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 recognised as a key nutrient for muscle physiology. When discussing proteins for football, the common response is that proteins are needed for muscle growth and repair, which is indeed true. However, proteins do not play just a structural role: they also have many other functions. For instance, proteins participate in transporting molecules such as oxygen; they speed up chemical reactions in metabolic pathways; they are part of the immune system, and they may trigger 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 it 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, which are summarised in this unit.

Proteins are essential for movements generated by muscle contractions. Muscle contractions are possible thanks to the action of certain proteins, actin and myosin, which interact to produce mechanical force within skeletal muscles.

Antibodies produced by the immune system are also proteins, which can recognise and neutralise 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 function to facilitate/speed chemical reactions in the player's 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.

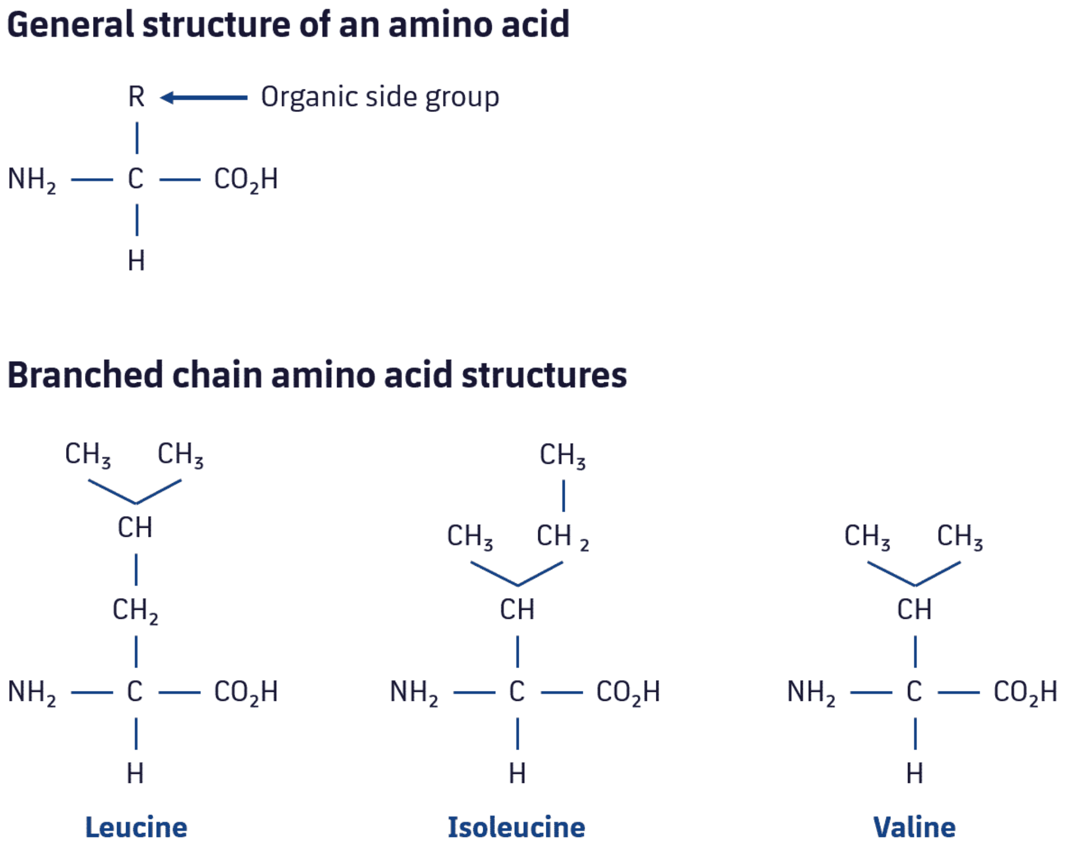
Figure 1. General protein functions in a player's body

Source: own source.

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



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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 & Tipton, 2000). In the post-absorptive state, protein synthesis is reduced, and protein breakdown is accelerated.

