Module 2. The role of protein in a player's diet

Unit 2.1 An Introduction to Protein

Protein is provided in the players' diet by both plant and animal-based foods. Within the field of sport nutrition, protein is widely recognized as a key nutrient for muscle physiology. When discussing proteins for football, the common response is that they are needed for muscle growth and repair, which is indeed true. However, proteins do not play just a structural role but they also have many other functions. For instance, proteins participate in transporting molecules such as oxygen; speed up chemical reactions in metabolic pathways; are part of the immune system; and may act as triggers to drive muscle adaptation following football training and matches.

DID YOU KNOW
The complete set of proteins expressed by an organism is called proteome and the study of the proteome is called proteomics.

Protein function

Protein is a component of every cell in the human body and is necessary for players' growth, development and health. As such, proteins have a diverse range of functions in the player's body. It is impossible to cover all the roles of proteins in detail, but it is important to understand some key functions, summarised in this unit.

Proteins are essential for movements generated by muscle contractions. Muscle contractions are possible thanks to the action of the proteins actin and myosin, which interact to produce mechanical force within skeletal muscles. Antibodies produced by the immune system are also proteins, which can recognize and neutralize microorganisms (pathogens) that may cause disease. The antibodies degrade and destroy the foreign substances in the body to maintain players' health.

Many proteins also play a role in the movement and transport of substances into and out of cells. For example, oxygen, required for aerobic metabolism, is transported by a protein called haemoglobin and fatty acids are transported in the plasma by a protein called albumin. The transport of many molecules such as glucose, calcium, sodium and potassium into and out of cells is reliant on complex protein structures that create channels or pumps in cell membranes.

Proteins also act as hormones secreted by the endocrine system, enabling cells to communicate with other cells in the body. For example, insulin is a protein secreted by beta cells of pancreas in response to the ingestion of food. Insulin then travels via the circulation towards target organs such as liver, adipose tissue or skeletal muscle, promoting the uptake of glucose in those tissues.

Another key role of proteins is related to the enzymatic activity. Enzymes are proteins and they function to facilitate/speed chemical reactions in the players' body. For example, phosphofructokinase is a key enzyme in the metabolism of carbohydrates and citrate synthase is a protein involved in the first reaction of the TCA cycle. Thus, proteins play a key role in energy production. Some of the key functions of protein in the players' body are listed in Figure 1.

Figure 1: General protein functions in players' body

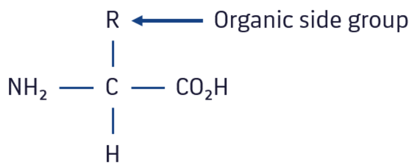
Source: Prepared by the author.

Protein structure

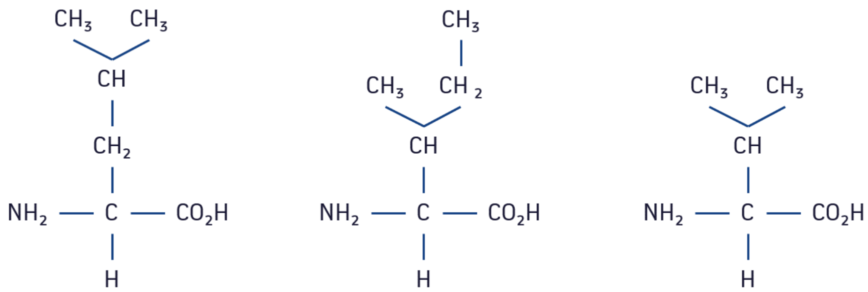
Protein is made up of hundreds or thousands of smaller units, called amino acids. The amino acids are linked to one another in long chains. Thus, amino acids are the building blocks of proteins. Each amino acid is composed of carbon, hydrogen, oxygen and nitrogen. The general formula for amino acids is RCH(NH2)COOH, where C is carbon, H is hydrogen, N is nitrogen, O is oxygen, and R is a group, varying in composition and structure, called a side chain. What makes amino acids different from each other is the R group. The side chain determines the amino acids overall size, structure and function.

Figure 2: General Amino acids and branched-chain amino acid structure

General structure of an amino acid



Branched chain amino acid structures



Leucine **Isoleucine** **Valine**

Source: Prepared by author.

Amino acids fall into two categories: essential and non-essential. Essential amino acids are required for normal body functioning, but they cannot be made by the body and must be obtained from food in the player's diet. Of the twenty amino acids, nine are considered "essential" and eleven are "non-essential". Non-essential amino acids can be made by the body from essential amino acids consumed in food or in the normal breakdown of body proteins.

All amino acids in the blood and extracellular fluids represent an amino acid "pool" that can vary in size and composition depending upon the body's nutritional state, the plasma amino acid availability and the hormonal milieu. Immediately following the ingestion of a meal, the amino acid pool in muscle is expanded since the delivery of amino acids to muscle exceeds its capacity to turn them into protein (Rennie and Tipton 2000). In the post-absorptive state, protein synthesis turns down and protein breakdown is accelerated.

Figure 3: Amino acid classification

Essential amino acids	Non-essential amino acids
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine

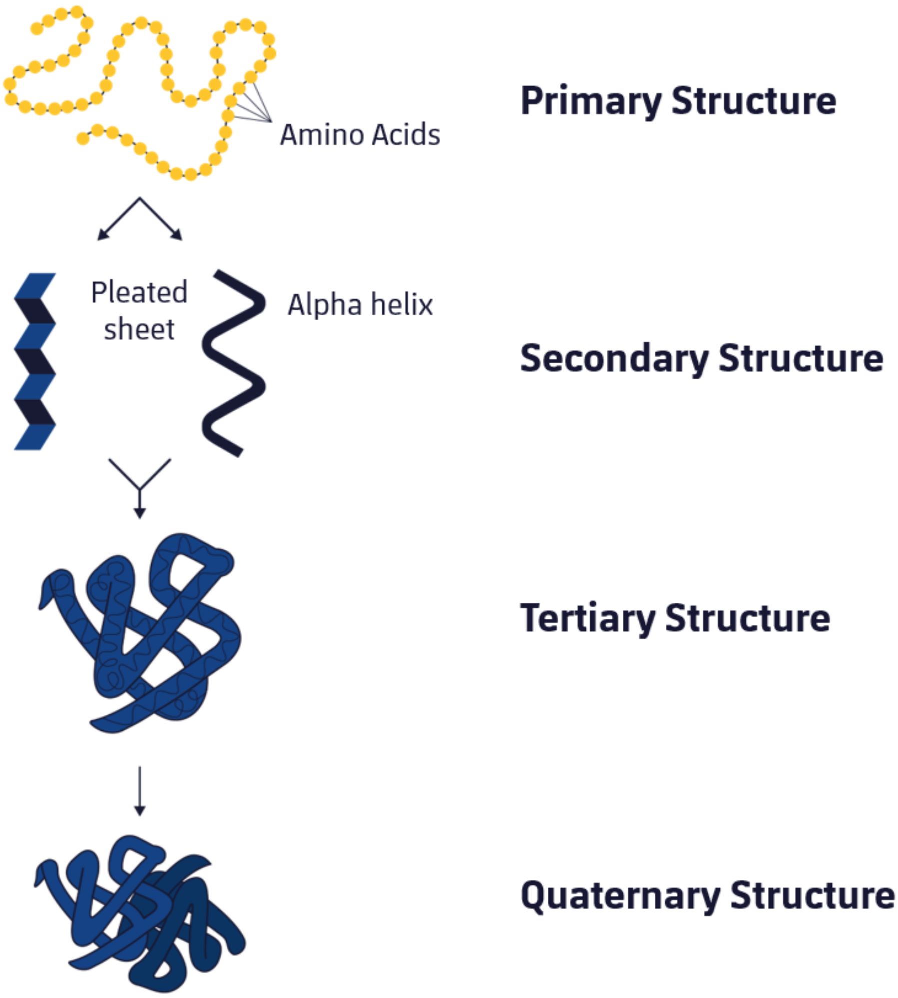
Valine	Proline Serine Tyrosine

Source: Prepared by the author.

