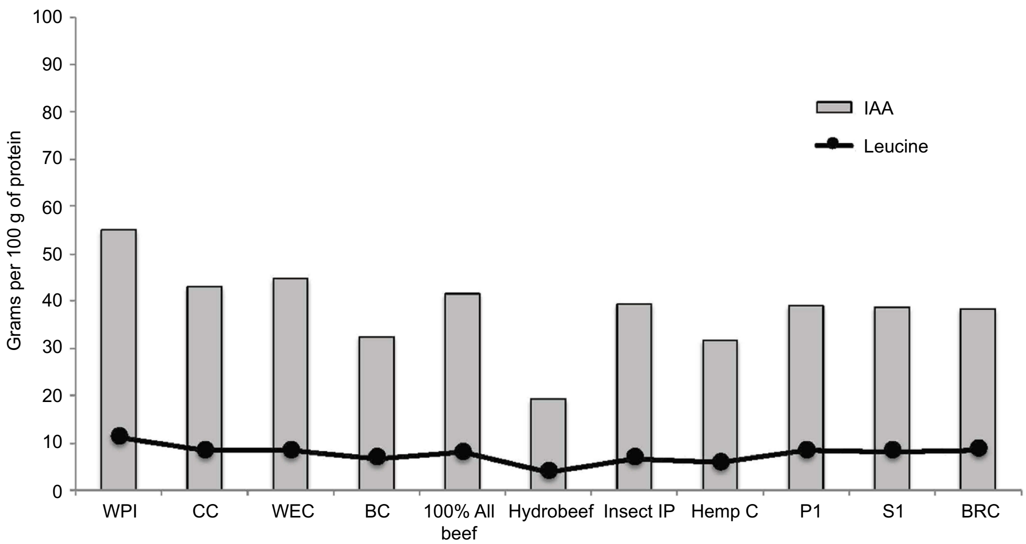

Figure 1 Amino acid profile of different protein sources describing the proportion of indispensable amino acids (IAA) and leucine in different animals and plant protein sources. Modified from: Phillips SM. The impact of protein quality on the promotion of resistance exercise-induced changes in muscle 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; PI, pea isolate protein; SI, 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 MPS[33] 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, Rutherfurd et al[30] 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 athletes[34] 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 isoenergic 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 adults[41] 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/kg[43] along with a minimum daily protein intake >1.6[6] to ~2.0 g/kg[44] 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 al[46] 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 IAA[50] 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 individuals[43] it can be considered a minimal threshold amount to increase MPS in the early post-exercise conditions. In this regard, Churchward-Venne et al[52] 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 unliteral 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 volume[54] and the amount of exercised muscle mass[55] has shown to be crucial in altering the dynamics of the MPS response to protein feeding during the post-exercise time.[56] Witard et al[40] 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 al[55] 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

Previous[4] and more recent meta-analysis[6] 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

