
Dietary Protein to Support Muscle Hypertrophy

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Abstract

Intact protein, protein hydrolysates, and free amino acids are popular ingredients in contemporary sports nutrition, and have been suggested to augment post-exercise recovery. Protein and/or amino acid ingestion stimulates skeletal muscle protein synthesis, inhibits protein breakdown and, as such, stimulates muscle protein accretion following resistance and endurance type exercise. This has been suggested to lead to a greater adaptive response to each successive exercise bout, resulting in more effective muscle reconditioning. Despite limited evidence, some basic guidelines can be defined regarding the preferred type, amount, and timing of dietary protein that should be ingested to maximize post-exercise muscle protein accretion. Whey protein seems most effective in stimulating muscle protein synthesis during acute post-exercise recovery. This is likely attributable to its rapid digestion and absorption kinetics and specific amino acid composition. Ingestion of approximately 20 g protein during and/or immediately after exercise is sufficient to maximize post-exercise muscle protein synthesis rates. Coingestion of a large amount of carbohydrate or free leucine is not warranted to further augment post-exercise muscle protein synthesis when ample protein is already ingested. Future research should focus on the relevance of the acute anabolic response following exercise to optimize the skeletal muscle adaptive response to exercise training.

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Introduction

Next to a certain genetic predisposition and regular participation in a well-designed training regimen, nutrition plays a key factor in determining

physical well-being and exercise performance capacity. Good nutritional practice becomes even more important as athletes approach their limits with respect to training volume and intensity. This has renewed the interest among athletes, coaches, and exercise physiologists in the role of nutrition and nutritional modulation on physical performance. Specific nutritional interventions, including the use of specifically designed sports nutrition products, are widely used by athletes in an effort to compensate for the metabolic demands imposed upon by intense exercise training and/or competition. To improve endurance exercise performance capacity, dietary interventions generally aim to maximize endogenous carbohydrate availability before, during and/or after exercise. For this purpose, numerous carbohydrate-based sports drinks, energy bars and gels have been developed. In contrast, to enhance performance capacity in resistance type exercise tasks, nutritional interventions generally focus on stimulating muscle protein synthesis and/or reducing protein breakdown to augment the skeletal muscle adaptive response. This brief review will discuss the impact of dietary protein administration during acute post-exercise recovery and its proposed impact on the muscle adaptive response to exercise training. Consequently, this review will define some basic guidelines regarding the preferred type, amount, and timing of dietary protein administration to maximize post-exercise muscle protein accretion. Finally, limitations of the presented research and suggestions for future research to more successfully apply dietary protein administration to improve skeletal muscle reconditioning during exercise training will be discussed.

Post-Exercise Muscle Protein Synthesis and Breakdown

For muscle hypertrophy to occur, muscle protein synthesis rates must exceed muscle protein breakdown rates over a given period of time. Resistance exercise training is an effective interventional strategy to stimulate muscle protein synthesis. A single bout of resistance type exercise has been reported to stimulate skeletal muscle protein synthesis for up to 24–48 h [1, 2]. Resistance type exercise also stimulates muscle protein breakdown rates, albeit to a lesser extent than protein synthesis, and thus resistance type exercise effectively improves muscle protein balance. However, net protein balance remains negative in the absence of nutrient intake. The ingestion of carbohydrate and protein during post-exercise recovery further augments muscle protein synthesis and inhibits protein breakdown, resulting in net muscle protein accretion. Nutrition therefore forms a key factor in determining the effect of exercise training on muscle hypertrophy.

Carbohydrate ingestion during post-exercise recovery is an effective intervention to inhibit exercise-stimulated muscle protein breakdown, but does not seem to affect muscle protein synthesis [3, 4]. Though post-exercise protein

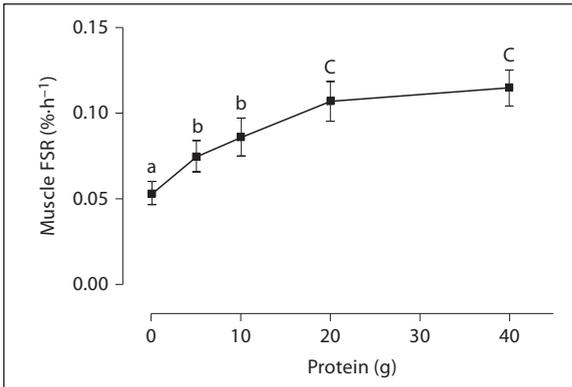


Fig. 1. Dose-response relationship between the amount of protein ingested and post-exercise muscle protein synthesis rates. Values represent means \pm SEM. Means with different letters are significantly different from each other. FSR = Fractional synthesis rate. Figure reproduced from Moore et al. [20].