Amino acids can join to form chains via peptide bonds between two adjacent amino acids. It is common to consider chains of 50 amino acids or more as proteins, whereas chains of less than 50 amino acids are commonly referred to as peptides or oligopeptides. Each type of protein has its own unique sequence of amino acids, which determines each protein's structure and its specific function.

It is the combination of amino acids in the peptide sequence that allows for the diversity of protein function. Apart from their primary structure, proteins can be organized into secondary structures, by folding in two common forms known as the alpha helix or beta pleated sheets. Proteins complexity increases as they organize into tertiary structures in order to acquire a three-dimensional shape (Figure 4). This shape is critical for proteins to have functionality, in part, due to effective functional interactions between parts of the protein. A quaternary structure is when two or more tertiary structures bond together.

Figure 4: Protein structure



Source: Prepared by Author.

Branched-chain amino acids

The branched-chain amino acids are the most abundant form of amino acids, accounting for 20% of amino acids found in skeletal muscles. The branched-chain amino acids, isoleucine, leucine and valine, are essential amino acids that have received a great deal of attention in sports and exercise nutrition recommendations. This is because the branched chain amino acids can be directly used by the skeletal muscles and may be important in the context of enhancing the anabolic response of muscles after exercise (Tipton 2017). The structure of the three branched chain amino acids is shown in Figure 2).

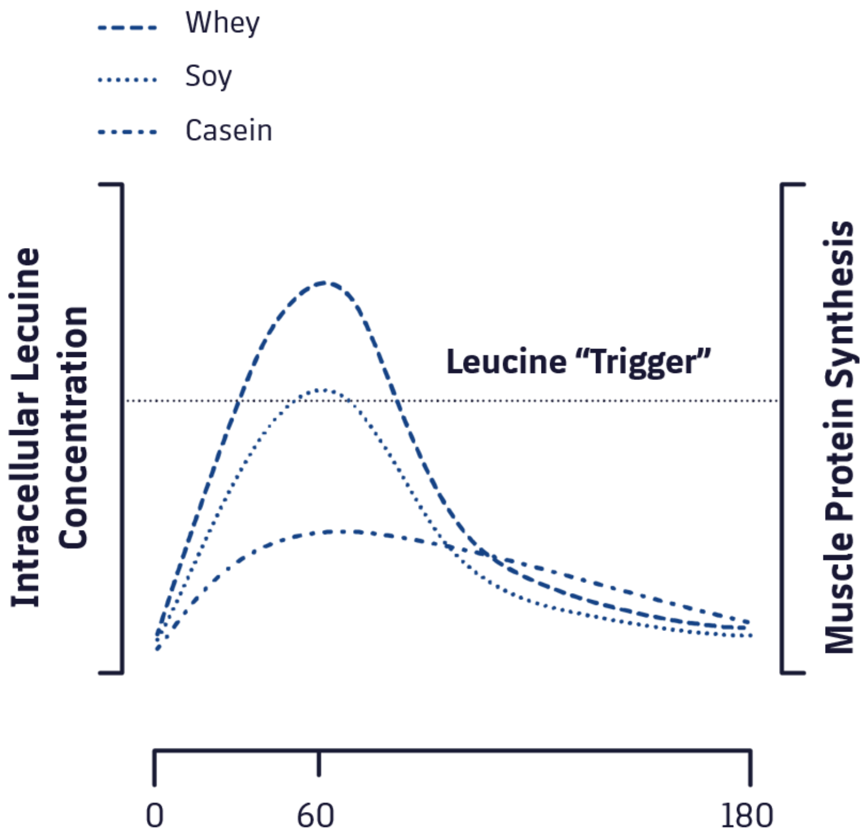
Branched-chain amino acid supplementation, due to the leucine content, has been shown to stimulate the molecular pathways leading to enhanced muscle protein synthesis. However, it is not clear if branched-chain amino acid supplements alone are effective for optimal stimulation of muscle protein synthesis following exercise. It is likely that consuming branched-chain amino acids without sufficient amounts of the other essential and non-essential amino acids will maximise the muscle protein synthetic response (Tipton 2017).

Supplementation with branched-chain amino acids has been reported to inhibit muscle protein breakdown at rest, but to date there is no evidence to suggest that they inhibit muscle protein breakdown after exercise (Tipton 2017). There is some evidence to support the use of branched-chain amino acids is an effective nutrition intervention to improve recovery from exercise-induced muscle damage (Howatson, Hoad et al. 2012). However, not all studies support these observations (Areces, Salinero et al. 2014), thus, more research is required to recommend branched-chain amino acids specifically for the recovery from football exercise. Finally, there is little evidence regarding branched-chain amino acid ingestion and muscle protein synthesis following endurance exercise. Therefore, again there is no reason to recommend specific branched-chain amino acid supplementation during or following football training or matches.

Leucine

All amino acids are believed to have a role, to a lesser or larger extent, on amino acid-induced stimulation of muscle protein synthesis (Volpi, Kobayashi et al. 2003). However, leucine has emerged as a key nutritional regulator of muscle protein synthesis (Koopman, Wagenmakers et al. 2005, Philips 2013). Studies have reported a greater stimulation of muscle protein synthesis when amino acid mixtures are enriched with leucine (Koopman, Wagenmakers et al. 2005) and proteins naturally rich in leucine in comparison to “low-leucine” proteins (Tang, Moore et al. 2009). These observations lead authors to speculate that a “leucine trigger” or “leucine threshold” may exist. Specifically, the “leucine threshold” represents the concentration of leucine required in the peripheral circulation for a maximal rate of muscle protein synthesis (Burke, Hawley et al. 2012). Thus, based on the assumption that the quantity of protein to be sufficient to produce a maximal synthetic response is approximately 20 g (egg protein), the corresponding dose of leucine to “Trigger” a response would equate to approximately 2.0-2.5 g (Moore, Robinson et al. 2009). The dose and quantity of protein is most likely different for players with lower body masses and following exercises that engage more or less muscle.

Figure 5: Leucine "trigger" hypothesis



Source: modified from (Phillips 2013).

Dietary protein

Amino acids are obtained by ingesting dietary protein. Players typically ingest about 10-15% of their total energy intake in the form of dietary proteins. The current international Recommended Dietary Allowance (RDA) for protein in healthy individuals is 0.8 g per kg of body mass (Albertus, Tucker et al. 2005, Institute of Medicine (IOM) Food and Nutrition Board 2005), but this is likely higher for football players (Unit 2).

Animal, dairy, and some plant proteins are considered high-quality proteins that confer health and metabolic benefits based on the digestible levels of the essential amino acids they contain (Pasiakos, Agarwal et al. 2015).

Legumes, nuts, seeds and certain grains such as quinoa are some of the plant foods with higher protein content. Soybeans in particular are much higher in protein and essential amino acids than other plant foods (Michelfelder 2009). However, with the exception of soy, most plant proteins are limited in one or more of the following amino acids: lysine, threonine, tryptophan, cysteine or methionine (Comerford and Pasin 2016). Conversely, most animal protein sources, with the exception of collagen/gelatine, contain adequate quantities of all the essential amino acids. To date, much of the research on nutrition has been on isolated protein sources. We know that, when players eat "meals", they typically contain a combination of foods. It is important to note that combinations of various plant-based protein isolates or blends of animal and plant-based proteins can provide protein characteristics that closely reflect the typical amino acid profiles/characteristics of animal-based proteins (Gorissen, Crombag et al. 2018). Some examples of protein-rich sources are provided in Figure 6 and the leucine content of common foods is listed in Table 1.

