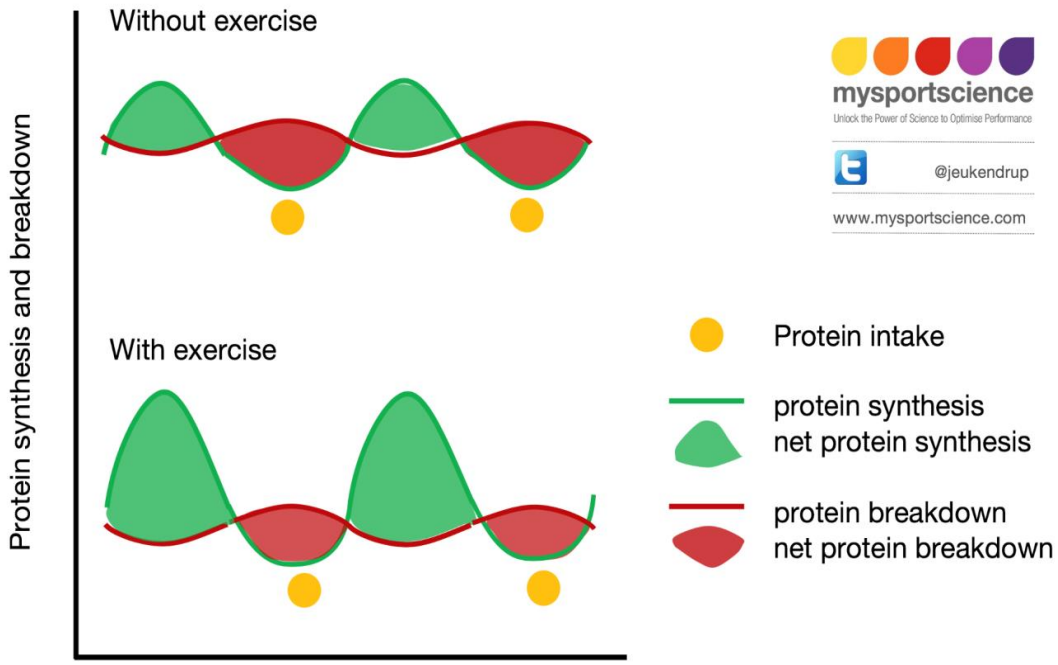
3.2 Optimising protein synthesis

3.2.1 Optimising Protein Synthesis

In resting conditions, higher amino acid concentrations in plasma have a stimulatory effect on protein synthesis (Bennet et al. 1990, 1991). Immediately after exercise this effect of increased availability of amino acids on protein synthesis is exaggerated compared with the resting condition (Biolo et al. 1997). Amino acids and exercise thus seem to have an additive effect on net protein synthesis. Note, however, that in these early studies, amino acids were infused and plasma amino acid concentrations were elevated to extremely high levels (much higher than those observed after oral ingestion of amino acid mixtures or protein). Intravenous infusion is not a practical method for athletes, and infused amino acids bypass the liver. The liver normally extracts over 20% and up to 90% of all amino acids after absorption from the gut (a phenomenon known as first-pass splanchnic extraction). Therefore, whether similar effects are to be expected after oral ingestion of amino acids is not clear. A follow-up study investigated this question (Tipton et al. 1999). In this study, a relatively large amount of amino acids was ingested after resistance exercise. Following exercise, muscle protein balance was negative after placebo ingestion, but when amino acids were ingested, the net balance was positive mainly because of increased muscle protein synthesis. From this study and a limited number of other studies, one can conclude that ingestion of amino acids or protein after exercise enhances net protein synthesis. (Jeukendrup & Gleeson, 2018).

Figure 4: Protein synthesis and breakdown are ongoing processes



Source: Jeukendrup, from mysportscience.com. Protein synthesis and breakdown are ongoing processes. After feeding, protein synthesis is stimulated. After a few hours, this returns back to net protein breakdown so that the whole body is in protein balance. With exercise and feeding, protein synthesis increases more than protein breakdown, and net protein accretion occurs. From mysportscience.com.