balance will improve following the ingestion of carbohydrate, net protein balance will remain negative [3]. The inhibitory effect of carbohydrate ingestion on post-exercise muscle protein breakdown has largely been attributed to the concomitant rise in circulating plasma insulin concentrations. However, even though elevated plasma insulin levels have been reported to stimulate net muscle protein anabolism, these properties are evident only in the presence of increased amino acid availability [5]. Recent studies support the contention that insulin is not a major regulatory factor determining muscle protein balance and identify amino acid availability as being the main stimulus for muscle protein synthesis under normal, resting conditions [6].

It has been established that protein/amino acid administration effectively stimulates muscle protein synthesis. Biolo et al. [7] demonstrated that hyper-aminoacidemia, following intravenous amino acid infusion, increased post-exercise muscle protein synthesis rate and suppressed the exercise-induced increase in protein breakdown. Thereafter, Tipton et al. [8] showed that post-exercise ingestion of 40 g of either mixed amino acids (MAA) or essential amino acids only (EAA) also effectively stimulated muscle protein synthesis. Follow-up studies assessed the impact of smaller amounts of EAA with and without carbohydrate and showed that these were also effective in stimulating post-exercise muscle protein synthesis, resulting in a positive net protein balance during acute post-exercise recovery [9, 10]. Numerous other studies have shown that amino acid and/or protein administration increases muscle protein synthesis rates following resistance type exercise [3, 9–16]. Furthermore, amino acid and/or protein administration has also been shown to increase mixed muscle protein synthesis rates following endurance type exercise [17–19].

Amount of Dietary Protein

Though it has been well established that protein ingestion effectively stimulates muscle protein synthesis rates both at rest and following exercise, there is still considerable debate regarding the exact amount and type of protein and the desired timing of protein ingestion to maximize post-exercise muscle protein synthesis. Tipton et al. [8] showed that post-exercise ingestion of 40 g MAA or EAA effectively stimulated muscle protein synthesis. Since the ingestion of 40 g MAA or 40 g EAA resulted in a similar net protein balance, it was suggested that it might not be necessary to ingest nonessential amino acids during immediate post-exercise recovery. Follow-up studies assessed the impact of only 6 g EAA with and without carbohydrate and showed that this amount was also effective in stimulating post-exercise muscle protein synthesis. However, ingestion of such a small amount of EAA after exercise resulted in a positive net protein balance for up to 2 h only, after which net protein balance became negative again [9]. This suggests that ingestion of such an amount of amino acids is not sufficient to remain in an anabolic state. Recently, Moore et al. [20] conducted a dose-response study to investigate the relationship between protein ingestion and post-exercise muscle protein synthesis. The fractional synthetic rate of mixed muscle protein increased with the ingestion of greater amounts of protein, reaching maximal stimulation after 20 g intact (egg) protein, which provided approximately 8.6 g EAA. The authors speculated that athletes should ingest this amount of dietary protein 5–6 times daily to maximize skeletal muscle protein accretion on a habitual basis.

Source of Dietary Protein

Various studies have reported improved post-exercise protein balance and/or greater muscle protein synthesis rates following the ingestion of whey protein [21], casein protein [21], soy protein [22], casein protein hydrolysate [12, 23], egg protein [20], and whole-milk and/or fat-free milk [22, 24]. It seems obvious to question which source of dietary protein would be most effective to promote post-exercise muscle protein anabolism. While research comparing the efficacy of different proteins on the post-exercise protein synthetic response is slowly emerging, it is presently not possible to identify a specific protein source that is most effective for promoting post-exercise muscle protein accretion. The issue is further complicated by the fact that numerous parameters modulate the muscle protein synthetic response to protein ingestion during post-exercise recovery. The type, intensity, and duration of exercise prior to protein ingestion, the duration of the recovery period that is being assessed, the amount and timing of protein administration, the amino acid composition of the protein, and the digestion and absorption kinetics of the protein source (or mixed meal)

provided, may all modulate the postprandial muscle protein anabolic response. To date, few studies have tried to assess differences in the post-exercise protein anabolic response to the ingestion of different types of protein.