Table 1. Amino acid classification

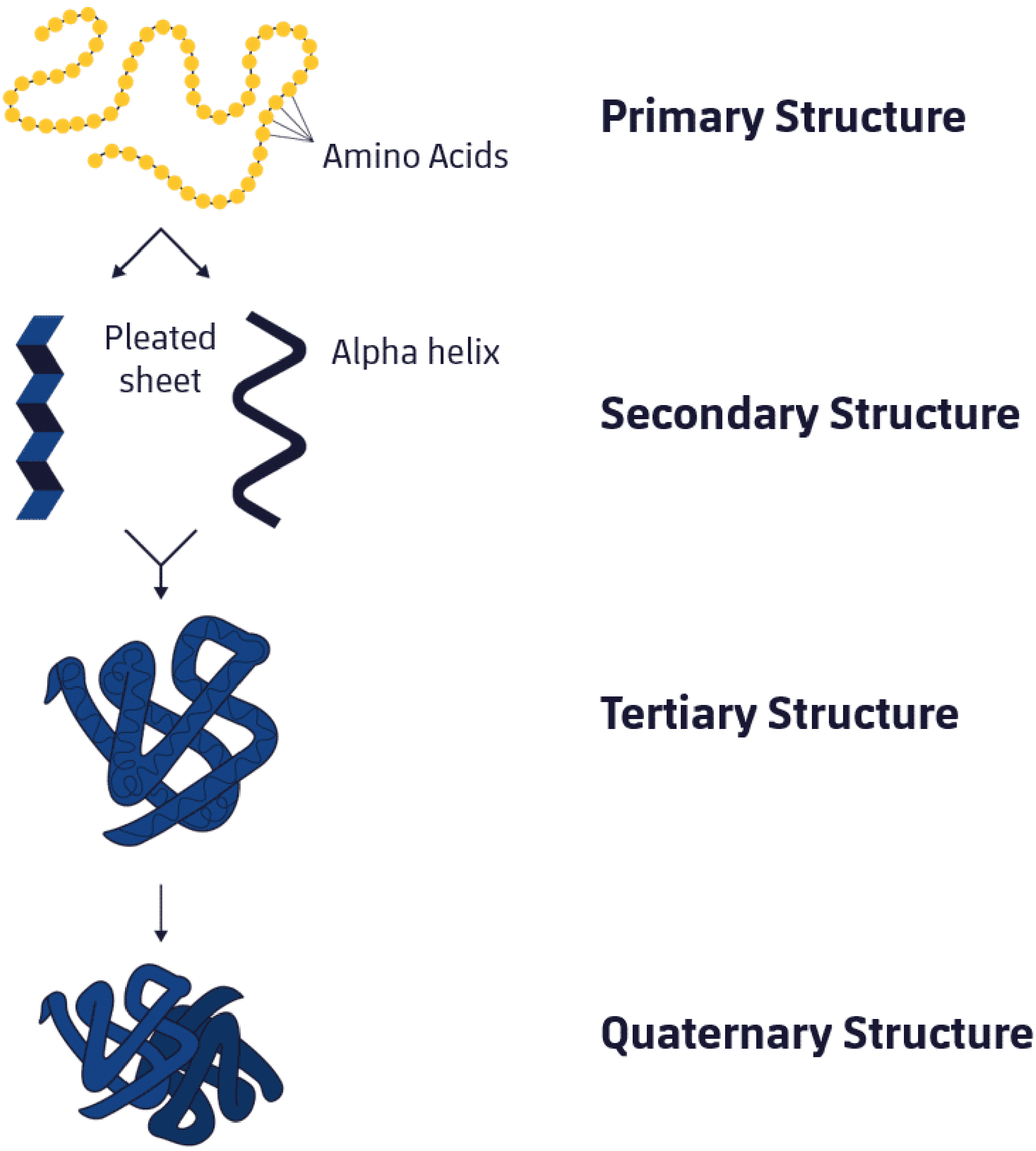
Essential amino acids	Non-essential amino acids
	Alanine
Histidine	Arginine
Isoleucine	Asparagine
Leucine	Aspartate
Lysine	Cysteine
Methionine	Glutamate
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
	Tyrosine

Source: own source.

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 organised into secondary structures, by folding in two common forms known as the alpha-helix or beta pleated sheets. Proteins' complexity increases as they organise into tertiary structures to acquire a three-dimensional shape (see figure 3). 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 3. Protein structure



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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 4.

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; Churchward-Venne *et al*., 2012).

Supplementation with branched-chain amino acids has been reported to inhibit muscle protein breakdown at rest; nevertheless, 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 *et al*., 2012). However, not all studies support these observations (Areces *et al*., 2014); thus, more research is required to recommend branched-chain amino acids, specifically for the recovery from football exercise.

Did you know?

An artificial intelligence (AI) programme called AlphaFold has been used to predict the structures of almost every protein made by the human body. This tool has accelerated the mapping and subsequent understanding of the human proteome.

Leucine

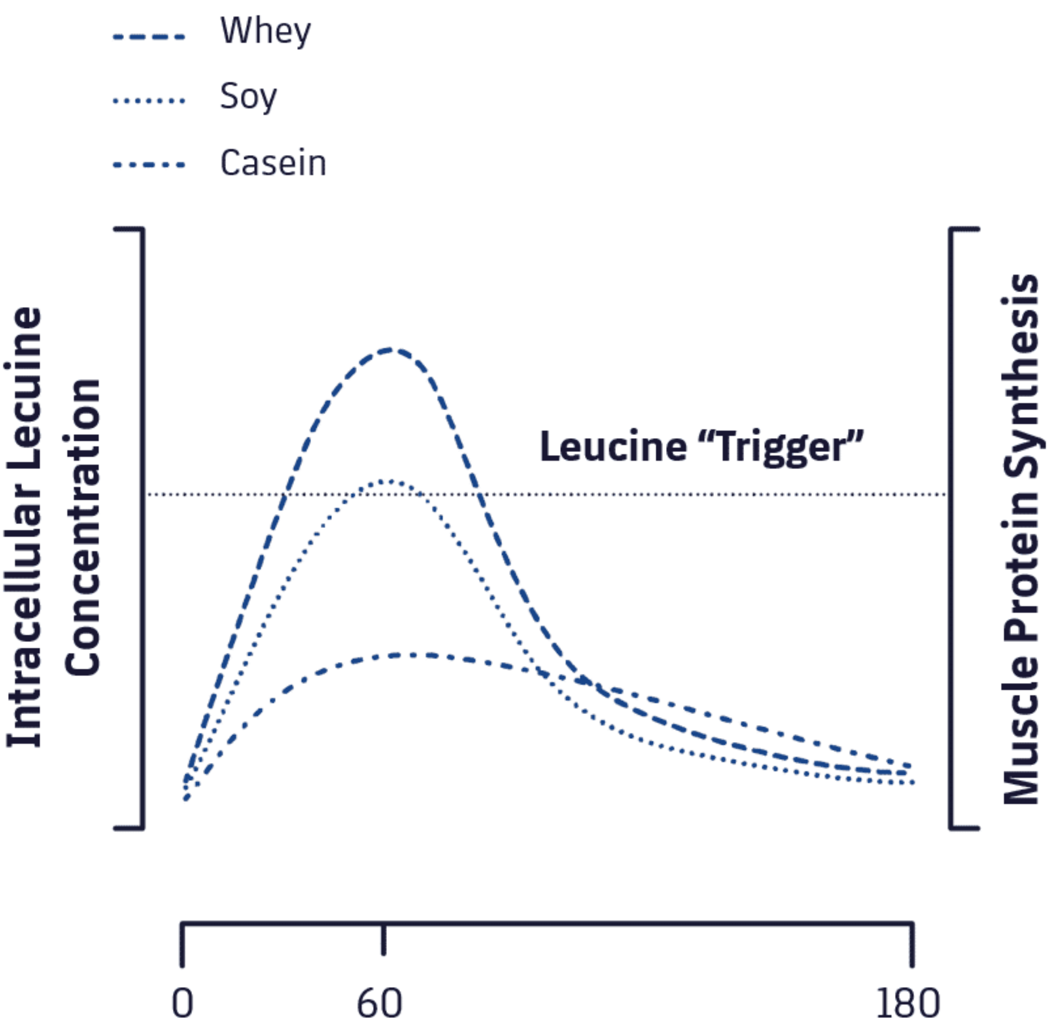
All amino acids are believed to have a role, to a lesser or larger extent, on amino acid-induced stimulation of muscle protein synthesis (Volpi *et al*., 2003). However, leucine has emerged as a key nutritional regulator of muscle protein synthesis (Koopman *et al*., 2005; Philips, 2013). Studies have reported a greater stimulation of muscle protein synthesis when amino acid mixtures are enriched with leucine (Koopman *et al*., 2005) and proteins naturally rich in leucine in comparison to 'low-leucine' proteins (Tang *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 *et al*., 2012). The dose and quantity and type of protein recommended for football is discussed in Unit 2.