Adaptation is the longer term process that takes place when training stress produces signals that initiate a cascade of reactions ultimately resulting in increases in net protein synthesis. Over time, the repeated small increases in protein synthesis of specific proteins will result in improved function. With nutrition we can influence the signals, and potentially enhance them, so that there is more stimulus for protein synthesis (this will be discussed in the sections on periodised nutrition). The process of protein synthesis, however, is highly dependent on nutrition itself and especially protein intake, which is also important. Here we will discuss how we can enhance net muscle protein synthesis. We will discuss the optimal amount of protein, the timing of intake, the type of protein that should be ingested, and whether there are other nutrients that improve or impair the process and so on.

Amount of Protein

Let's start with an important fact: most athletes exceed even the highest recommendations for protein intake on a daily basis. This makes the discussion about daily protein requirements mostly an academic discussion with little practical relevance (Tipton & Witard, 2007). However, the questions about the amount of protein in each meal required to optimize protein synthesis is still highly relevant (this point will also be discussed under "timing").

One of the first studies by Bohe Low, Wolfe, and Rennie (2003) demonstrated that extracellular, and not intracellular, amino acids concentrations are important for the rate of protein synthesis. Protein ingestion increases extracellular amino acid concentrations, but there appears to be a plateau above which protein synthesis is not further stimulated. This became the basis of a number of excellent studies to see what the optimal amount of protein is in a single meal. In other words: how much protein needs to be ingested before this plateau in protein synthesis is reached? An often quoted study by Moore et al. (Moore et al., 2009) compared the intake of 5, 10, 20 and 40 grams of protein after resistance exercise. Protein synthesis was measured over the following 4 hours. The study suggested that 20 grams was better than 10 grams, and 10 grams was better than 5 grams. However, 40 grams of protein had very similar effects to 20 grams. On the basis of these results, the recommendation is often to ingest around 20 grams of protein per meal. More may not be needed. Other studies have found similar results: 20 to 25 g of protein would be required, or 8 to 10 g of essential amino acids to optimise protein synthesis (Moore et al., 2009; Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009; Witard et al., 2014), regardless of training status (Witard et al., 2014). Ingesting larger amounts of protein does not further stimulate muscle protein synthesis (Moore et al., 2009; Witard et al., 2014). These studies were performed using whey protein as the source protein, but similar results were obtained "using whole food (lean minced beef) in young men and women (Symons et al., 2009). In this study a moderate (~ 30 g protein) amount was just as effective as a high (~ 90 g protein) amount for stimulating muscle protein synthesis" (Symons et al., 2009). (Jeukendrup, 2016a, https://bit.ly/2Dx2KAo). An excess of protein will result in higher rates of protein oxidation (Moore et al. 2009; Witard, 2014) and an increase of urea production (Witard et al., 2014). These findings together indicate that there is a limit to the rate at which amino acids can be used for muscle protein synthesis. Sometimes this is referred to as the "muscle full effect" (Atherton et al., 2010) (Jeukendrup, 2016a, https://bit.ly/2Dx2KAo).

Moore et al. (2015) performed a regression analysis of all existing dose response studies and came up with an estimate for protein dose that is expressed

> per kilogram of body weight. It was concluded that the dose of protein beyond which there was no further increase in muscle protein synthesis in young men was 0.325 g/kg bw per meal. To account for inter-individual variability they proposed the addition of two standard deviations to this estimate, yielding an optimal dose of protein of 0.4 g/kg bw per meal" (Jeukendrup, 2016a, https://bit.ly/2Dx2KAo).

So far, this message seems consistent and straightforward and has also been universally adopted in sports.