Milk protein and its main isolated constituents, whey and casein, seem to offer an anabolic advantage over soy protein for promoting muscle hypertrophy [22, 25, 26]. Casein and whey protein seem to have distinct anabolic properties, which are attributed to differences in digestion and absorption kinetics [21, 27–29]. Whereas whey protein is a soluble protein that leads to fast intestinal absorption, intact casein clots in the stomach delaying its digestion and absorption and the subsequent release of amino acids in the circulation [30]. The fast, but transient rise in plasma amino acid concentration after whey protein ingestion can lead to higher protein synthesis and oxidation rates [21, 27–29]. Despite these differences, Tipton et al. [21] found no difference in net protein balance during recovery from resistance type exercise following casein versus whey consumption. In addition to intrinsic differences in digestion and absorption rate, it has been suggested that whey protein can more effectively stimulate protein synthesis due to its greater leucine content when compared to casein [25]. However, the latter may be questioned as Koopman et al. [12, 23, 31] showed that coingestion of free leucine did not further increase muscle protein synthesis rate when an ample amount of casein hydrolysate is ingested during post-exercise recovery. Additional studies are warranted to assess the impact of the digestion and absorption kinetics of a protein source and its amino acid composition on stimulating muscle protein synthesis rates following exercise. In accordance, the influence of the timing of protein administration should be considered when defining nutritional strategies to augment post-exercise muscle protein accretion.

Carbohydrate Coingestion

Post-exercise nutritional interventions should aim to enhance recovery and facilitate the adaptive response to regular exercise training. In the endurance trained athlete, rapid restoration of depleted muscle glycogen stores is generally a priority to enhance post-exercise recovery and thereby maintain performance capacity. Therefore, endurance trained athletes mainly focus on carbohydrate ingestion for post-exercise recovery. Recently, coingestion of relative small amounts of protein and/or amino acids has become popular among these athletes, mainly because this can further accelerate muscle glycogen repletion when less than optimum amounts of carbohydrate (<1.0 g/kg bodyweight per hour) are ingested.

As protein and/or amino acid ingestion has been proven essential to allow net muscle protein accretion following exercise [3, 12, 13, 23], athletes involved in resistance type exercise training often ingest large quantities of protein and

carbohydrate after cessation of exercise (i.e. traditional ‘weight gainers’) to augment net muscle protein accretion. Coingestion of carbohydrate during post-exercise recovery has been shown to improve net leg amino acid balance [3], which has been attributed to the concomitant increase in circulating plasma insulin concentrations [4]. In accordance, elevated plasma insulin levels can increase net muscle protein anabolism *in vivo* in humans [32, 33]. However, insulin should not be regarded as a primary regulator of muscle protein synthesis as insulin exerts only a modest effect on muscle protein synthesis in the absence of elevated amino acid concentrations. In a recent attempt to assess whether carbohydrate coingestion is required to maximize post-exercise muscle protein synthesis, we observed no additional benefit of the coingestion of either a small or large amount of carbohydrate on post-exercise muscle protein synthesis rates under conditions where ample protein is ingested [11]. Though carbohydrate coingestion does not seem to be required to maximize post-exercise muscle protein synthesis rates, it is likely that some carbohydrate can attenuate the post-exercise rise in muscle protein breakdown rate, thereby improving net protein balance [3, 4]. Furthermore, as muscle glycogen content can be reduced by 30–40% following a single session of resistance type exercise [34], some carbohydrate coingestion may be preferred when these athletes wish to allow full muscle glycogen repletion to maintain optimum exercise capacity.

Timing of Dietary Protein Ingestion

Besides the amount and type of protein ingested, the timing of protein ingestion seems to represent an important factor in stimulating post-exercise muscle anabolism. Levenhagen et al. [35] reported an improved post-exercise net protein balance after consuming a supplement that contained protein, carbohydrate and fat immediately after cessation of exercise as opposed to 3 h later. Furthermore, recent studies suggest that carbohydrate and protein coingestion prior to and/or during exercise may further augment post-exercise muscle protein accretion [16, 36]. Tipton et al. [16] showed that amino acid ingestion prior to, as opposed to after, exercise further augments net muscle protein accretion during subsequent recovery. The stimulating effect of protein or amino acid supplementation prior to exercise on muscle protein synthesis after exercise has been attributed to a more rapid supply of amino acids to the muscle during the acute stages of post-exercise recovery. However, it could also be speculated that protein ingestion prior to and/or during resistance type exercise already stimulates muscle protein synthesis during exercise, thereby creating a larger time frame for muscle protein synthesis to be elevated. In a recent study, we confirmed that coingestion of protein with carbohydrate before and during 2 h of intermittent resistance type exercise stimulates muscle protein synthesis during