Did you know?

Professor Kevin D. Tipton (1961-2022) was a leader in the field of protein metabolism and exercise. He was a great scientist and valued mentor for many students in the field of sports nutrition. Much of our understanding and recommendations on protein ingestion are based on his research.

Figure 4. Leucine 'trigger' hypothesis



Source: adapted 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 *et al*., 2005; Institute of Medicine (IOM) Food and Nutrition Board, 2005), but this is likely higher for football players (Collins *et al*., 2021 [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 *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, except for soy, most plant proteins are limited in one or more of the following amino acids: lysine, threonine, tryptophan, cysteine, or methionine (Comerford & 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 *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 5. Examples of foods rich in protein

Source: own source.

Table 2. Sources of leucine in food

Food	Leucine g/100 g
Soy protein concentrates	4,92
Soybeans, raw	2,97
Minced 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: adapted from the Composition of Foods Raw, Processed, Prepared USDA National Nutrient Database for Standard Reference, 2008. Release 21. http://www.ars.usda.gov/nutrientdata

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 before swallowing (Remond *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 *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.

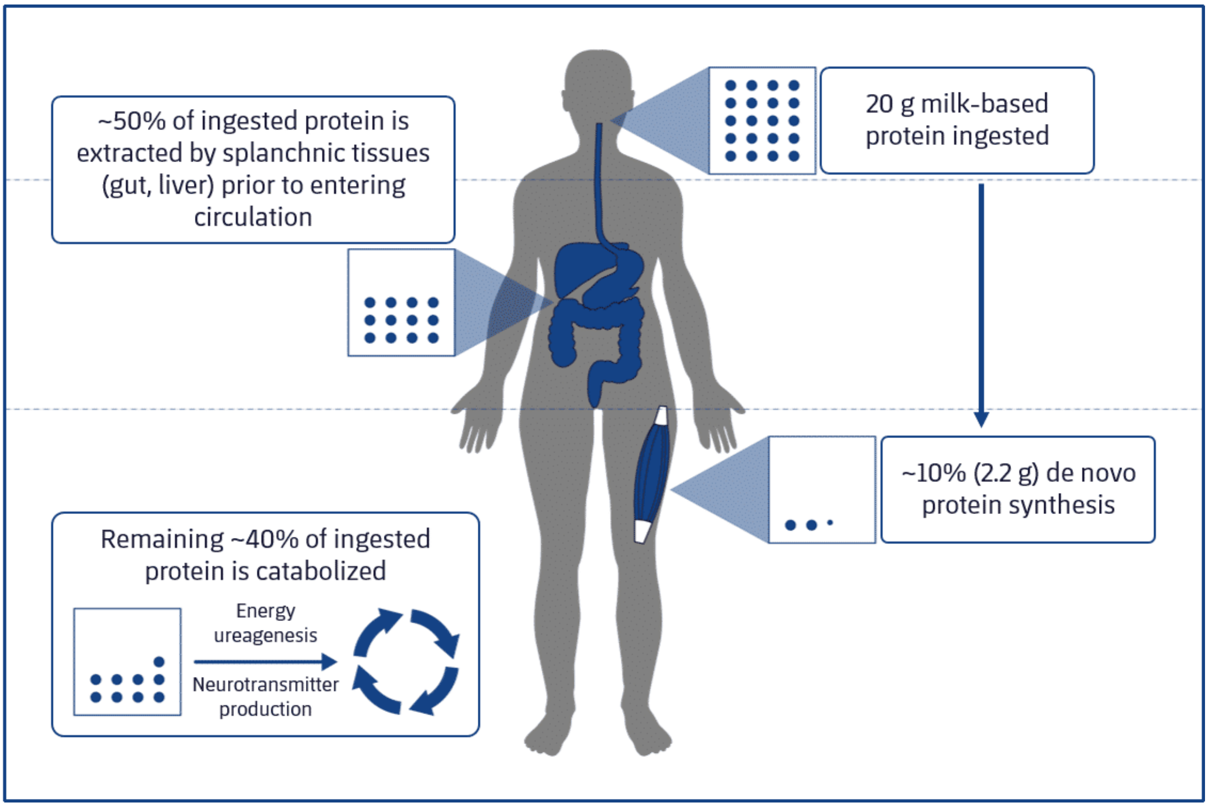
Many of the amino acids absorbed following protein digestion (~40-50%) are used by the intestinal cells for energy or synthesis of proteins, whereas the remainder of amino acids are released into the hepatic portal vein before being taken up by the liver (Stokes *et al*., 2018). The liver is the other 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 use (Groen *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 *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 used to build new skeletal muscle tissue (Stokes *et al.*, 2018) (see figure 7).