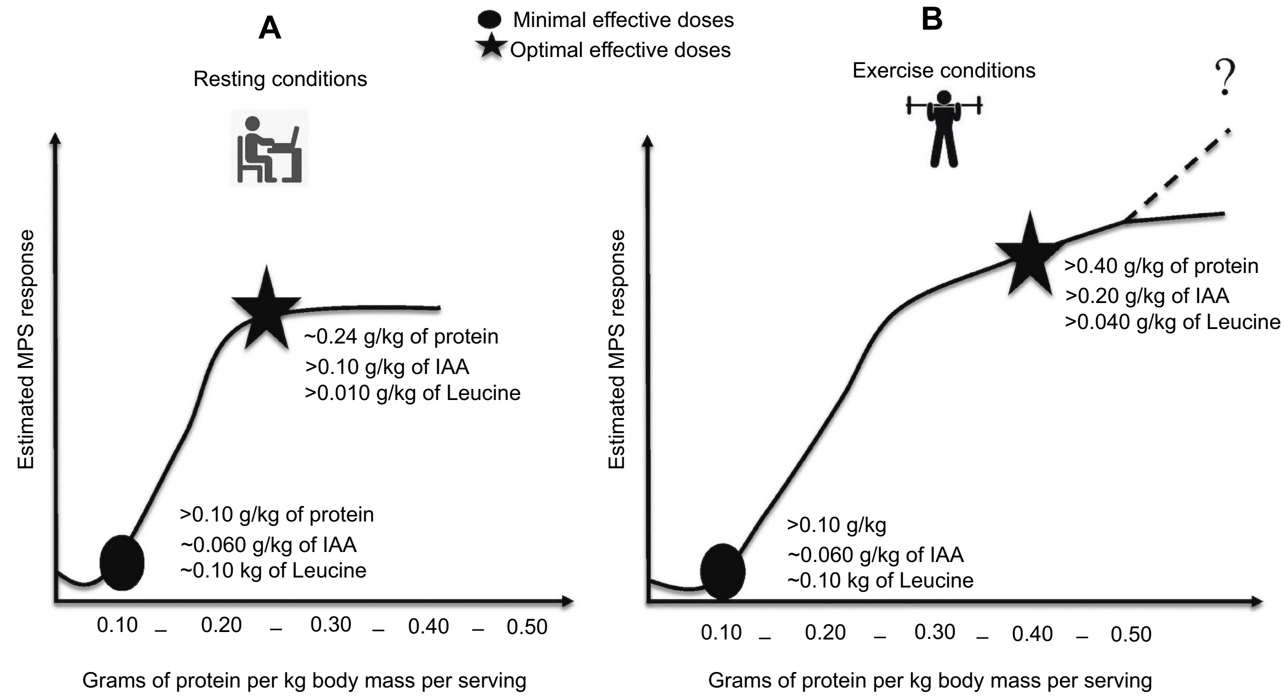

Figure 2 Theoretical model, showing the effect of a minimal and an optimal single dose (g/kg of body mass) of high-quality protein, IAA and leucine for stimulating muscle protein synthesis at rest (**A**) and after training (**B**).

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-Zabala[2] 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 al[6] 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-Zabala[2] 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 al[28] 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 study[56] 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 al[56] 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] Kerksick et al[57] 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 al[75] 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 al[68] 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 adaption 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 >010/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 or 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 adaptions 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.