Figure 6: Examples of foods rich in protein

Source: Prepared by the author.

Table 1: Sources of Leucine in Food

Food	Leucine g/100g
Soy protein concentrates	4.92
Soybeans, raw	2.97
Ground beef, lean	1.76
Peanuts	1.67
Salami, pork	1.63
Fish, salmon	1.62
Wheat germ	1.57
Almonds	1.49
Chicken (broilers or fryers, thigh, meat only, raw)	1.48
Chicken egg (yolk, raw, fresh)	1.40
Oat	1.28
Pinto beans, cooked	0.76
Lentils, cooked	0.65
Chickpea, cooked	0.63
Corn, yellow	0.35
Cow milk (whole, 3.25% milk fat)	0.27
Rice (brown, medium-grain, cooked)	0.19
Human milk	0.10

Source: modified from the National Nutrient Database for Standard Reference. U.S. Department of Agriculture, 2009.

Protein digestion and absorption

During digestion, proteins are broken down into free amino acids that are absorbed in the blood. Proteins are broken down into their amino acid subunits in a process called hydrolysis. The full complexities of protein hydrolysis are beyond the scope of this course. In summary, protein digestion begins in the mouth. Protein foods, especially meat, require adequate chewing prior to swallowing (Remond, Machebeuf et al. 2007). Protein hydrolysis begins in the stomach where the ingested food is exposed to the first in a series of proteases, the enzymes required for protein digestion. Proteases hydrolyse the peptide bonds, which cement the structure of the protein in food together. In the stomach, the protein is exposed to hydrochloric acid and pepsin. Pepsin is a proteolytic enzyme, which requires the acidic environment of the gastric juice to hydrolyse the protein. The delivery of protein to the intestine is governed by factors commonly reported to influence gastric emptying.

Peptide fragments and free amino acids are absorbed almost exclusively in the small intestine. The proximal jejunum is the major site of amino acid and peptide absorption. However, other sections of the small intestine also have significant transport capacity (Silk, Grimble et al. 1985). In the intestine, pepsin is denatured by the alkaline secretions of the pancreas. However, protein continues to undergo hydrolysis by the action of endopeptidases trypsin and chymotrypsin. Trypsinogen, also contained in pancreatic juice, activates enterokinase located along the wall of the intestinal brush boarder membrane. Most amino acids are transported across the brush border membranes of intestinal cells by either sodium-dependent or sodium-independent transporters.

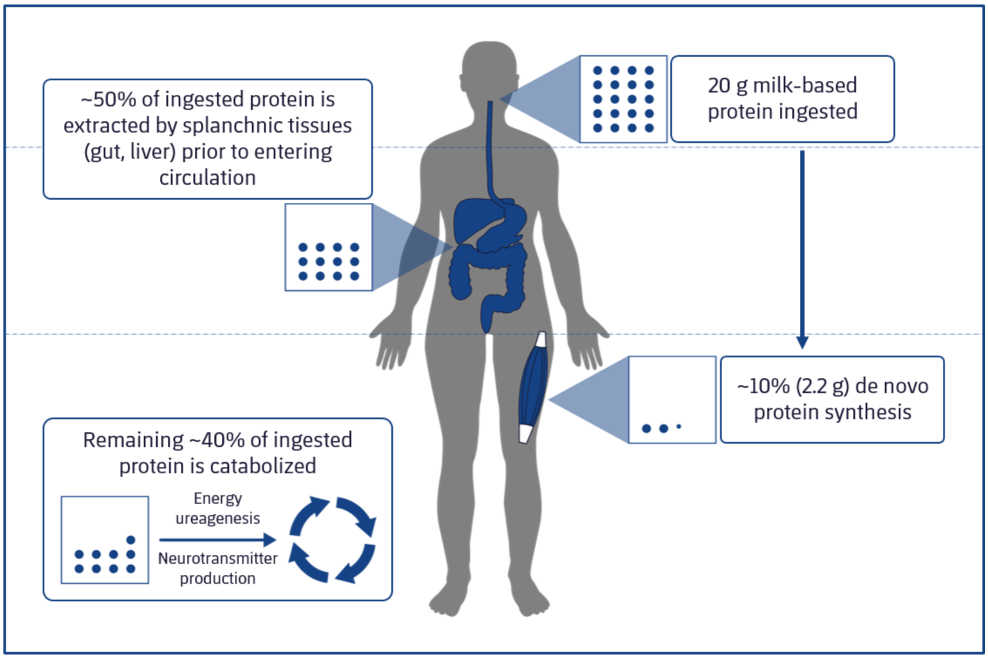
Many of the amino acids absorbed following protein digestion (~40-50%) are used by the intestinal cells for energy or synthesis of proteins, whereas the remainder of amino acids are released into the hepatic portal vein prior to being taken up by the liver (Stokes, Hector et al. 2018). The liver is the other key organ for amino acid metabolism in the body since more than 50% of the amino acids absorbed in the intestines are taken up and processed by the liver for local metabolism, that is, for the production of hepatic and liver-derived blood proteins. Overall, approximately 50% of the amino acids in a protein-containing meal are extracted by the splanchnic tissues whereas the rest are released into the plasma circulation for extra-splanchnic utilization (Groen, Horstman et al. 2015).

DID YOU KNOW

The splanchnic tissues are those organs in the abdominal cavity, including the liver, small and large intestine, stomach and pancreas

Although skeletal muscle is a large reservoir for the retention of amino acids, not all the amino acids released into plasma become incorporated into new skeletal muscle tissue. In a study employing an intrinsically-labelled tracer approach, Groen et al. (2015) demonstrated that approximately only 2.2 g or 10% of the amino acids provided to young men following the ingestion of 20 g of casein protein were used for protein synthesis. The use was low even though approximately 55% of the ingested amino acids were available in the peripheral circulation following splanchnic extraction (Groen, Horstman et al. 2015). The remaining amino acids are broken down and serve as substrates for a range of metabolic processes from energy production and urea synthesis and, to a minor extent, neurotransmitter production. Thus, interestingly, only 10% of the ingested protein is utilised to build new skeletal muscle tissue (Stokes, Hector et al. 2018) (Figure 7).

Figure 7: A summary of protein digestion and absorption and the corresponding availability and use of protein following the ingestion of 20 g.



Source: Modified from Stokes, Hector et al. 2018.

Summary

- Proteins in the player's body are made up of smaller units, called amino acids.
- Proteins have a huge variety of roles and functions in the player's body.
- Proteins can be provided to the body via animal and plant-based foods.
- Amino acids can be classified as "essential" and "non-essential", with essential amino acids needing to be ingested via the diet.

Unit 2.2 Protein and Football

All proteins are integral and/or functional proteins. Contractile fibres within muscles and enzymes needed for metabolism in the player's body are examples of proteins. An average 70-kg male player contains approximately 12 kg of protein and 220 g of free amino acids. Proteins are constantly broken down into amino acids and these amino acids then become available for the synthesis of other proteins. New amino acids are delivered through the player's diet, whilst excess amino acids are metabolised either to fatty acids or glucose. Muscle is an important tissue responsible for 25-30% of whole-body protein metabolism. Many of the adaptations we are interested in to support football performance occur within the muscle.