Figure 6. A summary of protein digestion and absorption and the corresponding availability and use of protein following the ingestion of 20 g



Source: adapted from Stokes *et al.*, 2018.

Summary

- Proteins in the player's body are made up of smaller units, called amino acids.
- Proteins have numerous 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

The physical demands of football training and matches stresses the player's musculoskeletal and tendinous tissues. Therefore, these protein-containing structures require constant repair and remodelling to either maintain or improve their integrity and function. Muscle is an important tissue being responsible for 25-30% of whole-body protein metabolism. As such, football players will benefit from ingesting quantities of protein that are higher than that needed by the general population. New amino acids are delivered through nutrition, whilst excess amino acids are either oxidised or metabolised to fatty acids or glucose. The recommended daily allowance for protein in Europe is 0.8 g/kg BM/day. However, increasing protein intakes up to 1.6–2.2 g/kg BM/day may be required to enhance training adaptations (Morton *et al.*, 2018), speed recovery and support player immunity (Walsh *et al.*, 2019).

Protein turnover

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. Many of the adaptations we are interested in to support football performance occur within the muscle.

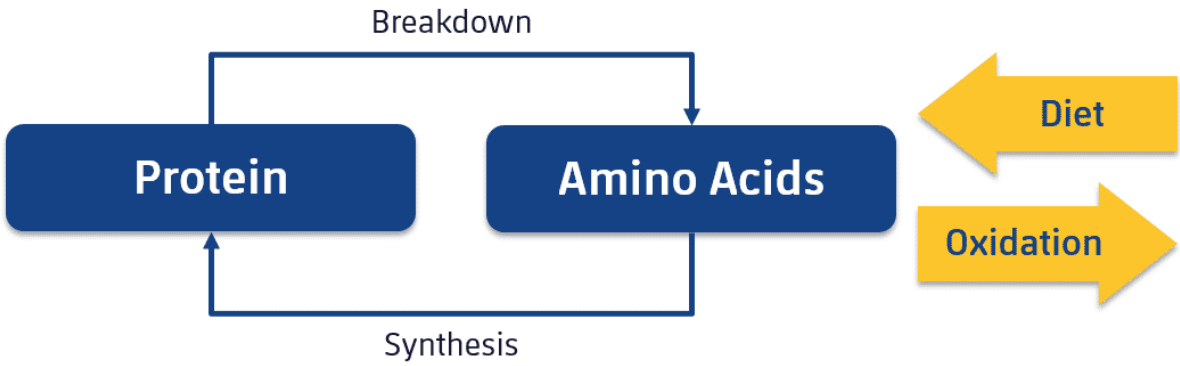
Proteins inside the player's body are continuously synthesised and degraded in a process called protein turnover. It is necessary for cellular components to be replaced and finely regulated in response to changes in cellular environment. This process of protein turnover is highly dynamic and, as such,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

Skeletal muscle has a turn-over rate of approximately 1-2% per day. Therefore, the leg muscles, for example, used to transport the player, pass the ball, and score a goal, would theoretically be completely ‘new’ in a 6-week period. Thus, over the competitive season, a player's entire muscular system may be broken down and rebuilt approximately six times (Rollo & van Loon, 2018).

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 (Phillips, 2013).

Research has mostly focused on protein synthesis, but both processes (breakdown and synthesis) are important (see figure 8). 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 & Van Loon, 2011).

Figure 7. Summary of protein turnover



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Factors influencing muscle protein synthesis

In a fasted state, protein breakdown is greater than protein synthesis (Biolo *et al.*, 1995). In response to protein or amino acid feeding, protein synthesis will increase, and the net protein balance will become positive (Biolo *et al.*, 1997). It is the contribution of these fed and fasted periods to overall protein balance that dictates long-term muscle remodelling and adaptation (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, as well as other potential injuries (Res, 2014). The main factors that affect the muscle protein synthetic response after football exercise are listed, then discussed below:

- The demands of football
- The quantity of protein ingested
- The timing of protein intake
- The frequency of protein intake

- The type of protein ingested
- Co-ingestion of protein with other nutrients

The demands of football

Exercise training for football is an important stimulator of protein synthesis, with the exercise mode dictating the type of proteins that are synthesised (Phillips & Van Loon, 2011). 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 a dedicated review by Hoff & 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 complement the player's training programme to improve markers of performance and to reduce the risk of injury (Hoff, 2005; Zouita *et al.*, 2016).

