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Achieving Optimal Protein Synthesis and Muscle Function: Less Processing May Be Beneficial

Short Review

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Abstract: It is well recognized that adequate protein intake, especially the amino acid leucine, stimulates muscle protein synthesis and therefore supports muscle growth and function. It has been suggested that the protein intake required to reach maximal protein synthesis has been suggested to be between 20-40g of high-quality protein per meal. Therefore, a protein source high in quality leucine may provide an enhanced benefit over other protein sources. This may be particularly beneficial for exercise recovery, injury recovery or age-related muscle loss prevention. Aging leads to higher protein needs, due to anabolic resistance in protein synthesis. Therefore, high quality leucine rich proteins are beneficial. One option is the native whey protein. Native whey protein is produced by low temperature, cold filtration of fresh milk which leaves proteins intact and results in a higher leucine content than other proteins such as whey protein concentrate. Typical whey protein is derived as a by-product of cheese production or from heat treated cow's milk. It is the purpose of this review to discuss the benefits of high-quality protein on muscle protein synthesis and compare the effects of native whey protein to standard whey and other proteins on protein synthesis and muscle function after exercise and in elderly individuals.

Key Words: native whey protein; sarcopenia; leucine

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Introduction

Maintaining a balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) is critical for optimizing athletic performance [1], enhancing recovery from exercise training [2], supporting recovery from injury [3] and helping to offset muscle loss often experienced with aging [4]. Therefore, strategies to promote protein synthesis that develop into long term improvements in skeletal muscle mass are warranted, particularly when the associated age-related clinical conditions such as diabetes, loss of muscle function, risk of falls, as well as decreased metabolic function are considered [5]. It has been reported that consuming high quality proteins rich in the essential amino acids (EAA) such as whey protein rich in leucine is ideal for stimulating protein synthesis [6,7]. However, anabolic sensitivity to amino acid availability is blunted with age, which can result in a diminished acute synthetic response following meal ingestion in older individuals when compared to younger subjects [8]. Therefore, older adults require a higher amount of EAA [9,10] especially leucine [4] than younger adults to stimulate protein synthesis. It should be noted that acute post meal changes in protein synthesis don't always translate to long term adaptations especially if all of the EAAs are not present [11]. Thus, identifying food sources containing adequate amounts of high quality proteins rich in leucine and all of the EAAs provides many potential benefits for athletes and aging individuals. One such protein source is milk, particularly the whey protein found in milk. Whey protein has been shown to stimulate MPS to a greater extent than casein and soy protein at rest and following exercise in young and older individuals with differences attributed to potential digestion rates and/or leucine content [12-15]. The processing of the whey protein can impact its quality [16], therefore the processing of the whey protein source should be considered when choosing a high quality whey protein. Native whey protein is produced by low temperature, cold filtration of fresh milk which leaves proteins intact and results in a higher leucine content than other high-quality proteins such as whey protein concentrate [17,18]. This is in comparison to commonly consumed whey proteins which are often derived from processes which result in a percentage of low of intact proteins [19]. It is the purpose of this review to discuss the benefits of high-quality proteins on muscle protein synthesis and compare the effects of native whey protein to standard whey and other proteins on protein synthesis and muscle function after exercise and in elderly individuals.