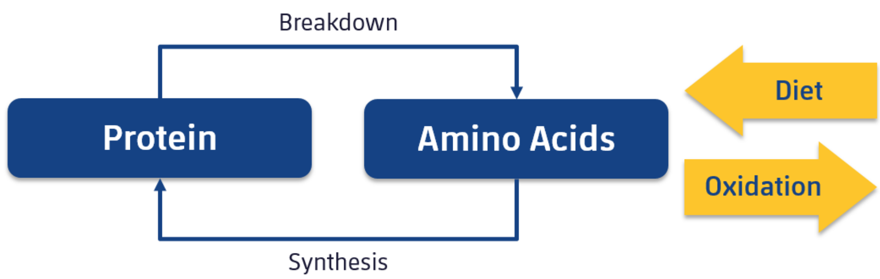
Protein turnover

Proteins inside the player's body are continuously synthesised and degraded in a process called protein turnover. It is necessary for cellular components to be replaced and finely regulated in response to changes in cellular environment. This process of protein turnover is highly dynamic and, as such, energy demanding, accounting for approximately 20% of daily basal energy expenditure.

Whether net protein synthesis occurs depends on the balance of protein synthesis and degradation. If protein degradation rates are greater than the rates of synthesis, there will be a reduction in protein content; conversely, muscle protein content can only increase if the rate of synthesis exceeds that of degradation (Lanham-New, MacDonald et al. 2011).

Research has mostly focused on protein synthesis but both processes (breakdown and synthesis) are important (Figure 1). It is believed that protein synthesis is the main driver of positive protein balance because protein breakdown does not change as much. On the other hand, it is also believed that protein degradation is important to remove damaged proteins and allow new proteins to be synthesised. The key to rapid recovery of football players is increasing protein turnover (synthesis as well as breakdown), not just reducing breakdown (Phillips and Van Loon 2011).

Figure 8: Summary of Protein Turnover



Source: Prepared by author.

The process of protein synthesis

In a fasted state, protein breakdown is greater than protein synthesis (Biolo, Maggi et al. 1995). In response to protein or amino acid feeding, protein synthesis will increase and the net protein balance will become positive (Biolo, Tipton et al. 1997). It is the contribution of these fed and fasted periods to overall protein balance that dictates muscle remodelling over time (Phillips 2004).

Football exercise will increase both muscle protein breakdown and muscle protein synthesis. However, in the absence of proteins in the diet, net protein balance will remain negative. Therefore, protein intake is important after matches and hard training sessions in order to achieve a positive net protein balance. In addition to general effects of exercise, football involves many decelerations (eccentric contractions) and contact between players, and it is well-known that this can result in muscle damage. Protein ingestion is, therefore, advised to aid the repair of muscle tissue and other potential injuries (Res 2014).

There are a number of factors that affect the muscle protein synthetic response post-exercise, including:

- The exercise (football) stimulus
- The quantity of protein ingested
- The type of protein ingested
- The timing of intake
- The frequency of intake
- Co-ingestion of other nutrients

Football stimulus

Exercise training for football is an important stimulator of protein synthesis, with the exercise mode dictating the type of proteins that are synthesised (Phillips and Van Loon 2011). In general, dedicated resistance exercise will increase muscle mass, whereas endurance exercise will stimulate mitochondrial biogenesis and the quality of the existing muscle without an increase in mass (or size) (Cermak, Res et al. 2012). The key variables in any type of exercise are the effort, the time under tension, the volume, the frequency of bouts, the metabolic stress and the training age of the individual. For more details on the specific effects of resistance exercise on strength gains, please see dedicated reviews (Hoff and Helgerud 2004). The effects of protein intake are relatively small compared to the effects of exercise, but worthwhile nevertheless. In football, an outdated view is that resistance-type exercise will result in the player becoming “too muscular”. Modern approaches use an appropriate resistance exercise to compliment the players’ training program to improve markers of performance as well as reduce the risk of injury (Hoff 2005, Zouita, Zouita et al. 2016).

Quantity of protein for football

Several studies have investigated the dose response relationship between protein intake and protein synthesis. The first study was completed by Moore et al. (2009) who gave whole egg protein to participants in doses ranging from 5 to 40 g (5, 10, 20 and 40 g) (Moore, Robinson et al. 2009). Protein synthesis post resistance exercise increased linearly with increasing dose from 5 to 20 g, but 40 g did not result in a further increase in protein synthesis. Thus, it was concluded that approximately a twenty-gramme portion was the dose required to reach optimal protein synthesis. These findings were later confirmed in a study investigating protein intake in young healthy males (Witard, Jackman et al. 2014). Although there was not enough strong evidence, it seemed reasonable to assume that the requirements were dependent on muscle mass and, therefore, recommendations were sometimes expressed in kilogrammes of body mass. Moore et al. (2015) used a statistical approach to arrive at a figure of 0.25 g of protein per kg of the player's body mass per meal (Moore, Churchward-Venne et al. 2015). However, Macnaughton et al. (2016) raised new questions. In this study, participants performed whole body exercise as opposed to single muscle groups as in the previous studies. It was reported that 40 g of protein resulted in greater protein synthesis than 20 g (Macnaughton, Wardle et al. 2016). Therefore, recommendations so far may have underestimated the requirements for optimal protein synthesis following exercise that engages whole body muscle mass. Thus, although general recommendations of 20-25 g are relevant to most footballing occasions, when players are engaged in whole body exercise, using a large percentage of their muscle mass, protein intake may need to be increased to 30-40 g or 0.3-0.5 g/kg/meal. It is important to recognize that intakes of 30-40 g of protein and beyond represent a large protein "load". Some players may struggle to consistently ingest this quantity of protein through food choices or supplementation. In these situations, some protein will be better than no protein. Players can work towards implementing recommendations, but a variety of options should be presented to meet the protein intake and suit the player's individual preference.

Historically there has been a dogma regarding the health implications of ingesting a large quantity of protein in the diet or supplementing the diet with amino acids. A systematic review and meta-analysis of available studies concluded that there is no evidence for long-term health implications or no damage to the kidneys or kidney function with high-protein diets (Devries, Sithamparapillai et al. 2018).

Timing of protein for football

It is often suggested that it is critical to ingest proteins immediately post-exercise when there is "a window of opportunity" for optimal protein synthesis. Indeed, studies have suggested that post-exercise rates of protein synthesis are high when protein is ingested immediately after exercise, but this window may not be as critical as often suggested (Morton, McGlory et al. 2015). It has been shown that, after resistance exercise, muscle protein breakdown remains elevated for 24 hours and muscle protein synthesis for 48 hours, while muscle protein synthesis in response to a meal has been shown to be elevated for up to 24 hours (Burd, West et al. 2011). It has also been shown that similar synthesis results can be obtained by ingesting protein just before exercise or even during exercise (Tipton 2007, Beelen, Koopman et al. 2008). Therefore, timing may not be as critical as often suggested but, from a purely pragmatic point of view, it still seems to make sense to start protein ingestion directly after exercise for optimal recovery, especially if limited time is available until the next match or important training session. Immediately post exercise (training or match) is also a time when players are still in a controlled environment and nutritionists, trainers, coaches or other staff can make sure that players ingest at least a certain amount of fluids, carbohydrates and proteins to start the 3 R's of recovery (Rehydration, Refuel, Repair).

It is important to note, that "immediately" after exercise is not realistic for most football situations. During a match, for example, when a player has been substituted, the player's blood will still be re-distributed away from the gastrointestinal system to the player's muscle. Thus, it is advised to wait 5-10 minutes to allow the player to "recover" from exercise and to allow blood flow back to the gastrointestinal system before protein is ingested. This principle is the same for training situations as well as immediately after a match.

As post-exercise nutritional requirements are multifactorial (rehydration, glycogen resynthesis, myofibrillar/mitochondrial protein synthesis), it is important to note that post-exercise protein intake can be achieved without compromising the rehydration of the player (James, Mattin et al. 2014). For football, a convenient method to achieve the recovery requirements is to provide protein in "shakes" or beverages post exercise. This way of delivering protein post exercise has several key advantages: (1) fluid and carbohydrate ingestion can be achieved simultaneously; (2) it is easier to ingest versus solid food; (3) composition and flavour of shake can be modified; (4) nutritionists can easily check to see if the player has adhered to the recovery strategy.