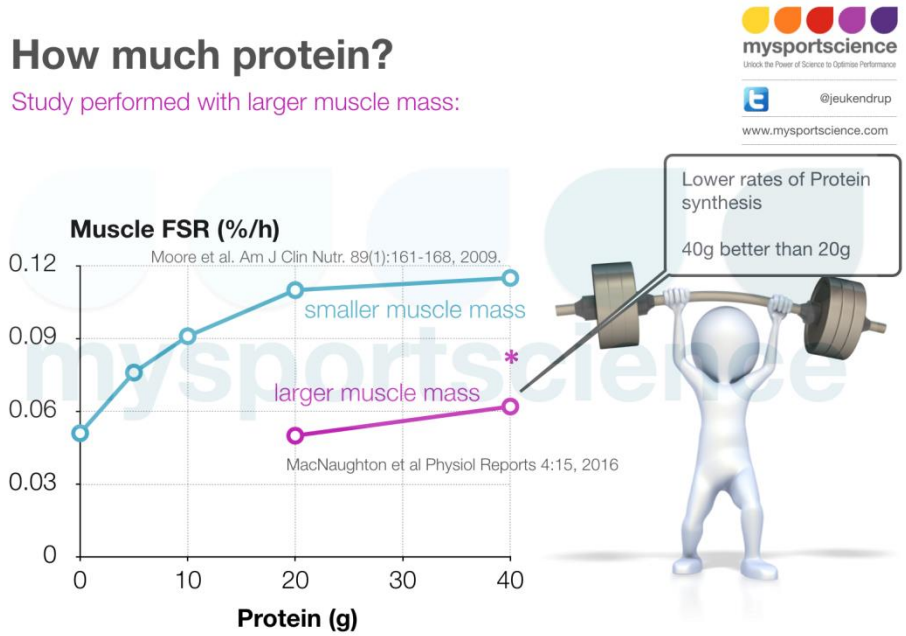
However, it is also important to be critical and keep in mind how these results were obtained. The studies, although carefully conducted with the latest techniques extensively validated, have some limitations. They are usually performed with resistance exercise in a small muscle group, and muscle biopsies are taken from this small muscle group (for example m. vastus lateralis) before and after a 4-5-hour period. Protein synthesis is then calculated from the amino acids incorporated into this muscle group. Muscle groups that have been inactive will see little or no amino acid incorporation in this period.

Recently, a study by Macnaughton et al. (Macnaughton et al., 2016) argued that when a larger muscle mass is involved (as would be the case for most athletes and most practical situations), larger amounts of protein may be required to achieve the optimal rates of protein synthesis. Their subjects performed resistance exercise with all main muscle groups and then the same measurements were performed. There were two main observations:

> The first one was that protein synthesis was lower in this study than in previous studies. This is possibly because the same amount of protein now had to be shared with a larger amount of muscle. Secondly, they observed that ingesting 40 g of protein resulted in greater protein synthesis than 20 g of protein. This is of course in contrast to previous studies. The authors discuss that perhaps when larger muscle groups are trained, more protein is required. (Jeukendrup, 2016a, https://bit.ly/2Dx2KAo).

Because this is only a single study, we will need to see how the guidelines develop in the future, but it is possible that the guidelines will have to be adapted upwards if more studies show that with training of large muscle groups higher protein intakes will provide better results (Jeukendrup, 2016a).

Figure 5: How much protein is needed to increase muscle FSR?



Muscle fractional synthetic rate (FSR) in two studies (Jeukendrup A. E., 2016a). In blue Moore et al. (Moore et al., 2009) and in purple Macnaughton et al. (Macnaughton et al., 2016).
Source: Jeukendrup, 2016a, https://bit.ly/2Dx2KAo

When a smaller muscle mass was used FSR was higher but plateaued after about 20 grams of ingested protein. Training a larger muscle mass resulted in lower FSR values but doubling protein intake modestly further increased FSR. (Jeukendrup & Gleeson, 2018).

Types of Protein

Not all proteins are the same. The amino acid composition and the digestive properties of proteins can vary enormously and this can influence the delivery of amino acids and protein synthesis. Often proteins are classified as fast or slow (this refers to the speed at which proteins can be digested and their amino acids can be absorbed). Boirie and colleagues (Boirie et al., 1997) were probably the first to compare fast and slow proteins. They compared whey protein with casein or soy protein, which are the three most commonly consumed isolated protein sources. Both casein and whey are derived from whole milk, whereas soy is derived from plants.

> About 80% of milk protein is casein and the remaining 20% is whey. Whey and soy are fast proteins because the body digests and absorbs them relatively rapidly, resulting in a rapid increase in amino acid concentrations in the blood. Casein, on the other hand, is a slow protein: it takes longer to break down and absorb. (Jeukendrup A., & Gleeson, 2018).

Casein tends to clot in the stomach, slowing gastric emptying. Because of the differences in composition and delivery speed, whey, soy, and casein vary in their rates of protein synthesis.

In a series of studies on weightlifters at the lab of Professor Stuart Phillips, it was found that milk protein results in greater rates of protein synthesis and a greater increase in muscle mass compared with soy protein (Hartman, 2007; Wilkinson, 2007). It must be noted, however, that a protein that is optimal in one condition is not necessarily optimal in all conditions.