2. Protein Metabolism

The body's protein balance is maintained by achieving and maintaining an optimal balance between MPS and MPB. Therefore, a significant rise in MPS and/or a reduction in MPB which results in a positive net protein balance can ultimately lead to a gain of skeletal muscle proteins. Conversely, a reduction in MPS and/or increase in MPB can result in negative net protein balance and ultimately will result in a loss of skeletal muscle protein [1]. Furthermore, ingestion of specific amino acids, or isolated protein sources rich in specific amino acids, can influence protein synthesis at different rates than others. For example, consumption of whey protein, rich in leucine results in a rapid rise (within ~ 60 min) in blood leucine concentrations and an associated rise in protein synthesis, especially when consumed around the time of an exercise bout when compared to consuming the same amount of casein. It should be noted that this may be less applicable when consuming whole food sources [14]. Since there is no significant protein pool to maintain balance in times of increased need or lack of food, the balance is dynamic and influenced both acutely and chronically by several factors including diet, exercise, hormones, stress and aging [20]. For example, following ingestion of the essential amino acids (postprandial) a negative net balance indicative of the fasted (postabsorptive) state will switch to a positive balance, primarily due to an upregulation of protein synthesis [9]. While the balance is dynamic and will switch from positive to negative over the course of a day, the optimal scenario is to either have an overall positive balance for muscle protein growth or an equal balance for maintenance. While an overall positive balance may be advantageous, particularly for athletes wishing to achieve muscle hypertrophy or in those recovering from an exercise bout, a prolonged negative overall balance can be detrimental to health for many reasons. For example, a disruption in protein balance during aging can lead to sarcopenia, a slow age-related decline in skeletal muscle mass [21,22]. Or more specifically defined, a relative muscle mass less than 2 standard deviations below a sex-matched control group of younger adults aged 18 to 40 years of age [23].

3. Why protein balance is essential in sport

It is believed that resistance exercise creates a negative net protein balance whereby protein breakdown exceeds synthesis. Therefore, it is critical to consume adequate calories and protein to shift the balance to a positive one in order to enhance recovery which ultimately leads to training adaptations [24]. Thus, nutrition for recovery from exercise, and to prepare the body for the next bout of exercise, is considered important for optimizing performance [24]. Whey protein is one of the most commonly used dietary supplements to aid in muscle recovery. A recent systematic review reported that whey protein had a substantial effect on reducing myoglobin and creatinine kinase levels indicative of enhanced recovery [2]. Similarly, acute studies have found that post exercise consumption of protein (with intact leucine) alone or when combined with carbohydrate reduces markers of muscle damage and perceived soreness in athletes. These markers are often used to assess the occurrence of delayed onset muscle soreness (DOMS) [2]. Decreasing the occurrence and severity of DOMS is associated with recovery, enhanced recovery leads to increased training sessions, which ultimately translates to optimal performance [25].

Recovery is an important part of physical activity for all ages. For the aging athlete, utilizing nutritional strategies that may enhance recovery and feelings of well-being, as well as actual performance remains important [26].The protective effect against elevated or extended elevated markers of muscle damage is not just seen in the young, but is also recognized in the aging individual [27]. Coupled with this, it has been shown that when compared to isocaloric carbohydrate beverages the same beverage with added protein with intact leucine demonstrates a beneficial effect on protein synthesis and recovery [28]. A systematic review and meta-analysis including 49 studies of 1863 subjects concluded that post-exercise dietary protein supplementation along with resistance training significantly increased strength and muscle size, although the increase in muscle mass was slightly reduced in the elderly, most likely due to sub-optimal doses in the studies of elderly subjects [29]. This is not to say elderly individuals cannot gain lean body mass as a result of resistance training, it just supports the reports that they may require more protein per meal than younger individuals to maximally stimulate postprandial rates of MPS [30]. These reviews and studies strengthen the utility for protein and leucine-containing proteins to have ergogenic and practical value for athletes of all ages [28,29,31].

4. Age-Related Decline in Muscle Mass

There is great interest in identifying the factors associated with the age associated decline in muscle mass and muscle function, especially when the decline is rapid and severe. It has been linked to many age-related clinical conditions such as diabetes, loss of muscle function leading to increased risk of falls, as well as decreased metabolic function such as insulin resistance [5].