DID YOU KNOW

The blood flow (cardiac output) is redistributed during exercise. During intense exercise, approximately 80% of blood will be distributed to the working muscles. Blood flow to the gastrointestinal system will be reduced to approximately 5% of cardiac output compared to 25% at rest.

Frequency of protein intake for football

Very few studies have investigated the effects of frequency of meals on protein synthesis. In one study, smaller, more frequent whey-protein meals versus larger, less frequent intake of whey proteins were investigated. Areta et al. (2013) measured protein synthesis during a twelve-hour period after the ingestion of 2 x 40 g every 6 hours, 4 x 20 g every three hours or 8 x 10 g every one and a half hours. From this study, providing a meal that contains 20 g every 3 hours appeared to be optimal (Areta, Burke et al. 2013). It is important to note, though, that these studies are rather artificial because meals consist of pure proteins and are typically delivered as drinks. Protein synthetic responses are known to be affected by other nutrients and, thus, when normal meals are consumed (including carbohydrates and fats), responses may be quite different. For now, based on available evidence, it is advised that players ingest regular meals, every 3-4 hours, containing adequate protein (20-40 g).

Recent evidence suggests that one of the protein-containing meals should be before bedtime. Normally during sleep (a period of fasting) protein breakdown will exceed protein synthesis. Ingesting proteins (40 g of casein) just before bed has been shown to turn this period of sleep into a period of net protein synthesis (Res, Groen et al. 2012). A twelve-week training study showed that giving athletes 27.5 g of protein (with 15 g of carbohydrate) after training and before sleep improved muscle mass and strength gains compared with a placebo (Snijders, Res et al. 2015). This may be particularly important if training is completed late in the day. This is because a recent study has shown that training performed in the evening augments the overnight muscle protein synthetic response to pre-sleep protein ingestion, allowing more of the ingested protein-derived amino acids to be used for myofibrillar protein synthesis (Trommelen, Holwerda et al. 2016). It is important to note that Wall and colleagues have reported that pre-sleep protein ingestion does not compromise the muscle protein synthetic response to protein ingested the following morning (Wall, Burd et al. 2016). Professional players have been reported to ingest only 0.1 g of protein per kg body mass before bed, thus highlighting an important opportunity for improved nutritional choices that may improve training adaptations (Anderson, Orme et al. 2017). For example, Abbott and colleges investigated the effect of ingesting 40 g of casein or 40 g of carbohydrates 30 minutes before sleep in professional players. In this study, either the protein or the carbohydrate was provided after an evening match (kick off at 7:00 pm). It was found that casein ingestion accelerated functional recovery, measured by countermovement jump height, reactive strength index, muscle soreness and mood (Abbott, Brett et al. 2018). Thus, the available information would suggest that players may benefit from developing a routine that includes ingesting a drink or meal containing 30-40 g of casein protein before bed time (Trommelen, Kouw et al. 2018).

Type of protein for football

One of the important factors governing the muscle protein synthetic response may be the amino acid composition of the ingested source. Specifically, the protein should contain all essential amino acids (Unit 1). Perhaps the most important of these essential amino acids is leucine, which stimulates the mechanistic target of rapamycin complex-1, a key signalling protein considered the main trigger for increases in muscle protein synthesis. Animal protein contains more leucine compared to plant sources (van Loon 2012).

Whey protein can be quickly digested and absorbed and contains a relatively high proportion of leucine. For example, approximately 2.5 g of leucine is contained in 20 g of whey. Whey has also been shown to elicit superior muscle protein synthesis compared to soy or casein, when taken in isocaloric amounts (Tang, Moore et al. 2009). In this study, whey appeared to be better than soy and soy better than casein. Generally, plant-based proteins contain less leucine compared to whey, therefore, comparatively more plant-based protein may need to be ingested to maximize muscle protein synthesis. Whey protein (or good sources of it) is, therefore, considered the preferential protein to ingest directly after exercise. However, blends of protein (for example 1:2:1 proportion of whey:casein:soy) can be just as effective as long as leucine content is high (Reidy, Walker et al. 2013).

Research suggests that leucine above a certain concentration (in muscle) triggers protein synthesis. It may be important to reach this level for protein synthesis to be stimulated and, thus, in the absence of exercise, meals should contain this amount of leucine (which is believed to be around 3 g) (Churchward-Venne, Breen et al. 2014). Although branched-chain amino acids (leucine, isoleucine and valine) are often claimed to stimulate protein synthesis, there is no evidence of this. It is likely that isoleucine and valine compete with leucine for transport into cells (Hyde, Taylor et al. 2003). Branched-chain amino acids may, therefore, result in a competitive antagonism for uptake and may not be as effective as leucine alone. In summary, to maximize muscle protein synthesis post exercise the player may require either a high-leucine (~ 3 g) and essential amino acid intake or simply a sufficient quantity of protein, independent of the source (Gorissen, Crombag et al. 2018).

DID YOU KNOW
Isocaloric simply means having the same calorific/calorie value

Other nutrients

Other nutrients ingested with protein may affect the protein synthetic response. Studies have investigated the co-ingestion of protein with carbohydrate to determine the impact on recovery of muscle glycogen as well as the synthetic response. It appears that, when adequate carbohydrate is ingested, protein does not increase glycogen restoration and, conversely, when adequate protein is ingested, additional carbohydrate does not improve muscle protein synthesis (van Loon, Saris et al. 2000, Betts and Stevenson 2011). Nevertheless, it is intuitive to combine the ingestion of protein and carbohydrate following training and matches, to gain both the aforementioned recovery benefits. The fat content of a meal (and possibly, the fibre) can change the delivery of the amino acids and, thus, it has the potential to affect protein synthesis. These interactions between nutrients and the effects of mixed meals have been studied very little and extrapolations have to be made from relatively "unnatural" laboratory studies in which only protein was ingested. Interestingly though, studies are beginning to look at foods rather than ingredients. In the future, research will likely focus on meals rather than on foods. These studies will hopefully provide better understanding of the nutrient density on muscle protein synthesis (Abou Sawan, van Vliet et al. 2018). Unfortunately, it is too early to draw firm conclusions on what meals would be best to stimulate protein synthesis. Instead, it is advised to offer many different options of meals to meet protein requirements over the competitive season. Omega 3 fatty acids have also been mentioned as a way to increase the sensitivity of protein synthesis to protein ingestion and this will be discussed further in Module 3.

Daily protein requirements for football

Daily protein intake for football players has been advised to be in the range of 1.4-1.7 g/kg (Lemon 1994). However, these recommendations are based on nitrogen balance studies and, thus, minimal protein needs to stay in protein balance. Such intake recommendations are not based on what is optimal for the stimulation of muscle protein synthesis. There is still considerable debate about how much dietary protein is required for "optimal" football performance and player recovery (Phillips and Van Loon 2011).

In extreme situations, daily protein intake in excess of general recommendations has proven to be beneficial beyond enhancing muscle protein synthesis. When daily protein intake was elevated from 1.5 g/kg to 3 g/kg, tolerance to intensified training was increased (Witard, Jackman et al. 2011) and immune function was better maintained, resulting in less upper respiratory tract infections (Witard, Turner et al. 2014). In addition, elevated daily protein intake in the range of 2.3 g/kg has been shown to better maintain muscle mass in the face of an energy deficit (Mettler, Mitchell et al. 2010). Thus, increasing habitual intake of protein may be of benefit when players are engaged in intensive pre-season training, involved in frequent travel and/or during periods of fixture congestion.