References

1. Battermann W. Whey protein for athletes. *Dtsch Milchwirtschaft*. 1986;37(33):1010–1012.
2. Naclerio F, Larumbe-Zabala E. Effects of whey protein alone or as part of a multi-ingredient formulation on strength, fat-free mass, or lean body mass in resistance-trained individuals: a meta-analysis. *Sport Med*. 2016;46(1):125–137. doi:10.1007/s40279-015-0403-y
3. 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
4. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr*. 2012;96(6):1454–1464. doi:10.3945/ajcn.112.037556
5. Miller PE, Alexander DD, Perez V. Effects of whey protein and resistance exercise on body composition: a meta-analysis of randomized controlled trials. *J Am Coll Nutr*. 2014;33(2):163–175. doi:10.1080/07315724.2013.875365
6. Morton RW, Murphy KT, McKellar SR, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sport Med*. 2018;52(6):376–384. doi:10.1136/bjsports-2017-097608
7. Reidy PT, Rasmussen BB. Role of ingested amino acids and protein in the promotion of resistance exercise-induced muscle protein anabolism. *J Nutr*. 2016;146(2):155–183. doi:10.3945/jn.114.203208
8. Reidy PT, Fry CS, Igbinigie S, et al. Protein supplementation does not affect myogenic adaptations to resistance training. *Med Sci Sport Exerc*. 2017;49(6):1197–1208. doi:10.1249/MSS.0000000000001224
9. Hector AJ, Phillips SM. Protein recommendations for weight loss in elite athletes: a focus on body composition and performance. *Int J Sport Nutr Exerc Metab*. 2018;28:170–177. doi:10.1123/ijsnem.2017-0273
10. Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD. Protein considerations for optimising skeletal muscle mass in healthy young and older adults. *Nutrients*. 2016;8:181. doi:10.3390/nu8040181
11. Hoffman JR, Falvo MJ. Protein- which is the best? *J Sport Sci Med*. 2004;13:118–130.
12. Marshall K. Therapeutic applications of whey protein. *Altern Med Rev*. 2004;9(2):136–156.
13. Etzel MR. Manufacture and use of dairy protein fractions. *J Nutr*. 2004;134:996S–1002S. doi:10.1093/jn/134.4.996S
14. McGregor RA, Poppitt SD. Milk protein for improved metabolic health: a review of the evidence. *Nutr Metab*. 2013;10(1):46. doi:10.1186/1743-7075-10-46
15. Morr CV, Ha EYW. Whey protein concentrates and isolates: processing and functional properties. *Crit Rev Food Sci Nutr*. 1993;33:431–476. doi:10.1080/10408399309527643
16. Calbet JA, MacLean DA. Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans. *J Nutr*. 2002;132(8):2174–2182. doi:10.1093/jn/132.8.2174
17. West DW, Burd NA, Coffey VG, et al. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *Am J Clin Nutr*. 2011;94(3):795–803. doi:10.3945/ajcn.111.013722
18. Holmer-Jensen J, Mortensen LS, Astrup A, et al. Acute differential effects of dietary protein quality on postprandial lipemia in obese non-diabetic subjects. *Nutr Res*. 2013;33(1):34–40. doi:10.1016/j.nutres.2012.11.004
19. Hamarsland H, Laahne JAL, Paulsen G, Cotter M, Børsheim E, Raastad T. Native whey induces higher and faster leucinemia than other whey protein supplements and milk: a randomized controlled trial. *BMC Nutr*. 2017;3:10. doi:10.1186/s40795-017-0131-9
20. Hamarsland H, Nordengen AL, Nyvik Aas S, et al. Native whey protein with high levels of leucine results in similar post-exercise muscular anabolic responses as regular whey protein: A randomized controlled trial. *J Int Soc Sports Nutr*. 2017;14:43. doi:10.1186/s12970-017-0202-y
21. Mitchell CJ, D'Souza RF, Fanning AC, Poppitt SD, Cameron-Smith D. Short communication: muscle protein synthetic response to microparticulated whey protein in middle-aged men. *J Dairy Sci*. 2017;100(6):4230–4234. doi:10.3168/jds.2016-12287
22. Renard D, Lavenant L, Sanchez C, Hemar Y, Horne D. Heat-induced flocculation of microparticulated whey proteins (MWP); consequences for mixed gels made of MWP and β-lactoglobulin. *Colloids Surfaces B Biointerfaces*. 2002;24:73–85. doi:10.1016/S0927-7765(01)00246-6
23. Dissanayake M, Liyanaarachchi S, Vasiljevic T. Functional properties of whey proteins microparticulated at low pH. *J Dairy Sci*. 2012;95:1667–1679. doi:10.3168/jds.2011-4823
24. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids*. 2009;37:1–17. doi:10.1007/s00726-009-0269-0
25. Wu G. Functional amino acids in nutrition and health. *Amino Acids*. 2013;45:407–411. doi:10.1007/s00726-013-1500-6
26. FAO Expert Consultation. *Dietary Protein Quality Evaluation in Human Nutrition*; Report of an FAO Expert Consultation Rome Italy 2013. Available from: http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf. Accessed July 24, 2019.
27. Wolfe RR. Update on protein intake: importance of milk proteins for health status of the elderly. *Nutr Rev*. 2015;73(Suppl 1):41–47. doi:10.1093/nutrit/nuv021
28. Joy JM, Lowery RP, Wilson JM, et al. The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J*. 2013;12(1):86. doi:10.1186/1475-2891-12-86