Initial studies suggested that age related sarcopenia was due to a decline in basal MPS [32,33], elevated MPB [34] or a combination of the two, leading to an overall negative balance and thus muscle loss. Fractional synthesis rate (FSR) of muscle protein was reported to be 20-30% less in the elderly compared with the young. Markers of myofibrillar proteolysis were increased by as much as 50% in the elderly as compared with younger adults [34]. This seems like a reasonable explanation for the sarcopenia, however, it was questioned because if there were a simultaneous decline in resting basal FSR and an increase in myofibrillar breakdown, the rate of muscle wasting would be greater than is typically measured in healthy people (0.5-1.5% per year between 50-80 years old) [35]. This combined with inconsistent findings as to whether or not MPS is decreased and MPB increased has led to the general agreement that muscle protein balance isn't compromised in healthy elderly individuals but that perhaps elderly individuals may be less efficient at utilizing the amino acids ingested [1].

4.1. Anabolic Sensitivity

It has been suggested that the anabolic sensitivity to amino acid availability is blunted with age, which can result in a diminished acute synthetic response following meal ingestion in older individuals when compared to younger subjects [8]. The term anabolic resistance is generally used to describe reduced anabolic responses to an anabolic stimuli, such as to EAA and leucine [36-38]. The resistance of elderly muscles to a physiological dose of amino acids has been reported by several studies [4,9,10]. Specifically, it was reported that while elderly and young responded similarly to a bolus ingestion of 15g of EAA, the elderly responded more slowly but remained in positive net balance for a longer period of time [9]. However, when protein and carbohydrate were ingested together, elderly respond with a diminished anabolic response compared with younger subjects [38]. Older adults require a higher amount of EAA [9,10], especially leucine [4] than younger adults to stimulate MPS.

Although it is not known exactly what causes anabolic resistance it has been suggested that it could be the gradual decline in physical activity that often occurs with aging or a change in the inflammatory response that could interfere with protein turnover [1]. Additionally, links between reduced anabolic sensitivity to amino acids and impaired insulin action have been shown in older individuals [38]. What causes it is yet to be agreed upon. However, exercise [39] and a leucine enriched amino acid mixtures may help to offset it [40].

4.2. Protein Threshold

Evidence has suggested that a minimum threshold concentration of amino acids needs to be reached to induce an adequate anabolic response in skeletal muscle protein synthesis [36] and that this threshold may be higher in older individual. It should be noted that essential amino acids are primarily responsible for the amino acid stimulation of muscle protein synthesis in the young and elderly [6]. This would mean that older people would require higher concentrations of essential amino acids to elicit maximum anabolic responses compared to the amount needed by younger people [1]. Therefore, protein recommendations have been examined and it has been suggested that aging adults would benefit from consuming a daily protein intake of 1.0 to 1.5 g/kg/day [41-44], this is above the current Recommended Dietary Allowance (RDA) for protein of .8g/kg/day [45]. When considering the evidence discussed above that amino acid availability is blunted with age, research was conducted to determine the per meal protein intake required to maximally stimulate MPS in healthy older and younger men. In a retrospective analysis of multiple studies Moore et al. reported that healthy older men are less sensitive to low protein intakes and require a greater relative protein intake (~.40g/kg), in a single meal, than young men (~.24g/kg) to maximally stimulate postprandial rates of MPS [30]. For both groups this is a per meal suggestion, other studies have reported that MPS is optimized when daily protein is distributed evenly at each meal over a 24 hour period [46,47]. This translates to a per meal protein intake goal of ~25–30 g/meal. This is assuming the protein is of high quality.