Fast proteins such as whey and soy induce a larger rise in muscle protein synthesis, but protein synthesis rates also drop more rapidly than casein (Tang et al., 2009). Whole-body protein synthesis is stimulated more with whey protein whereas whole-body protein breakdown is suppressed with ingestion of casein (Boirie et al., 1997). After ingestion of isolated casein, soy, and whey protein (all providing 10 g of essential amino acids), the rise of muscle protein synthesis over the following 3 h was the greatest with whey protein (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). Interestingly, soy protein had higher muscle protein synthesis than casein (Tang et al., 2009). Thus, it appears that the most effective protein source is whey, followed by soy and casein respectively.

One of the most important factors that determines the anabolic response is the amount of the amino acid leucine. Leucine is both a building block for protein as well as a signalling molecule to stimulate translation–initiation pathways, resulting in increased rates of protein synthesis (K. D. Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999). Without going to deep into the mechanisms: leucine stimulates the mechanistic target of rapamycin complex-1 (mTOR), a key signalling protein, and triggers a rise in muscle protein synthesis (McGlory, Devries, & Phillips, 2017). Therefore, ingested proteins with a high leucine content would be advantageous in triggering a rise in muscle protein synthesis. Thus, protein quality (reflected in leucine content, essential amino acid content and protein digestibility) has an impact on changes in muscle protein synthesis which and could ultimately affect skeletal muscle mass. Whey protein contains 10.0 g of leucine per 100 g which is more than more leucine per gram than casein (8.2 g/100g) or soy (5.9 g/100g). Foods that contain a relatively high content of leucine are shown in Table 2. Following ingestion of protein with a relatively high leucine content such as whey, the rapid and relatively large aminoacidemia may be increasing essential amino acid and specifically leucine delivery to the muscle, to a certain threshold that triggers a stimulation of muscle protein synthesis and the associated anabolic pathways.

(Jeukendrup, & Gleeson, 2018).

> Participants given 25 g of whey protein or only a quarter of this amount of whey (6.25 g) combined with 5 g leucine showed an increased MPS at rest and following a bout of resistance exercise to a similar extent despite the four-fold lower protein dose (Churchward-Venne et al., 2014). It appears that the leucinemia (and quite possibly the ensuing intramuscular leucine concentration) is the driver of the muscle protein synthesic response and thus" the anabolic process during recovery from exercise. (Morton, McGlory, & Phillips, 2015, https://bit.ly/2DKCSB3).

The leucine "threshold" amount to "trigger" the stimulation of muscle protein synthesis appears to be ~ 3 g of leucine per meal (Churchward-Venne et al., 2014), which may be the main factor determining the recommended amount of protein per meal of ~ 25 g per meal or ~ 0.4 g/kg bw per meal mentioned earlier.

> It should also be noted that the addition of the two other BCAAS, isoleucine and valine, does not further improve muscle protein synthesis (Churchward-Venne et al., 2014) and so it makes sense to only supplement with leucine (or consume dietary proteins that are known to have a high leucine content) as all three BCAAs share the same transporter and consumption of a mixture of BCAAs would be expected to result in antagonism for uptake from the gut and into the muscle and thus be not as effective as leucine alone in stimulating muscle protein synthesis. Despite the continued popularity of BCAA supplements among the athletic population there is very little evidence for their efficacy in promoting muscle protein synthesis or gains in muscle mass and current advice is to ingest intact dietary proteins that have a high leucine content and are rapidly digested. The slower and more protracted aminoacidemia accompanying the ingestion of casein, may be more effective at sustaining muscle protein synthesis and possibly at attenuating negative net protein balance during sleep or over longer periods of time but this requires confirmation by future studies. (Jeukendrup & Gleeson, 2018).