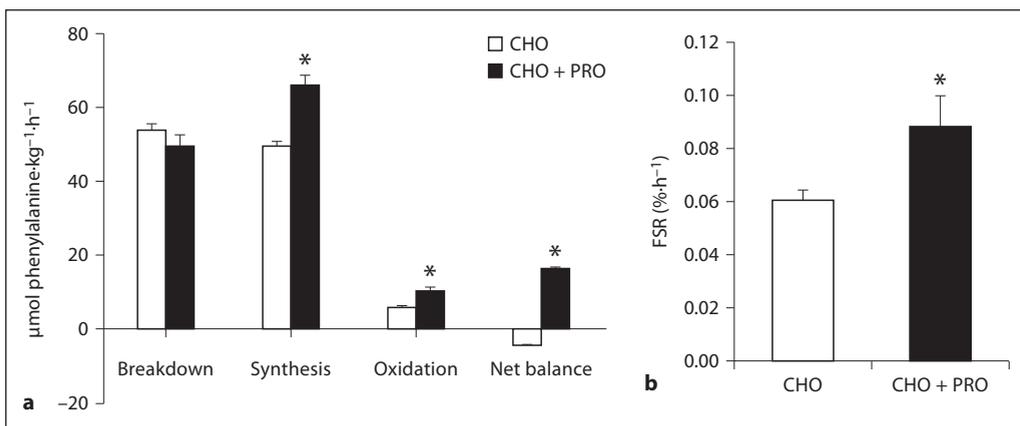


Fig. 2. Protein ingestion prior to and during resistance type exercise stimulates muscle protein synthesis during exercise. **a** Whole-body protein breakdown, synthesis, and oxidation rate as well as net protein balance. **b** FSR of mixed muscle protein during exercise conditions following carbohydrate (CHO) or CHO plus protein (CHO + PRO) ingestion. Values represent means \pm SEM. Asterisk denotes significant difference vs. CHO. Figure reproduced from Beelen et al. [36].

exercise [36]. It was speculated that the observed impact of protein coingestion on mixed muscle protein synthesis during exercise is restricted to intermittent, resistance type exercise activities [36]. It remains to be determined if protein ingestion before and/or during exercise also increases muscle protein synthesis during more continuous, endurance type, exercise. Preliminary findings in our lab seem to indicate that even during moderate intensity endurance type exercise muscle protein synthesis rates are stimulated in the working muscle by protein coingestion prior to and during exercise [unpubl. obs.]. More work is needed to address the relevance of the potential to stimulate muscle protein synthesis during as opposed to only after exercise.

Acute versus Long-Term Anabolic Response

Sports nutrition research has traditionally focused on the acute ergogenic properties of various nutritional compounds and/or products. However, the importance of chronic nutritional manipulation to augment the adaptive response to exercise training is receiving increasing attention. The applicability of specific nutritional interventions to improve post-exercise recovery represents a major factor allowing the athlete to maintain performance capacity as well as to improve the adaptive response to more prolonged exercise training. In this regard, more studies are warranted to assess the adaptive response to an acute

bout and/or successive bouts of exercise in a setting representative of real-life conditions. Most recovery studies have assessed the impact of nutritional intervention on muscle protein synthesis following a single bout of exercise performed in an overnight fasted state. The latter is not representative of exercise training or competition in which athletes generally practice standard pre-competition dietary guidelines. In addition, most recreational athletes exercise in the evening and have dinner before or after exercise training. In this respect, the benefits of post-exercise nutrition remain largely uninvestigated.

What is the impact of protein and/or amino acid ingestion following exercise on subsequent overnight recovery? For obvious methodological considerations post-exercise muscle reconditioning has hardly been studied during overnight sleep. Recently, we evaluated the impact of exercise performed in the evening on muscle protein synthesis during subsequent overnight recovery [37]. We observed an increase in muscle protein synthesis during the first few hours of post-exercise recovery when protein was being ingested. However, muscle protein synthesis rates during subsequent overnight sleep were unexpectedly low, with values being even lower than most basal, postabsorptive values. Clearly, many people misinterpret the outcome of classic studies like Phillips et al. [2] by suggesting that the post-exercise increase in muscle protein synthesis rate persists for up to 48 h. In accordance, Moore et al. [38] recently reported much greater post-exercise muscle protein synthesis rates after 3 compared with 5 h of post-exercise recovery in the fed state. Clearly, though post-exercise protein ingestion stimulates muscle protein synthesis during the acute stages of post-exercise recovery, these muscle protein synthesis rates are not maintained during subsequent overnight recovery and/or other conditions where amino acid availability is particularly low. Clearly, more research is required to determine the impact of nutrition on post-exercise overnight recovery.