Increasing the players protein intake can easily be achieved through normal protein-rich foods, such as meat, eggs, legumes, and dairy, which provide additional nutrients (Phillips 2014). Recent dietary surveys suggest that professional players readily meet such protein requirements. Indeed, in professional players from the English Premier League, daily protein was consistent across a seven-day in-season training period and was approximately 2-2.5 g/kg body mass per day (Anderson, Orme et al. 2017). This intake (approximately 200 g per day) was greater than that reported (<150 g/day) previously by adult players from the Scottish leagues (Maughan 1997). With dietary planning, protein supplements are probably not needed for most players, though they provide a convenient (especially in the post-training periods) and easily digestible alternative to foods.

Football players have been reported to skew their pattern of daily protein intake. It appears that, at least in English football, absolute amount of protein was consumed in the following hierarchical order: dinner>lunch>breakfast>snacks. This pattern would not theoretically favour an optimal stimulation of protein synthesis on each meal occasion. However, it is important to note that players report an overall consumption of ~0.3-0.4 g/kg BM at main meals (Anderson, Orme et al. 2017), which is consistent with recommendations.

On occasions, when players may be managing body mass through energy restriction, protein requirements are likely increased (Phillips 2014). During these occasions, it is unlikely that players will lose fat and gain muscle simultaneously (Churchward-Venne, Murphy et al. 2013). Instead, it is the preservation of muscle mass and a reduction in fat mass, which results in improved body composition profile. For this reason, it is prudent to recommend a higher protein intake dependent on the training load and other metabolic stresses, such as weight loss or rehabilitation from injury (Milsom, Barreira et al. 2014, Wall, Morton et al. 2015). Therefore, the daily protein intake for football players may range between 1.5 and 3 g/kg/day. Protein recommendations should be refined with individual player considerations of total energy needs, specific training needs and feedback from training / competition performance.

Table 2: Protein guidelines for football

Occasion	Protein Targets	Principle
Daily intake	1.5-3.0 / kg BM / day	Meals containing 20-40 g protein ingested routinely (3 h intervals) during the day
Exercise	20-40 g high quality protein containing 2-3 g leucine	Ingested immediately post exercise to support adaptation
Sleep	30 g casein	Ingest prior to sleep

Source: prepared by author.

Protein for tendons and connective tissue

The tendons and connective tissues such as ligaments are vital for football performance, as it is these tissues which hold the muscular skeletal system together and stabilise movement around joints. Strains and sprains of soft tissues (tendons and ligaments) account for more than 60% of all injuries reported in the English Premier League (Hawkins, Hulse et al. 2001). The physiology of tendons and ligaments is different to muscle (Kjaer, Langberg et al. 2009). This is because tendons and ligaments have limited blood flow, and they are dependent on nutrient delivery though bulk fluid flow (Baar 2015). The turn-over of tendon is significantly lower than muscle.

Early evidence suggests there are opportunities for remodelling of tendon tissue which can be augmented by nutrition. This is because ingested protein may add to the successive rings of collagen that surround the core, making the structure stronger. Specifically, the ingestion of gelatin has been reported to be effective in increasing circulating concentrations of the amino acids glycine, proline, hydroxyproline, and hydroxylysine (Shaw, Lee-Barthel et al. 2016). The ingestion of gelatin (15 g ingested with 50 mg vitamin C) 1 h prior to exercise increased blood markers (amino-terminal propeptide of collagen I) related to increased collagen synthesis (Shaw, Lee-Barthel et al. 2016). Although more research is required, the ingestion of gelatin is a promising nutritional intervention to improve both the function of connective tissues and speed the recovery from musculoskeletal injuries. Furthermore, this intervention may be of great relevance to those populations who experience a high incidence of ligament injury, such as female players (Celebrini, Eng et al. 2012, Celebrini, Eng et al. 2014, De Ste Croix, Priestley et al. 2015).

Given the low turnover rate of tendons, players will likely have the same "core" tendon protein between the ages of 17 years and 70 years of age (Heinemeier, Schjerling et al. 2013). Therefore, players will have the same tendons throughout their competitive career. However, as studies which have provided protein (proline) with vitamin C have reported improved collagen synthesis (Paxton, Grover et al. 2010), remodelling is possible throughout the season with appropriate loading and nutrition (Shaw, Lee-Barthel et al. 2016).

Summary

- Dietary protein is important for the recovery and adaptation process following football exercise.
- Players' daily protein intake (1.5-3 g/kg BM) should be relative to their body mass, the phase of the season and the amount of muscle mass used during exercise.
- To maximize muscle protein synthesis post exercise, the player will require a protein with a high leucine content or sufficient quantity of protein, independent of the source.
- Protein intake should be spread evenly throughout the day (every 3-4 hours approximately) and should be intensified post exercise.
- Protein feedings (~30 g casein) before bed offer an additional opportunity to speed up recovery and enhance adaptation.

Disclaimer: Ian Rollo is an employee of the Gatorade Sports Science Institute, a division of PepsiCo, Inc. The views expressed in this course are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

References

Abbott, W., A. Brett, E. Cockburn and T. Clifford (2018). "Presleep Casein Protein Ingestion: Acceleration of Functional Recovery in Professional Soccer Players." Int J Sports Physiol Perform: 1-24.

Abou Sawan, S., S. van Vliet, D. W. D. West, J. W. Beals, S. A. Paluska, N. A. Burd and D. R. Moore (2018). "Whole egg, but not egg white, ingestion induces mTOR colocalization with the lysosome after resistance exercise." Am J Physiol Cell Physiol **315**(4): C537-C543.

Albertus, Y., R. Tucker, A. St Clair Gibson, E. V. Lambert, D. B. Hampson and T. D. Noakes (2005). "Effect of distance feedback on pacing strategy and perceived exertion during cycling." Medicine and Science in Sports and Exercise **37**(3): 461-468.

Anderson, L., P. Orme, R. Naughton, G. Close, J. Milsom, D. Rydings, A. O'Boyle, R. Di Michele, J. Louis, C. Hambley, J. Speakman, R. Morgans, B. Drust and J. Morton (2017). "Energy intake and expenditure of professional soccer players of the English Premier League: evidence of carbohydrate periodization." International Journal of Sport Nutrition and Exercise Metabolism **4**: 1-25.

Anderson, L., P. Orme, R. J. Naughton, G. L. Close, J. Milsom, D. Rydings, A. O'Boyle, R. Di Michele, J. Louis, C. Hambley, J. R. Speakman, R. Morgans, B. Drust and J. P. Morton (2017). "Energy Intake and Expenditure of Professional Soccer Players of the English Premier League: Evidence of Carbohydrate Periodization." Int J Sport Nutr Exerc Metab: 1-25.

Areces, F., J. J. Salinero, J. Abian-Vicen, C. Gonzalez-Millan, C. Gallo-Salazar, D. Ruiz-Vicente, B. Lara and J. Del Coso (2014). "A 7-day oral supplementation with branched-chain amino acids was ineffective to prevent muscle damage during a marathon." Amino Acids **46**(5): 1169-1176.

Areta, J. L., L. M. Burke, M. L. Ross, D. M. Camera, D. W. West, E. M. Broad, N. A. Jeacocke, D. R. Moore, T. Stellingwerff, S. M. Phillips, J. A. Hawley and V. G. Coffey (2013). "Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis." J Physiol **591**(Pt 9): 2319-2331.

Baar, K. (2015). "TRAINING AND NUTRITION TO PREVENT SOFT TISSUE INJURIES AND ACCELERATE RETURN TO PLAY." Sports Science Exchange **28**(142): 1-6.