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 *et al.*, 2012). Concurrent exercise includes both resistance- and endurance-type elements to the training. Importantly, we know now that protein ingested after concurrent exercise (similar to football) is used to synthesise both endurance proteins (mitochondria) and contractile proteins (actin and myosin) (Churchward-Venne *et al.*, 2019). Therefore, protein ingestion is required to support those adaptations required to cope with the demands of the game.

The quantity of protein ingested

It is important to note that the quantity of protein required by the player can easily be achieved from a varied diet provided; the energy intake is sufficient to meet the demands of training (course 1). 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 *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 *et al.*, 2014).

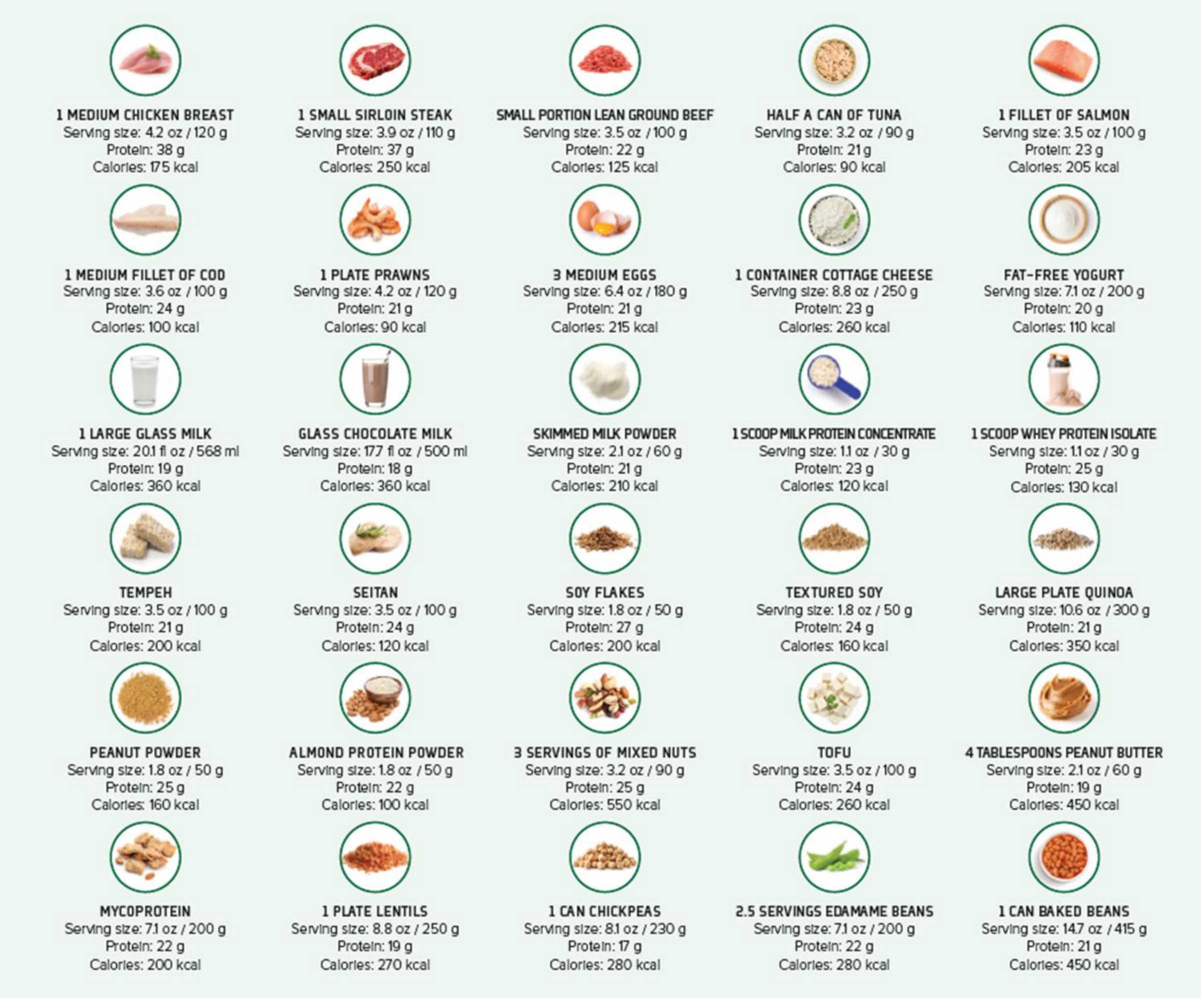
Moore *et al*. (2015) used a statistical approach to arrive at a figure of 0.25 g of protein per kg of the player's body mass per meal (Moore *et al*., 2015). However, Macnaughton *et al*. (2016) raised new questions. In this study, participants performed whole-body exercise as opposed to single muscle groups as in the previous studies. It was reported that 40 g of protein resulted in greater protein synthesis than 20 g (Macnaughton *et al*., 2016). Most recently, it has been shown that the ingestion of 100 g protein results in a greater and more prolonged (>12 h) anabolic response in comparison to the ingestion of 25 g protein (Trommelen *et al*., 2023). Therefore, quite simply, there is a dose-response relationship between the ingestion of dietary protein and plasma amino acid availability, with subsequent incorporation into muscle protein. From a purely scientific perspective, ingesting larger blouses of protein after exercise will further increase whole-body protein net balance, mixed-muscle, myofibrillar, muscle connective, and plasma protein synthesis rates (Trommelen *et al*., 2023).