5. Protein Quality

Traditionally protein quality has been used to define the ability of a protein to meet basic metabolic needs, however, there has been a call in the literature to expand this definition. Under traditional descriptions, to quantify protein quality, The Food and Agriculture Organization of the United Nations developed the protein digestibility corrected amino acid score (PDCAAS) [48]. The score is based on the amino acid profile, specifically on the relative amounts of dietary EAAs in the test protein, corrected for protein digestibility. The amount of potentially limiting amino acids is compared to a reference score, and the limiting amino acid determines the score [49]. Most high-quality proteins have a PDCAAS > 1.0. The limitation is that the scores are cut off at 1.0., which does not allow one to compare the high quality [50]. The Food and Agriculture Organization of the United Nations recently released a protein quality scoring method called the digestible indispensable amino acid score (DIAAS) [51]. The DIAAS differ from the PDCAAS protein quality score in that it is based on the relative digestible content of each EAAs and the amino acid requirement pattern. The second difference is that DIAAS is not truncated so that high quality proteins can be compared [50]. Using both methods, high-quality proteins include meat and dairy proteins [50]. Furthermore, proteins derived from meat and dairy have higher quality ratings than vegetable proteins in all methods used to quantify quality such as protein efficiency ratio (PER), biological value (BV), and net protein utilization (NPU) [52,53]. Regardless of the method to assess quality, the purpose in considering different methods is to expand the definitions and discussions of quality in order to better integrate the expanding knowledge of its physiological roles beyond just basic metabolic needs. In the context of that discussion, it is important to consider that multiple factors influence protein quality, "there are 2 important aspects of protein quality: 1) the characteristics of the protein and the food matrix in which it is consumed, and 2) the demands of the individual consuming the food, as influenced by age, health status, physiologic status, and energy balance" [49].

The concept of protein quality and of categorizing proteins based on EAA supports the idea that while total protein intake is important, perhaps most important for MPS is the amount of EAA [6], especially leucine [7]. Katsanos et al. [4] reported that ingestion of 6.7 g of an EAA mix containing 41% leucine (1.7 g over a 3.5 h period) stimulated MPS rates in the elderly to a greater extent than an EAA mixture with only 26% leucine, producing similar synthetic responses to those seen in young muscle. It has been suggested that added leucine is not necessary, even in elderly individuals, if the overall protein intake is higher than recommended (1.5g/kg/day or higher). However, this is not realistic for many elderly individuals for many reasons including increased satiety, financial constraints, poly pharmacia, clinical conditions, and access to food. Therefore, leucine supplementation may be ideal when intake is low.

A study was conducted to determine whether the increase in protein synthesis following a small, low protein and carbohydrate simulated meal could be chronically increased by 2-weeks of low-volume leucine supplementation (4g/meal:3 meals/day) in healthy, community-dwelling older adults habitually consuming close to the RDA for protein. The added leucine did improve both mixed-muscle protein synthesis and anabolic signaling in older adults [54]. Similarly, a study of 48 men in a parallel design reported that a low-protein (6.25 g) mixed macronutrient beverage was as effective as a high-protein dose (25 g) at stimulating increased MPS rates when supplemented with a high (5.0 g total leucine) amount of leucine [55]. Suggesting that a high leucine content may be advantageous even when suboptimal amounts of total protein are ingested. This further emphasizes the need to consider proteins in term of not just total protein or even EAA but also in terms of leucine content.

Milk proteins are considered among the highest quality proteins, in that they provide all of the EAAs. Casein and whey are the two main proteins in milk. Whey protein is rapidly digested and has a high leucine content and therefore leads to a rapid increase in leucinemia. Casein is more slowly digested and induces a moderate rise in plasma AA concentration [56,57]. Whey protein has been shown to induce greater muscle protein synthesis than casein. Only 15g of whey soluble milk proteins was necessary to increase mitochondrial and myosin muscle protein synthesis while 30g of casein was needed [15]. This supports the leucine trigger hypothesis that states that it is necessary that intracellular initiation factors, including p70S6K and 4EBP1, be activated for protein intake to fully stimulate protein synthesis [58,59] .

This is not to negate the importance of other amino acids. In fact, it has been suggested that while leucine acts as a trigger for muscle protein synthesis and transiently raises MPS, it will not result in sustained muscle growth without the availability of the other amino acids [58]. Therefore, it seems logical to incorporate foods that are both high in leucine as well as other EAA. One such source is whey protein.