Beelen, M., R. Koopman, A. P. Gijsen, H. Vandereyt, A. K. Kies, H. Kuipers, W. H. Saris and L. J. van Loon (2008). "Protein coingestion stimulates muscle protein synthesis during resistance-type exercise." Am J Physiol Endocrinol Metab **295**(1): E70-77.

Betts, J. A. and E. Stevenson (2011). "Should protein be included in CHO-based sports supplements?" Med Sci Sports Exerc **43**(7): 1244-1250.

Biolo, G., S. P. Maggi, B. D. Williams, K. D. Tipton and R. R. Wolfe (1995). "Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans." Am J Physiol **268**(3 Pt 1): E514-520.

Biolo, G., K. D. Tipton, S. Klein and R. R. Wolfe (1997). "An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein." Am J Physiol **273**(1 Pt 1): E122-129.

Burd, N. A., D. W. West, D. R. Moore, P. J. Atherton, A. W. Staples, T. Prior, J. E. Tang, M. J. Rennie, S. K. Baker and S. M. Phillips (2011). "Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men." J Nutr **141**(4): 568-573.

Burke, L. M., J. A. Hawley, M. L. Ross, D. R. Moore, S. M. Phillips, G. R. Slater, T. Stellingwerff, K. D. Tipton, A. P. Garnham and V. G. Coffey (2012). "Preexercise aminoacidemia and muscle protein synthesis after resistance exercise." Med Sci Sports Exerc **44**(10): 1968-1977.

Celebrini, R. G., J. J. Eng, W. C. Miller, C. L. Ekegren, J. D. Johnston, T. A. Depew and D. L. Macintyre (2014). "Effect of a novel movement strategy in decreasing ACL risk factors in female adolescent soccer players: a randomized controlled trial." Clin J Sport Med **24**(2): 134-141.

Celebrini, R. G., J. J. Eng, W. C. Miller, C. L. Ekegren, J. D. Johnston and D. L. MacIntyre (2012). "The effect of a novel movement strategy in decreasing ACL risk factors in female adolescent soccer players." J Strength Cond Res **26**(12): 3406-3417.

Cermak, N. M., P. T. Res, L. C. de Groot, W. H. Saris and L. J. van Loon (2012). "Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis." Am J Clin Nutr **96**(6): 1454-1464.

Churchward-Venne, T. A., L. Breen, D. M. Di Donato, A. J. Hector, C. J. Mitchell, D. R. Moore, T. Stellingwerff, D. Breuille, E. A. Offord, S. K. Baker and S. M. Phillips (2014). "Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial." Am J Clin Nutr **99**(2): 276-286.

Churchward-Venne, T. A., C. H. Murphy, T. M. Longland and S. M. Phillips (2013). "Role of protein and amino acids in promoting lean mass accretion with resistance exercise and attenuating lean mass loss during energy deficit in humans." Amino Acids **45**(2): 231-240.

Comerford, K. B. and G. Pasin (2016). "Emerging Evidence for the Importance of Dietary Protein Source on Glucoregulatory Markers and Type 2 Diabetes: Different Effects of Dairy, Meat, Fish, Egg, and Plant Protein Foods." Nutrients **8**(8).

De Ste Croix, M. B., A. M. Priestley, R. S. Lloyd and J. L. Oliver (2015). "ACL injury risk in elite female youth soccer: Changes in neuromuscular control of the knee following soccer-specific fatigue." Scand J Med Sci Sports **25**(5): e531-538.

Devries, M. C., A. Sithamparapillai, K. S. Brimble, L. Banfield, R. W. Morton and S. M. Phillips (2018). "Changes in Kidney Function Do Not Differ between Healthy Adults Consuming Higher- Compared with Lower- or Normal-Protein Diets: A Systematic Review and Meta-Analysis." J Nutr **148**(11): 1760-1775.

Gorissen, S. H. M., J. J. R. Crombag, J. M. G. Senden, W. A. H. Waterval, J. Bierau, L. B. Verdijk and L. J. C. van Loon (2018). "Protein content and amino acid composition of commercially available plant-based protein isolates." Amino Acids **50**(12): 1685-1695.

Groen, B. B., A. M. Horstman, H. M. Hamer, M. de Haan, J. van Kranenburg, J. Bierau, M. Poeze, W. K. Wodzig, B. B. Rasmussen and L. J. van Loon (2015). "Post-Prandial Protein Handling: You Are What You Just Ate." PLoS One **10**(11): e0141582.

Hawkins, R. D., M. A. Hulse, C. Wilkinson, A. Hodson and M. Gibson (2001). "The association football medical research programme: an audit of injuries in professional football." Br J Sports Med **35**(1): 43-47.

Heinemeier, K. M., P. Schjerling, J. Heinemeier, S. P. Magnusson and M. Kjaer (2013). "Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C." FASEB J **27**(5): 2074-2079.

Hoff, J. (2005). "Training and testing physical capacities for elite soccer players." J Sports Sci **23**(6): 573-582.

Hoff, J. and J. Helgerud (2004). "Endurance and strength training for soccer players: physiological considerations." Sports Med **34**(3): 165-180.

Howatson, G., M. Hoad, S. Goodall, J. Tallent, P. G. Bell and D. N. French (2012). "Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study." J Int Soc Sports Nutr **9**: 20.

Hyde, R., P. M. Taylor and H. S. Hundal (2003). "Amino acid transporters: roles in amino acid sensing and signalling in animal cells." Biochem J **373**(Pt 1): 1-18.

Institute of Medicine (IOM) Food and Nutrition Board, e. (2005). "Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. ." Washington, D.C. The National Academies Press. **https://fnic.nal.usda.gov/dietary-guidance/dri-nutrient-reports/energy-carbohydrate-fiber-fat-fatty-acids-cholesterol-protein**.

James, L. J., L. Mattin, P. Aldiss, R. Adebishi and R. M. Hobson (2014). "Effect of whey protein isolate on rehydration after exercise." Amino Acids **46**(5): 1217-1224.

Kjaer, M., H. Langberg, K. Heinemeier, M. L. Bayer, M. Hansen, L. Holm, S. Doessing, M. Kongsgaard, M. R. Krogsgaard and S. P. Magnusson (2009). "From mechanical loading to collagen synthesis, structural changes and function in human tendon." Scand J Med Sci Sports **19**(4): 500-510.

Koopman, R., A. J. Wagenmakers, R. J. Manders, A. H. Zorenc, J. M. Senden, M. Gorselink, H. A. Keizer and L. J. van Loon (2005). "Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects." Am J Physiol Endocrinol Metab **288**(4): E645-653.

Lanham-New, S. A., I. A. MacDonald and H. M. Roche (2011). Nutrition and Metabolism, Wiley.

Lemon, P. W. (1994). "Protein requirements of soccer." J Sports Sci **12 Spec No**: S17-22.

Macnaughton, L. S., S. L. Wardle, O. C. Witard, C. McGlory, D. L. Hamilton, S. Jeromson, C. E. Lawrence, G. A. Wallis and K. D. Tipton (2016). "The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein." Physiol Rep 4(15).

Maughan, R. J. (1997). "Energy and macronutrient intakes of professional football (soccer) players." Br J Sports Med **31**(1): 45-47.

Mettler, S., N. Mitchell and K. D. Tipton (2010). "Increased protein intake reduces lean body mass loss during weight loss in athletes." Med Sci Sports Exerc **42**(2): 326-337.

Michelfelder, A. J. (2009). "Soy: a complete source of protein." Am Fam Physician **79**(1): 43-47.

Milsom, J., P. Barreira, D. J. Burgess, Z. Iqbal and J. P. Morton (2014). "Case study: Muscle atrophy and hypertrophy in a premier league soccer player during rehabilitation from ACL injury." Int J Sport Nutr Exerc Metab **24**(5): 543-552.