So far, most work in the field aims to establish the impact of nutritional intervention on muscle protein accretion during the acute stages of post-exercise recovery. However, it should be noted that dietary interventions that optimize post-exercise muscle protein accretion do not necessarily translate into a more successful skeletal muscle adaptive response following more prolonged exercise training. Though a discussion on this topic would be beyond the scope of this review, it is evident that numerous intrinsic and extrinsic factors are responsible for orchestrating the long-term skeletal muscle adaptive response to exercise training, and we need to establish the impact of nutrition on these various processes. Specifically designed sports nutrition will have a major impact on optimizing the more prolonged adaptive response to one or several successive bouts of exercise. With regard to the application of protein and/or amino acids, it seems evident that differences in amino acid composition and specific differences in digestion and absorption kinetics would be of great relevance here. As a consequence, more specific designer proteins or protein mixtures will be defined and applied in more individualized sports recovery nutrition, with

specificity regarding the type, intensity, duration, and frequency of exercise and optimized for the concomitant priorities set for post-exercise recovery and subsequent muscle tissue reconditioning.

Conclusion

Protein ingestion following either resistance and/or endurance type exercise activities can be used as an effective nutritional strategy to inhibit protein breakdown, stimulate muscle protein synthesis and, as such, augment net muscle protein accretion. The latter has been suggested to lead to a more efficient adaptive response to each successive exercise bout, resulting in improved muscle tissue reconditioning. Whey protein seems most effective in stimulating muscle protein synthesis during acute post-exercise recovery: this is attributed to its rapid digestion and absorption kinetics and specific amino acid composition. About 20 g protein should be provided during and/or immediately after each exercise bout to allow maximum post-exercise muscle protein synthesis rates. Coingestion of large amounts of carbohydrate or free leucine do not further augment post-exercise muscle protein synthesis rates when ample protein is already ingested. Most research has assessed the impact of nutrition on acute post-exercise recovery, with exercise being performed in the morning following an overnight fast. The latter is hardly in line with normal everyday practice, where exercise is generally performed in the evening merely a few hours after the last meal. Future research should focus on the relevance of the acute anabolic response following exercise to optimize the skeletal muscle adaptive response to more prolonged exercise training.

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Discussion

Dr. Gibala: Relatively few studies have measured muscle protein breakdown (MPB) as it is technically difficult, but in the big picture how much are we missing by not measuring FBR simultaneously with muscle protein synthesis (MPS)? The consensus view is that MPS is the main regulated variable but the different proteins and amino acids can differentially effect the insulin response. What is your view on that?

Dr. van Loon: I think we are missing a significant piece of the puzzle, but as you note there are methodological difficulties, and we cannot accurately measure MPB. A second consideration is that the acute muscle protein anabolic response to exercise and/or food intake may not necessarily reflect what happens over the long term, and chronic transient changes in MPB may contribute considerably to the more prolonged adaptive response to exercise. I feel that protein breakdown is of even greater relevance over the first few days following exercise to allow the reconditioning process to occur. If we look at satellite cell activation and differentiation we see a large number of satellite cells appearing in skeletal muscle tissue 1–2 days after exercise. A few days later they have disappeared again, which implies that massive muscle reconditioning has occurred over these days. So, what is happening in that phase? That skeletal muscle adaptive response that occurs between 4 h and 5 days following exercise has not yet received much attention.

Dr. Gibala: Another methodological issue relates to the determination of muscle blood flow. This is also technically difficult to measure, but relatively small differences in flow can have considerable impact on determinations of muscle protein turnover. An

example is the paper by Kevin Tipton that received a lot of attention and suggested that protein feeding prior to exercise stimulated a greater rise in MPS, but this was largely attributable to the blood flow measures, if I recall.

Dr. van Loon: The delivery of the amino acids to the muscle is a key factor driving the muscle protein synthetic response to exercise and nutrition. The postprandial rise in circulating insulin stimulates muscle perfusion and, therefore drives the postprandial muscle protein synthetic response. Tipton has reported that protein ingestion prior to exercise may further augment muscle protein synthesis rates during post-exercise recovery. The latter was explained by the fact that protein ingestion prior to exercise allows the ingested amino acids to be available immediately following cessation of exercise. In contrast, when protein is ingested after exercise, it takes about 30 min for the ingested amino acids to become available. More recently, we showed that muscle protein synthesis rates can already be increased during exercise when protein is provided prior to exercise. The latter further increases the window of opportunity during which protein synthesis is increased following exercise. In short, there are various reasons why it might be advantageous to provide protein prior to and/or during exercise. However, the latter should be investigated in a condition where exercise is performed in a postprandial, as opposed to a postabsorptive, state. This would also be of more practical relevance, as no athlete will compete without having breakfast in the morning.