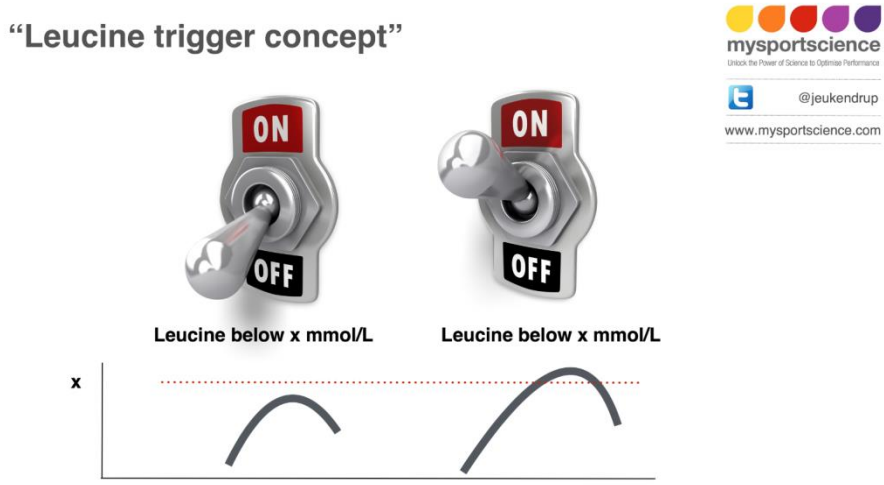
Table 2: Leucine Content of Various Foods

Food	Amount needed to supply 3 g Leucine	Leucine content (g/100kcal)
Whey Protein Isolate	25 g	2.90
Soy Protein Isolate	37 g	2.00
Greek Yogurt	300 g	1.75
Chicken Breast	170 g	1.70
Lean beef	170 g	1.30
Cheese	105 g	1.09
Egg	4 large eggs	0.94
Skimmed Milk	900 ml	0.93
Kidney Beans	525 g	0.65
Tofu	600 g	0.44
Raw Peanuts	180 g	0.29
Bread	770 g (14 slices)	0.07

Source: Jeukendrup & Gleeson, 2018.

"The leucine trigger concept. More rapidly digested protein that contains a relatively high proportion of leucine (e.g., whey) is most effective in promoting increases in muscle protein synthesis" (Jeukendrup & Gleeson, 2018).

Figure 6: Graphical representation of the leucine trigger concept



Source: Jeukendrup, mysportscience.com. It is thought that leucine concentrations in plasma need to be above a certain threshold before leucine triggers protein synthesis through mTOR signalling. The concentration required is unknown, but it appears that ingestion of 2-3 grams of leucine triggers protein synthesis.

Protein quality is the protein digestibility corrected amino acid score (PDCAAS) which is a method of evaluating the protein quality based on the amino acid requirements of and their ability to digest it. The PDCAAS rating was adopted by the US Food and Drug Administration (FDA) and the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) in 1993 as "the preferred 'best'" method to determine protein quality. The maximum score of 1.0 means that after digestion of the protein, it provides, per unit of protein, 100% or more of the essential amino acids required. (Schaafsma, 2000, https://bit.ly/2DPdwCO)

However, leucine content is probably more important than the PDCAAS of a protein source.

Timing of Protein Intake

The timing of food intake after exercise is important to the balance between protein synthesis and protein degradation. Studies have investigated protein ingestion immediately after exercise, delayed by 1 to 3 hours, or ingested before the exercise bout. In one study by Tipton et al. (Kevin D. Tipton et al., 2001) volunteers ingested 6 g of essential amino acids plus 35 g of carbohydrate immediately before the start and immediately after completion of an intense, leg resistance exercise bout. Amino acid uptake was greater when the nutrients were ingested before the exercise bout compared with afterwards

Thus, these data suggested that the anabolic response to exercise and amino acid and carbohydrate ingestion might be greater with pre-exercise ingestion versus post-exercise whether this is immediately post-exercise, 1 h postexercise, or 3 h postexercise. It is possible, however, that the study design affected the results: these studies used essential amino acids. When the study was “repeated with whey protein (and no carbohydrate), the difference between feeding before a bout of resistance exercise and after was not evident” (K. D. Tipton et al., 2007).

These observed differences are likely related to the delivery of amino acids to the muscle. Free amino acids ingested before exercise may result in increased amino acid delivery

(because of increased blood flow to active muscles during the exercise session) and lead to superior amino acid uptake compared with amino acids ingested after exercise. (Jeukendrup & Gleeson, 2018).

Apart from the delivery of amino acids, another factor may determine the anabolic response. As discussed above, leucine can serve as a signalling molecule and stimulate translation-initiation pathways resulting in increased protein synthesis.