These latest scientific studies may suggest that we have underestimated the protein requirements for optimal protein synthesis following football, that engages whole-body muscle mass for 90 min. However, a general and practical recommendation is for players to continue to ingest (0.25-0.30 g/kg BM) 20-30 g of protein following most intense footballing occasions, when engaged in whole body exercise (Collins *et al*., 2021).

This is because intakes of 30-40 g of protein (and beyond) represent a large protein 'load'. Most players would struggle to consistently ingest this quantity of protein after exercise. The ingestion of approximately 20 g of protein combined with carbohydrates and fluid, helps kick-start the recovery process, whilst achieving other nutrient needs. Thus, protein drinks provide a convenient and easily digestible alternative to foods, especially in the post-training period. This approach can then be followed up by secondary recovery strategies, specifically the ingestion of food before leaving the training ground or stadium. Following this approach, dietary surveys in professional players meet or exceed the 1.6–2.2 g/kg BM/day protein intake recommended for football (Anderson *et al*., 2017).

In any situation where players prefer to avoid protein shakes post exercise, the advice is to focus on other recovery priorities. These players' food choices from the options provided should be monitored to ensure they are making good choices, contributing towards protein recommendations.

Figure 8. Dietary sources providing ~20 g of protein



Source: adapted from Collins *et al.*, 2021.

Table 3. A guide to protein consumption per serve (meal occasion) based on body mass (BM)

Body Mass		Recommended Protein Intake (g) Per Serve, Depending on Body Mass	
kg	lb	0.25 g/kg BM	0.3 g/kg BM
60	132	15	18
65	143	16	20
70	154	18	21
75	165	19	23
80	176	20	24
85	187	21	26
90	198	23	27
95	209	24	29
100	221	25	30
105	232	26	32
110	243	28	33

Source: adapted from Collins *et al.*, 2021.

Timing of protein for football

It is often suggested that it is critical to ingest protein immediately post-exercise when there is a ‘window of opportunity’ for optimal protein synthesis. This is based on studies which have suggested that post-exercise rates of protein synthesis are higher when protein is ingested immediately after exercise. Nonetheless, this ‘window’ may not be as critical as often suggested (Morton *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 *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 *et al.*, 2008). Therefore, timing may not be as critical as thought. From a purely pragmatic point of view, it still makes 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 number of fluids, carbohydrates, and proteins to start the 3 Rs of recovery (rehydration, refuel, repair).

A practical consideration is that ingesting protein ‘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 offering protein to be ingested. This principle is the same for training situations as well as immediately after a match. Put yourself in the player's position. Next time you have a really hard training session, try the nutrition intervention you are recommending.

As post-exercise nutritional requirements are multifactorial (rehydration, glycogen resynthesis, myofibrillar/mitochondrial protein synthesis), it is important to remember that post-exercise protein intake can be achieved without compromising the rehydration of the player (James *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?

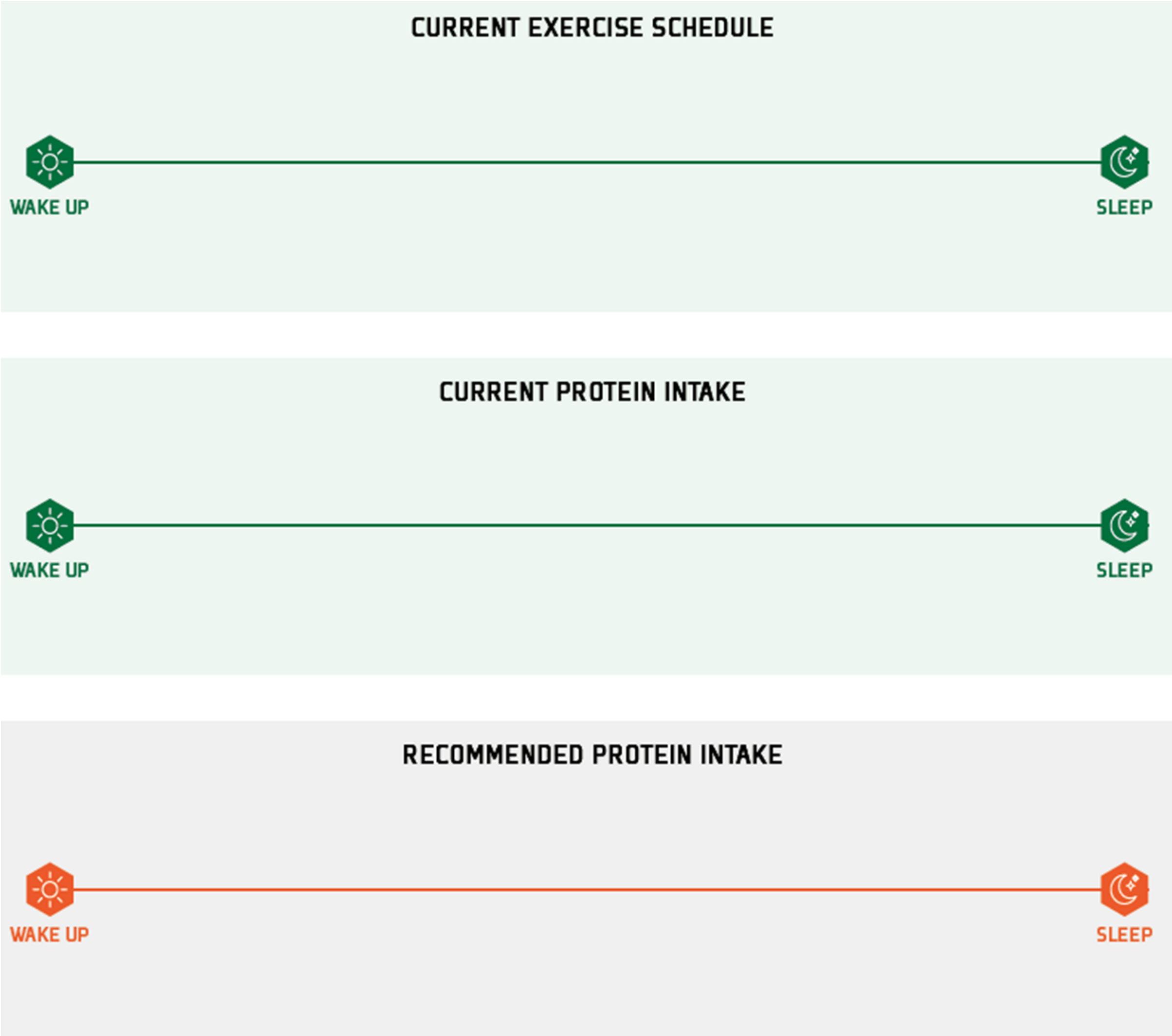
The blood flow (cardiac output) is redistributed during exercise. During intense exercise, approximately 80% of blood will be distributed to the working muscles. Blood flow to the gastrointestinal system will be reduced to approximately 5% of cardiac output compared to 25% at rest.