5.1. Benefits of Whey Protein

Milk contains two proteins, casein (about 80% of protein content) and whey (about 20% of protein content). Whey is a "by- product" of cheese production or can be specifically isolated through a complex filtration process of milk [60]. Milk has been shown to increase muscle protein synthesis to a greater extent than other protein sources, mostly due to whey protein [12-15,26]. Whey protein is commonly used in supplements consumed by athletes in order to support muscle protein synthesis for recovery and muscle growth and in medical nutrition to support increased protein demands for various clinical conditions. Whey protein isolate is classified as among the highest quality proteins with 11.7% leucine (g/100g of food); whey protein concentrate is also high quality but with 6.42% leucine. Followed by whole milk with .32% leucine, 3.5% of protein including 8.68% casein, however its slower absorption leads to a slightly lower quality rating in some quality rating methods [61]. Whey protein has been shown to stimulate MPS to a greater extent than casein and soy protein at rest and following exercise in young and older individuals with differences attributed to potential digestion rates and/or leucine content [12-15]. As discussed above, adding leucine to whey protein when whey protein is consumed in inadequate amount (<25g) can help to maintain the MPS advantages when it is not realistic to consume 25g of protein in a meal [55]. Therefore, a source of whey protein with a higher leucine content would be advantageous.

5.2. Native Whey Protein – Processing and Utility

5.2.1 Process

Whey protein concentrate is produced by coagulation of milk with the enzyme rennet or acid, resulting in separation of curds and whey, further ultrafiltration and drying produces whey protein concentrates containing ~25-80% protein [19]. In contrast, native whey protein is produced by filtration of fresh milk. The processing of milk itself may leave the milk proteins with altered nutrient composition. In fact, The processing methods like thermal treatment, chemical treatment, biochemical processing, physical treatments, nonconventional treatments, etc. may exert positive or negative influence on nutritional quality of milk proteins [16]. Thermal processing is an integral step in the manufacturing of almost all dairy products. The aim of the thermal treatment is to improve the microbial quality of milk by killing the microflora and thus, safeguarding against pathogens that can lead to food borne illnesses. Heat denaturation of milk protein fractions varies between the temperatures of 62 to 78 degrees Celsius. Commercial pasteurization is typically 72 degrees Celsius for 15 seconds and has been known to induce changes mostly in the reactive amino acids (i.e., during heating tryptophan and glutamic acid can form mutagenic derivatives, and arginine may be converted into citrulline and ornithine) [16]. It is possible that this altered amino acid profile secondary to the thermal and related processing may impact the overall nutrient quality.

The production method for native whey protein is cold filtration of a minimally processed fresh milk with unprocessed meaning not treated by, chemical or other typical dairy processing methods. The filtration process leaves the proteins in the milk more intact and more like the profile seen in raw unprocessed milk, this is in comparison to the processed whey protein commonly used in the marketplace. A more intact protein, a more natural protein may have advantages for digestion and utilization by the body [18].

This production method leaves proteins intact and results in a higher and more bioavailable leucine content than other high-quality proteins such as the common whey used in most supplements (WPC-80) and bovine milk. The higher leucine content may give native whey protein a greater MPS-stimulating ability than WPC-80. In addition, the chemical and heat-treatment that WPC-80 undergoes can render some amino acids unavailable for physiological utilization [17]. The PDCASS of Native whey protein is higher (139.9%) than traditional WPI (125.9%) or caseins (125.5%) (Internal data). The higher leucine content combined with greater digestibility impacts amino acid bioavailability. In a prospective, randomized, double-blind, crossover study, 10 healthy active young males ingested different whey and milk proteins after a resistance exercise session in order to characterize the blood amino acid response to the various milk proteins and to determine if there were any acute impacts on strength in the immediate 30 hour post-exercise period. Results indicated that native whey protein induced the greatest leucinemia (faster to concentration maximum and greater peak concentration) as compared to other whey protein supplements. The more robust leucine response to native whey protein may have clinical implications [18].