Dr. Gibala: Many different proteins are synthesized inside skeletal muscle. In the interest of time you were not able to get into the issue of protein subfraction responses, but there is some evidence that different fractions (e.g. mitochondrial, myofibrillar) do not respond in the same manner to a given exercise stimuli. Could you comment on this?

Dr. van Loon: It is obvious that different types of exercise affect the synthesis rates of different sets of proteins. Endurance type exercise will have a greater impact on the synthesis rate of mitochondrial proteins, whereas resistance type exercise activity will strongly stimulate the synthesis of myofibrillar protein. Of course, this is mainly driven by the type of exercise that is performed (i.e. endurance versus more resistance type exercise) and not by the timing, type or dosing regimen of protein intake.

Dr. Maughan: You said no athlete starts their training day without breakfast, but many ultra-distance runners won't eat breakfast before they race because they figure that 100 or 200 g of carbohydrate won't make much difference over 10–12 h of exercise. I suspect there will be a few triathletes on race day who take no food before the race. Certainly, some runners and also many swimmers will do at least some early morning training sessions in the fasted state.

Dr. van Loon: Some athletes train and/or compete in a fasted state. However, to optimize the acute muscle protein synthetic response during and/or after exercise, dietary protein is required. How this translates in a more prolonged adaptation to exercise training remains to be assessed.

Dr. Hawley: The protein literature suggests that an absolute amount of protein (20–25 g) is sufficient to maximally stimulate protein synthesis for individuals with a wide range of body masses. But will the needs of a 100-kg person be the same as someone with a 50-kg body mass and less muscle mass? Please explain how that can be.

Dr. van Loon: The amount of muscle tissue that was actually recruited during the exercise session will likely be the most important factor determining the optimum amount of dietary protein that is needed following exercise. However, at this point we

can only say that 20 g of protein appears sufficient to maximize MPS during acute post-exercise recovery in young, lean recreational athletes.

Dr. Hawley: Is that because the studies that have been conducted so far have not systematically compared the response of individuals who range in body size, e.g. a 120-kg man versus a 50-kg woman?

Dr. van Loon: Much of the work is based on men with a body mass of around 85 kg, although the range varies. As far as I know, there is presently only one study that has assessed a dose-response relationship between dietary protein ingestion during acute post-exercise recovery. More work is needed to assess the exact dose of protein that would be needed to optimize the (acute) muscle protein synthetic response to exercise in different populations.

Dr. Phillips: I want to ask about the pre-exercise provision of protein in the Tipton study. Can you explain to me why the authors were unable to reproduce these data in a subsequent study?

Dr. van Loon: I do not know. I can only state that we have recently shown that at least part of the effect that was reported by Tipton's first study is attributed to higher muscle protein synthesis rates during exercise following protein ingestion prior to, as opposed to after, exercise.

Dr. Phillips: Are there differences in the post-exercise muscle protein turnover response to different dietary proteins, i.e. whey versus casein versus soy? There is evidence that whey proteins seem most effective in stimulating muscle protein synthesis during acute post-exercise recovery, and this is attributed to its rapid digestion and absorption kinetics and specific amino acid composition.

Dr. van Loon: I think it depends on when you ingest the different proteins. Digestion and absorption kinetics are important, but if you ingest it prior to exercise then digestion and absorption are of less relevance. If you ingest casein only following exercise it will take at least 30–45 min before there is ample amino acid available in the blood. If you ingest it after exercise, it is probably best to take a protein hydrolysate or whey. If you ingest casein hydrolysate following exercise, it will likely be just as good as whey, so the digestion and absorption kinetics are important when you want the amino acids at a certain point of time, and that's preferably even before exercise.

Dr. Phillips: With regard to the effectiveness of leucine, in the manuscript you use the terminology when ample protein is consumed or adequate protein is consumed. What if a suboptimal protein dose is ingested, e.g. 10 g, and I were to add leucine together to the 20 g level that we think is important to saturate the MPS response. Could it then become ergogenic or give you back the same response?

Dr. van Loon: I deliberately used the word ample because I would like some room for the possibility of leucine to attenuate post-exercise muscle protein breakdown. However, when you provide such large amounts of dietary protein, it's more than sufficient to inhibit protein breakdown, at least on a whole body level. Coming back to the leucine, following exercise I don't see any value for additional leucine ingestion. Many authors have suggested that it has an advantage in the elderly in the postabsorptive phase. But during post-exercise recovery, we have found no evidence that additional leucine ingestion is of any benefit to the muscle protein synthetic response to exercise. Moreover, we have not been able to detect any clinical benefits of leucine supplementation in healthy elderly and elderly diabetics following 3 and 6 months of intervention, respectively. So, we can't reproduce the acute effects of leucine in elderly people in long-

term interventions. That is also one of the reasons why I question the validity of extrapolating findings from acute studies to predict the long-term effect of that regimen.