29. Babault N, Paizis C, Deley G, et al. Pea proteins oral supplementation promotes muscle thickness gains during resistance training: a double-blind, randomized, placebo-controlled clinical trial vs. Whey protein. *J Int Soc Sport Nutr*. 2015;12(1):3. doi:10.1186/s12970-014-0064-5
30. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. *J Nutr*. 2015;145:372–379. doi:10.3945/jn.114.195438
31. Millward DJ. Knowledge gained from studies of leucine consumption in animals and humans. *J Nutr*. 2012;142:2212S–2219S. doi:10.3945/jn.111.157370
32. Millward DJ. Amino acid scoring patterns for protein quality assessment. *Br J Nutr*. 2012;108:S3–S21. doi:10.1017/S0007114512002462
33. Anthony JC, Anthony TG, Kimball SR, Jefferson LS. Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr*. 2001;131(3):856S–8560S. doi:10.1093/jn/131.3.856S
34. Phillips SM. Dietary protein requirements and adaptive advantages in athletes. *Br J Nutr*. 2012;108(Suppl):S158–67. doi:10.1017/S0007114512002516
35. Devries MC, McGlory C, Bolster DR, et al. Protein leucine content is a determinant of shorter-and longer-term muscle protein synthetic responses at rest and following resistance exercise in healthy older women: A randomized, controlled trial. *Am J Clin Nutr*. 2018;107(2):217–226. doi:10.1093/ajcn/nqx028
36. Burke DG, Chilibeck PD, Davidson KS, Candow DG, Farthing J, Smith-Palmer T. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *Int J Sport Nutr Exerc Metab*. 2001;11(3):349–364.
37. Phillips SM, Tipton KD, Aarsland AA, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol*. 1997;273:E99–E107.
38. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol Metab*. 1997;273:E122–E129. doi:10.1152/ajpendo.1997.273.1.E122
39. Stokes T, Hector AJ, Morton RW, McGlory C, Phillips SM. Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. *Nutrients*. 2018;10–18. doi:10.3390/nu10020180
40. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr*. 2014;99(1):86–95. doi:10.3945/ajcn.112.055517
41. Moore DR, Churchward-Venne TA, Witard O, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol A Biol Sci Med Sci*. 2015;70(1):57–62. doi:10.1093/gerona/glu103
42. Devries MC, Phillips SM. Supplemental protein in support of muscle mass and health: advantage whey. *J Food Sci*. 2015;80(Suppl 1):A8–A15. doi:10.1111/1750-3841.12802
43. Morton RW, McGlory C, Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Front Physiol*. 2015;6:245. doi:10.3389/fphys.2015.00245
44. Jager R, Kerksick CM, Campbell BI, et al. International society of sports nutrition position stand: protein and exercise. *J Int Soc Sport Nutr*. 2017;14:20. doi:10.1186/s12970-017-0177-8
45. Moore RD, Robinson MJ, Fry JL, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr*. 2009;89:161–168. doi:10.3945/ajcn.2008.26401
46. Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR, Phillips SM. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. *Appl Physiol Nutr Metab*. 2007;32(6):1132–1138. doi:10.1139/H07-076
47. Trommelen J, Betz MW, van Loon LJC. The muscle protein synthetic response to meal ingestion following resistance-type exercise. *Sport Med*. 2019;49:185–197. doi:10.1007/s40279-019-01053-5
48. Bilsborough S, Mann N. A review of issue of dietary protein intake in humans. *Int J Sport Nutr Exc Metab*. 2006;16:129–152. doi:10.1123/ijsnem.16.2.129
49. Norton L, Wilson GJ. Optimal protein intake to maximize muscle protein synthesis. *AgroFood Ind Hi-tech*. 2009;20:54–57.
50. Reitelseder S, Agergaard J, Doessing S, et al. Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *Am J Physiol Endocrinol Metab*. 2011;300(1):E231–42. doi:10.1152/ajpendo.00513.2010
51. Hulmi JJ, Lockwood CM, Stout JR. Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. *Nutr Metab*. 2010;7:51. doi:10.1186/1743-7075-7-51
52. Churchward-Venne TA, Burd NA, Mitchell CJ, et al. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J Physiol*. 2012;590(Pt 11):2751–2765. doi:10.1113/jphysiol.2012.228833
53. Mitchell CJ, Zeng N, D'Souza RF, et al. Minimal dose of milk protein concentrate to enhance the anabolic signalling response to a single bout of resistance exercise; A randomised controlled trial. *J Int Soc Sports Nutr*. 2017;14:17. doi:10.1186/s12970-017-0175-x
54. Burd NA, Holwerda AM, Selby KC, et al. Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *J Physiol*. 2010;15(588):3119–3130. doi:10.1113/jphysiol.2010.192856
55. Macnaughton LS, Wardle SL, Witard OC, et al. The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep*. 2016;4:15. doi:10.14814/phy2.12893
56. Haun CT, Vann CG, Mobley CB, et al. Effects of graded whey supplementation during extreme-volume resistance training. *Front Nutr*. 2018;5:84. doi:10.3389/fnut.2018.00084
57. Kerksick CM, Rasmussen CJ, Lancaster SL, et al. The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *J Strength Cond Res*. 2006;20(3):643–653. doi:10.1519/R-17695.1
58. Hoffman JR, Ratamess NA, Kang J, Falvo MJ, Faigenbaum AD. Effects of protein supplementation on muscular performance and resting hormonal changes in college football players. *J Sport Sci Med*. 2007;6(1):85–92.
59. Hoffman JR, Ratamess NA, Tranchina CP, Rashti SL, Kang J, Faigenbaum AD. Effect of protein-supplement timing on strength, power, and body-composition Changes in resistance-trained men. *Int J Sport Nutr Exerc Metab*. 2009;19(2):172–184. doi:10.1123/ijsnem.19.2.172
60. Jeacocke NA, Burke LM. Methods to standardize dietary intake before performance testing. *Int J Sport Nutr Exerc Metab*. 2010;20(2):87–103.
61. Cribb PJ, Williams AD, Carey MF, Hayes A. The effect of whey isolate and resistance training on strength, body composition, and plasma glutamine. *Int J Sport Nutr Exerc Metab*. 2006;16:494–509. doi:10.1123/ijsnem.16.5.494
62. Cooper R, Naclerio F, Allgrove J, Jimenez A. Creatine supplementation with specific view to exercise/sports performance: an update. *J Int Soc Sports Nutr*. 2012;9. doi:10.1186/1550-2783-9-33.
63. Kreider RB, Earnest CP, Lundberg J, et al. Effects of ingesting protein with various forms of carbohydrate following resistance-exercise on substrate availability and markers of anabolism, catabolism, and immunity. *J Int Soc Sport Nutr*. 2007;4:18. doi:10.1186/1550-2783-4-18
64. Hulmi JJ, Laakso M, Mero AA, Häkkinen K, Ahtiainen JP, Peltonen H. The effects of whey protein with or without carbohydrates on resistance training adaptations. *J Int Soc Sports Nutr*. 2015;12:48. doi:10.1186/s12970-015-0109-4