Moore, D. R., T. A. Churchward-Venne, O. Witard, L. Breen, N. A. Burd, K. D. Tipton and S. M. Phillips (2015). "Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men." J Gerontol A Biol Sci Med Sci **70**(1): 57-62.

Moore, D. R., M. J. Robinson, J. L. Fry, J. E. Tang, E. I. Glover, S. B. Wilkinson, T. Prior, M. A. Tarnopolsky and S. M. Phillips (2009). "Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men." Am J Clin Nutr **89**(1): 161-168.

Morton, R. W., C. McGlory and S. M. Phillips (2015). "Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy." Front Physiol **6**: 245.

Pasiakos, S. M., S. Agarwal, H. R. Lieberman and V. L. Fulgoni (2015). Sources and Amounts of Animal, Dairy, and Plant Protein Intake of US Adults in 2007–2010. Nutrients. **7:** 7058-7069.

Paxton, J. Z., L. M. Grover and K. Baar (2010). "Engineering an in vitro model of a functional ligament from bone to bone." Tissue Eng Part A **16**(11): 3515-3525.

Philips, S. (2013). "Protein Consumption and Resistance Exercise: Maximizing Anabolic Potential." Sports Science Exchange **26**(107): 1-5.

Phillips, S. M. (2004). "Protein requirements and supplementation in strength sports." Nutrition **20**(7-8): 689-695.

Phillips, S. M. (2013). "Protein Consumption and Resistance Exercise: Maximizing Anabolic Potential." Sports Science Exchange **26**(107): 1-5.

Phillips, S. M. (2014). "A brief review of higher dietary protein diets in weight loss: a focus on athletes." Sports Med **44 Suppl 2**: S149-153.

Phillips, S. M. and L. J. Van Loon (2011). "Dietary protein for athletes: from requirements to optimum adaptation." J Sports Sci **29 Suppl 1**: S29-38.

Reidy, P. T., D. K. Walker, J. M. Dickinson, D. M. Gundermann, M. J. Drummond, K. L. Timmerman, C. S. Fry, M. S. Borack, M. B. Cope, R. Mukherjea, K. Jennings, E. Volpi and B. B. Rasmussen (2013). "Protein blend ingestion following resistance exercise promotes human muscle protein synthesis." J Nutr **143**(4): 410-416.

Remond, D., M. Machebeuf, C. Yven, C. Buffiere, L. Mioche, L. Mosoni and P. Patureau Mirand (2007). "Postprandial whole-body protein metabolism after a meat meal is influenced by chewing efficiency in elderly subjects." Am J Clin Nutr **85**(5): 1286-1292.

Rennie, M. J. and K. D. Tipton (2000). "Protein and amino acid metabolism during and after exercise and the effects of nutrition." Annu Rev Nutr **20**: 457-483.

Res, P. (2014). "RECOVERY NUTRITION FOR FOOTBALL PLAYERS." Sports Science Exchange **27** (129): 1-5.

Res, P. T., B. Groen, B. Pennings, M. Beelen, G. A. Wallis, A. P. Gijsen, J. M. Senden and V. A. N. L. LJ (2012). "Protein ingestion before sleep improves postexercise overnight recovery." Med Sci Sports Exerc **44**(8): 1560-1569.

Shaw, G., A. Lee-Barthel, M. L. Ross, B. Wang and K. Baar (2016). "Vitamin C-enriched gelatin supplementation before intermittent activity augments collagen synthesis." Am J Clin Nutr.

Silk, D. B., G. K. Grimble and R. G. Rees (1985). "Protein digestion and amino acid and peptide absorption." Proc Nutr Soc **44**(1): 63-72.

Snijders, T., P. T. Res, J. S. Smeets, S. van Vliet, J. van Kranenburg, K. Maase, A. K. Kies, L. B. Verdijk and L. J. van Loon (2015). "Protein Ingestion before Sleep Increases Muscle Mass and Strength Gains during Prolonged Resistance-Type Exercise Training in Healthy Young Men." J Nutr **145**(6): 1178-1184.

Stokes, T., A. J. Hector, R. W. Morton, C. McGlory and S. M. Phillips (2018). "Recent Perspectives Regarding the Role of Dietary Protein for the Promotion of Muscle Hypertrophy with Resistance Exercise Training." Nutrients **10**(2).

Tang, J. E., D. R. Moore, G. W. Kujbida, M. A. Tarnopolsky and S. M. Phillips (2009). "Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men." J Appl Physiol (1985) **107**(3): 987-992.

Tipton, K. (2017). "Branched-chain amino acid supplementation to support muscle anabolism following exercise." Sports Science Exchange **28,** (170): 1-6.

Tipton, K. D. (2007). "Role of protein and hydrolysates before exercise." Int J Sport Nutr Exerc Metab **17 Suppl**: S77-86.

Trommelen, J., A. M. Holwerda, I. W. Kouw, S. L. Halson, L. B. Verdijk and L. J. van Loon (2016). "Protein Ingestion Before Sleep Provides Precursors For Post-exercise Overnight De Novo Muscle Protein Synthesis: 88 Board #4 June 1, 9: 30 AM - 11: 30 AM." Med Sci Sports Exerc **48**(5 Suppl 1): 4.

Trommelen, J., I. W. K. Kouw, A. M. Holwerda, T. Snijders, S. L. Halson, I. Rollo, L. B. Verdijk and L. J. C. van Loon (2018). "Presleep dietary protein-derived amino acids are incorporated in myofibrillar protein during postexercise overnight recovery." Am J Physiol Endocrinol Metab **314**(5): E457-E467.

van Loon, L. J. (2012). "Leucine as a pharmaconutrient in health and disease." Curr Opin Clin Nutr Metab Care **15**(1): 71-77.

van Loon, L. J., W. H. Saris, M. Kruijshoop and A. J. Wagenmakers (2000). "Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures." Am J Clin Nutr **72**(1): 106-111.

Volpi, E., H. Kobayashi, M. Sheffield-Moore, B. Mittendorfer and R. R. Wolfe (2003). "Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults." Am J Clin Nutr **78**(2): 250-258.

Wall, B. T., N. A. Burd, R. Franssen, S. H. Gorissen, T. Snijders, J. M. Senden, A. P. Gijsen and L. J. van Loon (2016). "Presleep protein ingestion does not compromise the muscle protein synthetic response to protein ingested the following morning." Am J Physiol Endocrinol Metab **311**(6): E964-E973.

Wall, B. T., J. P. Morton and L. J. van Loon (2015). "Strategies to maintain skeletal muscle mass in the injured athlete: nutritional considerations and exercise mimetics." Eur J Sport Sci **15**(1): 53-62.

Witard, O. C., S. R. Jackman, L. Breen, K. Smith, A. Selby and K. D. Tipton (2014). "Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise." Am J Clin Nutr **99**(1): 86-95.

Witard, O. C., S. R. Jackman, A. K. Kies, A. E. Jeukendrup and K. D. Tipton (2011). "Effect of increased dietary protein on tolerance to intensified training." Med Sci Sports Exerc **43**(4): 598-607.

Witard, O. C., J. E. Turner, S. R. Jackman, A. K. Kies, A. E. Jeukendrup, J. A. Bosch and K. D. Tipton (2014). "High dietary protein restores overreaching induced impairments in leukocyte trafficking and reduces the incidence of upper respiratory tract infection in elite cyclists." Brain Behav Immun **39**: 211-219.

Zouita, S., A. B. Zouita, W. Kebsi, G. Dupont, A. Ben Abderrahman, F. Z. Ben Salah and H. Zouhal (2016). "Strength Training Reduces Injury Rate in Elite Young Soccer Players During One Season." J Strength Cond Res **30**(5): 1295-1307.