Dr. Zemel: I would like to explore the issues that Dr. Phillips raised regarding dose and type. Just to make sure I understand, if you do a dose-response curve, you get a plateau around 20 g of dietary protein after exercise, with 10 g being suboptimal. Also, if you look at different sources of protein, you see that soy tends not to be as effective as milk despite having an identical amount of protein, so the differences must be attributable to something, whether it is digestion, absorption or amino acid composition. However, the rest of the experiments that you showed were performed at this optimum level of protein and then superimposed with or without carbohydrate, with or without leucine, etc. So, I am still having trouble reconciling the difference between milk and soy if it's not leucine or perhaps an insulin response or a combination of factors. Can you comment on that?

Dr. van Loon: We have very limited data regarding the effect of different proteins ingested after exercise, so I have to rely on data from the postabsorptive phase where there is more evidence regarding the response to the ingestion of different doses and types of protein. We have compared casein with casein hydrolysate having exactly the same amino acid composition and with whey which has a higher leucine content but the same digestion and absorption kinetics as the casein hydrolysate. Whey is most effective for stimulating postprandial muscle protein synthesis, but following exercise I don't believe that small differences in the anabolic response to food intake are of much importance. The latter is explained by the fact that the stimulating effect of exercise is much stronger than the impact of small differences on insulin responses or the small amino acid responses that you see in the postprandial phase. And that's also evident from our recent work that shows that 30% more of the ingested protein is used for the de novo muscle protein synthesis when exercise is performed prior to protein ingestion. After exercise, we should be concerned about getting ample protein in time; the type of protein is likely to be of less relevance at that stage.

Dr. Zemel: I am puzzled about the acute protein signaling responses and how this correlates with long-term adaptation. You showed the only study where there is any relationship between the acute protein synthesis response and the long-term effect on hypertrophy. How good is protein synthesis after exercise as a marker of hypertrophy?

Dr. van Loon: That's a great question, and I can only say that so far every study shows that if you ingest protein following exercise you have a tremendous increase in net balance and a tremendous increase in protein synthesis. However, I would challenge everybody here to look through the literature and find studies that show that protein supplementation following exercise promotes net muscle protein gain more than not supplementing additional protein. The evidence for the additional benefits of protein supplementation to augment muscle hypertrophy is not very strong.

Dr. Maughan: I would go further and say that we need to be cautious in how we interpret an increase in protein synthesis in response to endurance training: endurance training does not result in increased muscle mass. The net change in muscle total protein content is very small and may even be negative. High loads with few repetitions will give you the biggest increases in muscle mass and best strength adaptation. How can we interpret the changes in protein synthesis and breakdown and how do the acute responses to a single exercise bout translate into a long-term training response?

Dr. van Loon: Differences in the impact of exercise and/or nutrition on the acute muscle protein synthetic response do not necessarily translate into long-term adaptive responses following more prolonged intervention. For example, though leucine coingestion has been reported to augment the postprandial muscle protein synthetic response to food intake, we could not detect any measurable changes in muscle mass following 6 months of leucine supplementation in elderly men. The latter is not surprising as there are numerous factors that modulate the long-term adaptive response to exercise and/or nutritional intervention. I would like to invite Dr. Phillips into this discussion since he has recent data showing differences in the acute muscle protein synthetic response to translate successfully into differences in the long-term adaptive response.

Dr. Phillips: We have data in which we tested two paradigms for contraction, 1 set versus 3 sets. We published the acute protein synthetic response, and it clearly showed that it was superior in 3 sets versus 1 set. We have also data that show when you lift at 30% of your maximum voluntary contraction to fatigue you get an equivalent stimulation of protein synthesis as if you were lifting at 90%. And so we have done a training study comparing those two different contraction paradigms, 1 set versus 3 sets or 3 sets of 30% versus work-matched 90%, and the long-term hypertrophy response is predicted by the acute response in both instances.

Dr. Zemel: Are the long-term muscular responses accompanied by similar performance changes?

Dr. Phillips: That's a rather different thing, performance, and we have known for a long time, for example, that the way you train for muscle mass is not necessarily the way you train to optimize performance, especially power.

Dr. Maughan: We should also remember that training does not only affect the muscles: there's a neural component too, and that may account for some of the differences.