It has been known for some time that resistance exercise alone results in a long-lasting elevation in muscle protein synthesis for at least 48 h and muscle protein breakdown for 24 h (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997). This means that even in the basal fasted state, there is a subsequent increase in the turnover of muscle proteins. In this situation muscle protein breakdown will exceed muscle protein synthesis and thus net tissue protein loss occurs. Exercise essentially primes the muscle to be more responsive, in terms of an increased muscle protein synthesis response to aminoacidemia following ingestion of a protein containing meal. This increased sensitivity lasts for at least 24 h (Burd et al., 2011) and possibly up to 48 h but diminishes over time. Therefore, it is most advantageous to ingest protein and generate hyeraminoacidemia in the postexercise period. It has also been suggested that pre-exercise protein ingestion may also prime the system and offer some advantage over a post-exercise supplementation strategy. However, ingesting 20 g of protein either before or 1 h after 10 sets of knee extensor exercise resulted in similar rates of amino acid uptake (K. D. Tipton et al., 2007). In other studies there was no benefit shown with pre-exercise amino acid feeding (L. M. Burke et al., 2012; Fujita et al., 2009) so the general scientific consensus now is that it is optimal to ingest protein immediately following a bout of resistance exercise. Moreover, it is possible that a feeding-induced preexercise or during aminoacidemia may blunt the subsequent post-exercise muscle protein synthesis response to amino acids due to an overlap in the aminoacidemic responses and the "muscle full" effect (Atherton et al., 2010). A recent meta-analysis examining protein timing and hypertrophy concluded that the ingestion of a post-exercise protein supplement shortly after resistance exercise positively influenced hypertrophy (Schoenfeld, Aragon, & Krieger, 2013) but that the amount of total protein intake was the strongest predictor of muscular hypertrophy and that protein timing did

not influence hypertrophy. Nonetheless, sensible, practical advice is that athletes should consider the postexercise period as a time for rehydration (to restore fluid and electrolyte losses), refueling (to restore glycogen), repair (of damaged muscle fibers), and remodeling (adaptation to the training stimulus) known collectively as the "4Rs" and that the appropriate amounts of fluid, electrolytes, carbohydrate, and protein should be ingested in order to accomplish the goals defined by the 4Rs. (Jeukendrup & Gleeson, 2018).

The timing and amount of protein ingestion over the course of a day that is needed to optimize training adaptation is still a matter of debate. In an acute study, an “intermediate” pattern of whey protein ingestion (4 × 20 g every 3 h) throughout a 12-h recovery period following a bout of resistance exercise was found to be more effective than ingestion of large boluses (2 × 40 g every 6 h) or a pulse (8 × 10 g every 1.5 h) protocol for stimulating muscle protein synthesis (Areta et al., 2013). (Morton et al., 2015, https://bit.ly/2DKCSB3).

These results are in agreement with the "muscle full" effect, when amino acid delivery is sufficient (~ 20 g), amino acids are no longer used for muscle protein synthesis and are instead mostly oxidized (Atherton et al., 2010; Moore et al., 2009; Witard et al., 2014).

However, it should be noted that many studies examining the impact of protein feeding on muscle protein synthesis either infused amino acids or provided oral amino acid mixtures or protein a bolus form. In real life people usually eat meals composed of a mixture of macronutrients (i.e., protein, carbohydrate and fat containing meals). The macronutrient composition and form of meal intake may influence both the meal-induced rise in hyperaminoacidemia and subsequent muscle and whole body protein synthesis. Future studies need to examine the influence of mixed macronutrient meals on rates of muscle protein synthesis and muscle protein breakdown over longer periods of time (Jeukendrup & Gleeson, 2018).