In a follow-up study which included a goal of examining how older males respond to various proteins, researchers utilized a cross over design to compare the effects of post exercise supplementation of 20g of WPC-80 or native whey protein on the acute (1-5 hours) MPS-response of elderly participants. Native whey protein increased blood leucine concentrations more than WPC-80 ($P < 0.05$), but not p70S6K phosphorylation or mixed muscle FSR. Both whey supplements increased blood leucine concentrations ($P < 0.01$) and P70S6K phosphorylation more than milk ($P = 0.014$). The authors concluded that native whey protein reached higher mixed muscle FSR and greater leucinemia as compared to WPC-80 and milk, while only being superior to milk for overall impact on MPS and phosphorylation of P70S6K [17].

A randomized double blind, placebo-controlled study comparing native whey protein to standard whey protein was conducted in 42 active young males who were randomly assigned to receive either 15 g of carbohydrates C, 15 g of NW (n = 17), 15 g of carbohydrates C 15 g of standard whey protein (SW; n = 15), or placebo (PLA; 30 g of carbohydrates; n = 10) for 5 days/week, while undergoing a 12-week electrostimulation (ES) training program of the knee extensors. Plyometrics were added for the second half of the training program. Anthropometrical characteristics, dimensions and neuromuscular properties of the knee extensor muscles and sprinting and jumping performances were measured to evaluate training adaptations, three testing sessions were conducted before (T0), and after 6 (T1) and 12 weeks (T2) of training. Concentric power recovery kinetics differed between groups ($p < 0.01$): Concentric power started to recover at 30 min in NW, whereas it recovers in 24 h in SW and 48 h in PLA. Muscle Training adaptations also differed between groups: Volountary contraction torque (MCV) increased between T0 and T2 in NW (+11.8%, $p < 0.001$) and SW (+7.1%, $p < 0.05$), but not PLA. Nevertheless, the adaptation kinetics differed: MVC increased in NW and SW between T0 and T1, but an additional gain was only observed between T1 and T2 in NW. Also, maximum voluntary activation level declined at T1 and T2 in PLA (-3.9%, $p < 0.05$), at T2 in SW (-3.5%, $p < 0.05$), but was unchanged in NW. The authors concluded that NW may promote faster recovery than SW after training [62]. While this study did not measure MPS, it offers suggestion that there may be a benefit to results in training adaptations that are not reflected in short term MPS measurements.

In a recent applied study, conducted by INSEP in France, researchers examined trained male rugby players during a simulated tournament for how recovery drinks would impact markers of muscle damage, exercise performance and perceived pain/soreness. The drinks tested were either a placebo (PLA, water), a carbohydrate drink (CHO, 80g of carbohydrates) or an iso-energetic carbohydrate-protein drink (P-CHO, 20g of Native whey protein and 60g of carbohydrates).The rugby simulated matches were three per day with two-hours rest between the games, repeated per protocol with six to seven-day washout between the test days. The results generally indicated that a recovery drink which included Native whey protein (with the carbohydrate) was generally superior than carbohydrate alone and placebo (water) for supporting exercise performance (running velocity) and definitively superior to placebo (water) and carbohydrate alone (equal in calories) for attenuating post-exercise muscle damage.[63].

6.0. Conclusion

There appears to be an ideal amount of protein to maximally stimulate post prandial protein synthesis, especially in elderly individuals, but it is not clearly defined. It is supported in the literature that the amount of leucine may make a difference by increasing MPS and this may matter more in elderly than the young. Therefore, the source of the protein may make a difference both because of its leucine content and because of other potential nutritional factors that may be influenced by processing. Consuming less protein for the same effect on protein stimulus may offer advantages especially for those who find it challenging to consume enough total protein. This may have particular value for elderly individuals, clinical patients, athletes and people trying to cut calories. Native whey protein may offer an advantage over commonly used whey protein supplements because it has a higher more bioavailable leucine content but also because it contains minors proteins such as lactoferrin and immunoglobulins and may therefore enable the consumer to achieve the same postprandial protein synthesis for less total protein. More research is needed to clearly define the unique advantages of native whey protein.

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