Dr. Haschke: I have another comment on protein quality. Most studies are done using common protein sources such as milk, egg, whey, casein and soy, but we don't know whether these are the best proteins under certain circumstances. Using an example from intensive care medicine, it is possible to spike a basic milk product with certain amino acids and then look which amino acid becomes limiting during synthesis. This has allowed the design of products which will come to the market quite soon. The same is true for example for premature babies. The premature baby doubles its body weight in 3 months from 1 to 2 kg. This is a much faster synthesis of protein than occurs in adult life, and we always thought that breast milk would be best. It's not, it serves as the best basis, but if you spike it with amino acids, the infants grow much faster. So, my question is whether you could further improve the protein quality?

Dr. van Loon: I still believe you can improve protein quality and the anabolic response to protein ingestion when it's not in a post-exercise phase. As I mentioned, the exercise response overwhelms almost everything. However, the postprandial muscle protein synthetic response may be further improved by changing dietary protein source, macronutrient composition of a meal, and/or by ingesting specific amino acids or amino acid combinations. But so far, at least in healthy or diabetic elderly, we don't see this being translated to clinical benefits after long-term intervention. However, with intensive care unit patients we see a lot of things happening with infusions over time to attenuate muscle protein breakdown, but in normal consumer nutrition I don't see much evidence for that. Of course, there are always interesting clues from basic nutrition research, e.g.

somebody finds an anabolic response to leucine and then everybody starts working on leucine but many other amino acids tend to get neglected. So, we started screening for the anabolic properties of different amino acids and amino acid combinations. We observed that the rise in the plasma concentrations of these amino acids and/or amino acid combinations shows strong correlations with the muscle protein synthetic response. So, there might be more.

Dr. Maughan: I used to speak to some body builders who told me they would set the alarm clock for every 2 h during the night and get up and have a dose of protein and go back to sleep. Are they doing the right thing or would they be better just getting some sleep?

Dr. van Loon: I think they are doing the right thing, and we have done some work looking at night-time muscle protein synthesis and whether it is limited by the availability of protein. A first study has shown that protein synthesis rates during the night are very low. It has been suggested that amino acids are released from the gut throughout the night, but we didn't find any evidence for that. So, what happens if you provide protein like the body builders do by simply taking a handful of branched chain amino acids and a large protein shake at 3 o'clock in the morning? There might be some rationale for that practice. However, waking up during the night to ingest protein might cause more problems than its proposed benefits, and there are other options to provide protein in a more sustained manner throughout the night.

Dr. Baar: Can I follow up on that? Some studies have looked at long-term amino acid provision and suggested that there is either an insulin resistance or a negative feedback that could have a negative effect and attenuate the response to amino acids. If you take protein every couple of hours or if you maintain a constant high level of amino acids in another way, might that have a negative effect on muscle protein synthesis?

Dr. van Loon: It depends completely on which study you look at. There are also studies that compared spread feeding versus pulse feeding, showing that pulse feeding has a greater anabolic response than spread feeding because nitrogen retention was shown to be much higher. However, these data are based on whole-body nitrogen retention and, as such, might not be representative of the muscle tissue. However, from the other point of view, in many clinical conditions people are being provided with parenteral nutrition constantly, and I am certain that if you apply pulse feeding you don't see the anabolic resistance or the insulin resistance that occurs with continuous feeding. Overall studies suggest that a temporary increase in amino acid provision is the best strategy to promote the anabolic response. This seems superior to continual provision at a more constant level.

Dr. Hawley: I just want to bring it back to the practical, bring it back to the theme of the conference 'Triggers for Adaptation'. I am intrigued with the endurance training response to protein. Should we recommend that instead of just focusing on increasing carbohydrate availability for glycogen resynthesis, endurance athletes should consume 20 g of protein as well?

Dr. van Loon: There is so much that we don't know about. How much protein should be ingested to optimize the acute muscle protein synthetic response to endurance type exercise, and how relevant is it? What kinds of proteins are being synthesized during endurance type exercise? If you believe it is important to stimulate muscle protein synthesis during or immediately after exercise, you should ingest about 20 g of dietary protein. If you do not provide sufficient dietary protein to allow the protein synthesis to

occur, you might impair the subsequent adaptive response. But, whether this would have any consequences for the more long-term adaptive response remains to be established. What's the minimum amount of protein required? It may be less than what we are presently providing.

Dr. Gibala: We need more of these long-term studies. They are difficult to do, but that's really what needs to be done.

Dr. Maughan: Measuring performance outcomes is a lot easier to do, and maybe we should do this first: if there is no performance outcome, you don't need to look for a mechanism.