Pre-sleep Protein

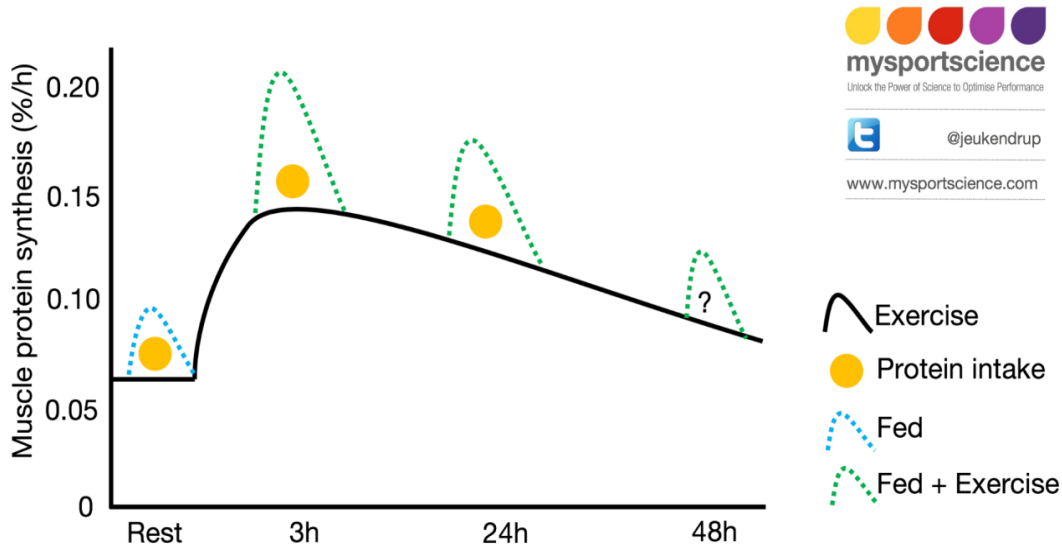
If we study protein synthesis and breakdown in humans over 24 h it becomes obvious that the protein turnover is low during sleep. This is because it is usually the only time of day that we are fasted for a longer period of time. There is no supply of amino acid and no leucine to turn on

protein synthesis. Therefore, researchers at Maastricht University suggested that if protein could be supplied during the night this could enhance overall protein synthesis and recovery. Initially, they studied this in the elderly, but a study by Res et al. (Res et al., 2012) in which healthy male subjects ingested 40 g of protein before bedtime showed that muscle protein synthesis was stimulated and net protein balance was improved overnight. The amount of protein ingested was large and not very practical. As a result, the study was followed up by another study. In this 12-week progressive resistance exercise training study "showed that a pre-sleep beverage containing 27.5 g protein, 15 g carbohydrate, and 0.1 g fat amplified muscle mass, muscle fiber area, and strength gains compared with a non-caloric placebo beverage" (Snijders et al., 2015, https://bit.ly/2r7KCpc). The critical reader, however, will note that the placebo control group in this study did not receive a protein supplement resulting in a 0.6 g/kg difference in total protein intakes. Consequently, it cannot be dismissed that the observed effects were due to the higher protein intake and not to the timing of intake. Nevertheless, the authors make a good case regarding why it may be more likely that the effects are a result of the timing rather than the amount.

> In summary, it seems that the timing of protein intake is an important variable to consider in optimizing skeletal muscle recovery and hypertrophy. It appears optimal to ingest protein in the postexercise period though the purported "anabolic window" for protein ingestion lasts at least 24 h. (Jeukendrup & Gleeson, 2018).

(~ 0.4 g.kg bw/meal) distributed throughout the day. Lastly, "ingesting larger doses of protein (~ 0.6 g/kg bw) before bedtime appears to augment both acute overnight muscle protein synthesis and chronic skeletal muscle adaptations" (Jeukendrup & Gleeson, 2018).

Figure 7: Protein Synthesis Stimulation After Resistance Training



Source: Adapted from Phillips 2013, https://bit.ly/2FRaekG. Resistance exercise stimulates a prolonged (~ 24-48 hour) increase in muscle protein synthesis. This increases the "anabolic potential" of protein ingestion. Consuming protein will always increase protein synthesis, but consuming it close to the exercise will result in greater increases. There are, thus, likely advantages of consuming protein during or soon after exercise.

Simultaneous Intake of Other Nutrients

Feeding a mixed diet not only provides substrates but also results in a favorable hormonal milieu for protein synthesis. Increased availability of glucose and amino acids also results in increased plasma insulin concentrations, which, in turn, may cause a reduction of protein breakdown and a small increase in protein synthesis. (Biolo, Williams, Fleming, & Wolfe, 1999, https://bit.ly/2S8RdeG).

Carbohydrate ingestion per se may not have an effect on protein synthesis after exercise. But carbohydrate ingestion elevates plasma insulin concentrations and thereby may reduce the breakdown of protein that normally occurs with resistance exercise. Insulin inhibits protein breakdown and also promotes amino acid (and glucose) uptake by some tissues including skeletal muscle. The combined ingestion of protein and carbohydrate seems to be preferred after exercise, at least for situations where the amount of protein ingested is quite small (most studies reporting this effect fed their subjects only about 6-12 g of protein or amino acids after exercise together with 30-100 g carbohydrate). The protein delivers the substrate (amino acids), and carbohydrate further increases the anabolic hormonal milieu required for net

protein synthesis.