65. Kerksick CM, Rasmussen C, Lancaster S, et al. Impact of differing protein sources and a creatine containing nutritional formula after 12 weeks of resistance training. *Nutrition*. 2007;23(9):647–656. doi:10.1016/j.nut.2007.06.015
66. Naclerio F, Larumbe-Zabala E, Larrosa M, Centeno A, Esteve-Lanao J, Moreno-Perez D. Intake of animal protein blend plus carbohydrate improves body composition with no impact on performance in endurance athletes. *Int J Sport Nutr Exerc Metab*. 2019;1–7. doi:10.1123/ijsnem.2018-0359
67. Willoughby DS, Stout JR, Wilborn CD. Effects of resistance training and protein plus amino acid supplementation on muscle anabolic, mass, and strength. *Amin Acids*. 2007;32:467–477. doi:10.1007/s00726-006-0398-7
68. Jakubowski JS, Wong EPT, Nunes EA, et al. Equivalent Hypertrophy and Strength Gains in HMB- or Leucine-supplemented Men. *Med Sci Sport Exerc*. 2019;51(1):65–74. doi:10.1249/MSS.0000000000001752
69. Cooper R, Naclerio F, Larumbe-Zabala E, Chassin C, Allgrove A, Jimenez A. Effects of a carbohydrate-protein-creatine supplement on strength performance and body composition in recreationally resistance trained young Men. *JOEP Online*. 2013;16(1):72–85.
70. Willems ME, Sallis CW, Haskell JA. Effects of multi-ingredient supplementation on resistance training in young males. *J Hum Kinet*. 2012;33:91–101. doi:10.2478/v10078-012-0048-y
71. Tipton KD, Wolf R. Exercise, protein metabolism and muscle growth. *Int J Sport Nutr Exc Metab*. 2001;11(1):109–132. doi:10.1123/ijsnem.11.1.109
72. Breen L, Philp A, Witard OC, et al. The influence of carbohydrate-protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *J Physiol*. 2011;589:4011–4025. doi:10.1113/jphysiol.2011.211888
73. Greenhaff PL, Karagounis LG, Peirce N, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab*. 2008;295:E595–E604. doi:10.1152/ajpendo.90411.2008
74. Glynn EL, Fry CS, Drummond MJ, et al. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am J Physiol Integr Comp Physiol*. 2010;299:R533–R540. doi:10.1152/ajpregu.00077.2010
75. Cribb PJ, Williams AD, Stathis CG, Carey MF, Hayes A. Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. *Med Sci Sport Exerc*. 2007;39(2):298–307. doi:10.1249/01.mss.0000247002.32589.ef
76. Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sport Nutr*. 2017;14:33. doi:10.1186/s12970-017-0189-4
77. 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. doi:10.1123/ijsnem.2017-0003
78. 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. doi:10.1093/nutrit/nux057