The frequency of protein intake

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 contained 20 g every 3 hours appeared to be optimal (Areta *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. Based on available evidence, it is advised that players ingest 3-4 meals, every 3-4 hours, containing adequate protein (20-40 g). If we consider the dose-response relationship to protein ingestion discussed previously, eating 20 g of protein on 5 occasions (breakfast, lunch, post exercise, dinner, before bed) achieves the large single dose of 100 g.

Evidence suggests that one of the protein-containing meals could be before bedtime. Protein ingestion before sleep increases both mitochondrial and myofibrillar protein synthesis rates during overnight recovery from exercise (Trommelen *et al*., 2023). Normally, during sleep (a period of fasting) protein breakdown will exceed protein synthesis. Ingesting proteins (30-45 g protein) just before bed has been shown to turn this period of sleep into a period of net protein synthesis (Res *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 *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 *et al*., 2016). It is important to note that Wall *et al*. (2016) have reported that pre-sleep protein ingestion does not compromise the muscle protein synthetic response to protein ingested the following morning (Wall *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 *et al*., 2017). For example, Abbott et al. (2018) 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 *et al*., 2018). Sports nutrition advice was initially focused on the ingestion casein protein before sleep. However, new evidence suggests that both whey and casein protein are equally effective in promoting an overnight synthetic response in the muscle (Trommelen *et al*., 2023). Thus, the available information would suggest that players may benefit from developing a routine that includes ingesting a drink or meal containing 30-45 g of protein before bedtime (Trommelen *et al*., 2018). This may be a particularly potent strategy to introduce during periods of fixture congestion or pre-season.

Figure 9. Dietary analysis tool to assess actual and optimal timing of protein intake



Source: own creation.

Type of protein for football

One of the most 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 *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 maximise 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 *et al.*, 2013).

Indeed, leucine concentrations above a certain concentration (in muscle) triggers protein synthesis. It may be important to reach this level for protein synthesis to be stimulated. Therefore, at rest, meals may ideally contain this amount of leucine (which is believed to be around 3 g) (Churchward-Venne *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 *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.

Interestingly, we have reported that the post-exercise myofibrillar and mitochondrial synthesis rates do not differ after co-ingestion of carbohydrate with 20 g of protein from whey, soy, or leucine-enriched soy protein (Churchward-Venne *et al.*, 2019). These results were obtained during 360 min of recovery from concurrent resistance- and endurance-type exercise in young, recreationally active men. Therefore, following football exercise, it is likely that the muscle is primed for adaptation, and as such the sports nutritionist priority is to provide a sufficient quantity of protein, which can be modified depending on the players' preference (Gorissen *et al.*, 2018).

Did you know?

Isocaloric simply means having the same calorific/calorie value.

Table 4. A guide to protein consumption per day based on body mass (BM)

		Increasing Exercise Intensities →								
Body Mass		Recommended Protein Intake (g) Per Day Based on Body Mass								
kg	lb	1.2 g/kg BM	1.3 g/kg BM	1.4 g/kg BM	1.5 g/kg BM	1.6 g/kg BM	1.7 g/kg BM	1.8 g/kg BM	1.9 g/kg BM	2.0 g/kg BM
60	132	72	78	84	90	96	102	108	114	120
65	143	78	85	91	98	104	111	117	124	130
70	154	84	91	98	105	112	119	126	133	140
75	165	90	98	105	113	120	128	135	143	150
80	176	96	104	112	120	128	136	144	152	160
85	187	102	111	119	128	136	145	153	162	170
90	198	108	117	126	135	144	153	162	171	180
95	209	114	124	133	143	152	162	171	181	190
100	221	120	130	140	150	160	170	180	190	200
105	232	126	137	147	158	168	179	189	200	210
110	243	132	143	154	165	176	187	198	209	220

Source: adapted from Collins *et al.*, 2021.