In a study by Miller et al. (Miller, Tipton, Chinkes, Wolf, & Wolfe, 2003) volunteers performed leg resistance exercise and then ingested one of three drinks: (amino acids (AA, ~6 g) only , carbohydrate (CHO, ~35 g) only, or AA (~6 g) + CHO (~35 g) combined) at 1 and 2 hours postexercise. Total net uptake of phenylalanine across the leg over 3 hours was greatest in response to AA + CHO and least in CHO. Stimulation of net uptake in AA + CHO was due to increased muscle protein synthesis. In the control (CHO only) condition, a net protein breakdown was observed, confirming that hyperinsulinemia alone (i.e., in the absence of ingested amino acids) does not stimulate protein synthesis. These results suggest that the ingestion of a relatively small amount of amino acids with a larger amount of carbohydrate can increase net muscle protein synthesis in the hours after resistance exercise. Similar data is available for endurance exercise. Although these results are interesting, more recent studies indicate that the dose of amino acids was suboptimal for the maximum rate of post-exercise protein synthesis. When 20 g of amino acids or protein is ingested after exercise there is a larger increase in protein synthesis compared with 0 or 6 g and the addition of large amounts of carbohydrate to 20 g of protein does not further increase net protein synthesis (Koopman et al., 2007).

Fat also seems to have an effect on protein synthesis because whole milk seemed to give responses different from those of fat-free milk (Elliot, Cree, Sanford, Wolfe, & Tipton, 2006). After a bout of resistance exercise, subjects consumed either fat-free milk or whole milk. Both milk drinks stimulated protein synthesis, but the greatest effect was seen with whole milk. The explanation for this is not immediately clear, but it was suggested that the fat in the whole milk would delay the delivery of the amino acids and provide a more sustained supply of amino acids for protein synthesis. More research is needed to look into the effects of added fat to protein synthesis. In fact more research is needed to understand the effects of normal meals that contain carbohydrate, fibre, fat, protein and other nutrients. Most studies have been performed using pure forms of protein, usually in isolated form and not in food form. Therefore, we will need to understand the behaviour of protein when ingested in and with normal foods (Jeukendrup & Gleeson, 2018).

Practical Recommendations to Maximize Hypertrophy with Resistance Exercise Training and Nutrition

- Engage in regular training bouts of high load resistance training.
- Lifting heavier loads over longer durations stimulates greater improvements in muscular strength.
- Lift to the point of contraction failure.
- Maintain energy balance.
- "Consider the postexercise period as a time for rehydration (to restore fluid and electrolyte losses), refueling (to restore glycogen), repair (of damaged muscle fibers), and remodeling (adaptation to the training stimulus) known collectively as the "4Rs". (Jeukendrup & Gleeson, 2018).

Ensure that appropriate amounts of fluid, electrolytes, carbohydrate, and protein are ingested in order to accomplish the goals defined by the 4Rs.

- "Timing of protein intake is an important variable to consider in optimizing skeletal muscle recovery and hypertrophy. It appears optimal to ingest protein in the postexercise period in sufficient doses (~ 0.4 g · kg b.w.$^{-1}$. meal^{-1})" and up to 3 other meals distributed throughout the day with the aim of achieving a total dietary protein intake of 1.4-1.6 g · kg b.w.$^{-1}$. Eating fewer meals with more protein is not as good as eating this optimal amount. Ingesting a larger dose of protein (~ 0.6 g · kg b.w.$^{-1}$. meal^{-1}) "before bedtime appears to augment both acute overnight muscle protein synthesis and chronic skeletal muscle adaptations.
- The protein content of the postexercise meal should be predominantly in the form of rapidly digested high quality proteins with a high leucine content. Such proteins include whey, and skimmed milk and eggs.
- Other protein consumed during the day should be mostly lean high quality protein containing all the essential amino acids in approximately equal proportions. This will predominantly be protein from animal sources including beef, ham, lamb, poultry and fish but can be supplemented with soy, beans, cheese, nuts and bread. (Jeukendrup & Gleeson, 2018).

- Remember that some protein can also be obtained from soy, beans, nuts and bread.

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