Co-ingestion of protein with other nutrients

The complete properties of foods (food synergies) and their influence on post-exercise muscle protein remodelling and repair has received little attention. When players ingest dietary protein, it is usually provided with more than just its constituent amino acids. Dietary protein typically contains other non-protein nutritive components that are likely to interact with nutrients, modulate nutrient behaviour, and/or act directly as signalling molecules. For example, yogurts may contain living organisms or live and active cultures, which synergistically enhance the health impact of the food source.

Did you know?

The food matrix describes the overall physical form of food and includes how various food components are structured and may interact.

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 *et al*., 2000; Betts & 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 at ingredients. By doing so, these studies will provide a better understanding on the importance of the nutrient density on muscle protein synthesis (Abou Sawan *et al*., 2018). For example, omega 3 fatty acids can increase the sensitivity of protein synthesis in response to protein ingestion (Module 3). Furthermore, there has been direct evidence that a food matrix rich in dietary proteins, vitamins, minerals, and other macronutrients (whole egg) improves the post-exercise muscle protein synthetic response when compared to eating protein isolates (egg whites) (van Vliet *et al*., 2017). Unfortunately, it is too early to draw firm conclusions on which meals would be best to stimulate protein synthesis. Thus, it is advised to build menus and provide foods which offer the more or less of the macronutrients discussed in this course. These menu building skills will be discussed in Course 4.

Daily protein intakes in football

Increasing a player's 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 *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 *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 *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 et al., 2014; Wall *et al*., 2015). Therefore, the daily protein intake for football players may range between 1.6 and 2.4 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 (Collins *et al*., 2021).

Table 5. Protein requirements for training

Occasion	Protein Targets	Principle
Daily intake	1.6-2.4 / kg BM / day	Meals containing 20-40 g protein ingested routinely (3 h intervals) during the day
Post Exercise	20-40 g high quality protein containing 2-3 g leucine	Ingested post exercise to support adaptation
Sleep	30 g	Ingest prior to sleep

Source: own source.

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 *et al*., 2001). The physiology of tendons and ligaments is different to muscle (Kjaer *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 was believed to be significantly lower than muscle. However, new evidence revealed that the basal protein synthesis rates in various musculoskeletal tissues including tendon, bone, cartilage, ligament are within the same range of skeletal muscle protein synthesis rates (Smeets *et al*., 2019). This 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 of the tendon, making the structure stronger.

Dietary collagen contains large amounts of glycine and proline and, therefore, has been proposed to provide the precursors required to facilitate connective tissue protein synthesis (Holwerda & van Loon LJC, 2022). Indeed, the ingestion of 15 g of gelatine has been reported to be effective in increasing circulating concentrations of the amino acids' glycine, proline, hydroxyproline, and hydroxylysine (Shaw *et al*., 2016). The ingestion of gelatine (15 g ingested with 50 mg vitamin C) 1 h before exercise also corresponded to increased blood markers (amino-terminal propeptide of collagen I) as an indirect indicator of collagen synthesis (Shaw *et al*., 2016). However, despite these early results large variability between individuals to this dietary intervention have been observed (Lis & Baar, 2019). Furthermore, studies that have directly biopsied muscle and connective tissue have reported that 30 g of collagen (nor 30 g of whey) protein ingestion after exercise did not further increase muscle connective protein synthesis rates in both male and female recreational athletes (Aussieker *et al*., 2023). Nonetheless, co-ingesting a small amount of collagen (5 g) with whey protein (25 g) after exercise is sufficient to prevent the decline in plasma glycine availability during recovery from lower body resistance-type exercise (Aussieker *et al*., 2024).

Although more research is required, the ingestion of gelatine is a promising nutritional intervention to improve both the function of connective tissues and speed the players' recovery from musculoskeletal injuries. Furthermore, this intervention may be a relevant sports nutrition strategy for footballers who experience a high incidence of ligament injury, such as female players (Celebrini *et al*., 2012; Celebrini *et al*., 2014; De Ste Croix *et al*., 2015). Timing appears to be an important consideration. Therefore, should this intervention be introduced, it is advised to ingest 15 g of collagen with vitamin C approximately one hour before training or matches (Paxton *et al*., 2010; Shaw *et al*., 2016). After training or matches other protein sources would be preferential, however including small amounts of collagen would not be detrimental.

Common misconceptions about protein

As dietary protein supplementation has gained in popularity, so have misconceptions, misinformation, and conflicting advice. Therefore, it is important for the sports nutritionist in football to provide accurate information. This knowledge will ensure players make informed decisions and staff provide sound advice. Historically, there has been 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 *et al*., 2018). Furthermore, there is no evidence that high-protein intake increases body fat mass in well-trained players. Instead, increases in fat mass are more likely the result of excess energy intake from carbohydrates and fats. There is no evidence that a high-protein diet is deleterious to player bone health, and may be beneficial. Finally, vegan or vegetarian players can meet their total daily energy and protein needs. However, it is important to note that these players will need to consume a greater quantity (+20–40% more) of plant protein to meet protein targets discussed in this module during periods of fixture congestion or pre-season (Antonio *et al*., 2024).

Summary

- Dietary protein is important for the recovery and adaptation process following football training and matches.
- Players' daily protein intake (1.6-2.4 g/kg BM) should be relative to their body mass, the phase of the season, and the amount of muscle mass used during exercise.
- The ingestion of protein (0.25-0.30 g/kg BM) following training and matches is recommended to support the remodelling of myofibrillar and mitochondrial proteins in the muscle.
- Protein intake should be spread evenly throughout the day (every 3-4 hours approximately) and it should be intensified post exercise.
- Protein feedings (~30-45 g) before bed offer an additional opportunity to support player recovery and enhance adaptation.

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