

OPTIMISING MUSCLE RECOVERY
AND MAXIMISING MUSCLE GROWTH
*- the key to meeting the immediate biological demands
of the muscle after intense anaerobic exercise*

A Scientific Literature Review.
Dr Sam Godfrey

Published by:

CellsUnited Limited

©2017 CellsUnited Limited. All rights reserved.

V1.0 – 11/08/17

CONTENTS

ABSTRACT	1
1. Introduction	3
2. Objectives	3
3. The implications of hypertrophy in sport	4
4. Muscle equilibrium	5
5. The vital importance of nutrition for enhanced muscle protein synthesis (MPS)	7
6. The timing of nutrition	8
7. The necessity of rapid hyperacidaemia	9
8. The digestive barrier to MPS	10
9. A comparison of alternative ways of reaching the window of anabolic potential	14
10. The optimum dietary protein source	15
REFERENCES	18

Sam Godfrey is a professional science writer who evaluates and reviews research literature for a wide range of audiences and publications. He gained his PhD in biochemistry from the University of Kent and undertook post-doctoral studies at Imperial College, London in immunology and neuroscience.

ABSTRACT

At no time in competitive sport has the need for fast recovery and improved physical performance been greater. Strategies that promote effective recovery along with increases in muscle size and strength (hypertrophy) are of great importance to the modern athlete.

Hypertrophy is the process which enables athletes to increase their physical strength and extend the limits of their performance. The biological processes that drive hypertrophy also govern recovery.

The key to muscular recovery and strength gains centres on the mechanistic target of rapamycin (mTOR). mTOR is the master controller responsible for the synthesis of new muscle proteins and is activated by high intensity exercise, also by the arrival of key nutrients in the bloodstream.

The maximum potential for muscle protein synthesis (MPS) peaks approximately 30-60 minutes after exercise with the potential then diminishing over time. The initial period, often described as “the 30 minute window”, is the optimal period for amino acids to be introduced into the bloodstream. The physiology of the human digestive system means that conventional protein requiring digestion, even consumed immediately after exercise, is not able to reach the muscles before this MPS potential is in decline.

The simplest and most effective way to increase MPS is through optimising the diet. All human bodily proteins are constructed from combinations of the 20 amino acids in the standard genetic code, known as the proteinogenic amino acids. The presence of high levels of these amino acids in the blood alone (known as hyperacidaemia) is known to boost MPS. Leucine is known to be the most effective stimulant of MPS but must be part of a balanced intake for effective muscle recovery and growth.

The optimum physiological condition to promote fast recovery and maximum muscle gain requires combining MPS activation through anaerobic exercise with MPS activation from appropriate nutrition.

For athletes looking to maximise the benefits of a drastically increased positive protein balance, timing is vital. Although the period of increased MPS following exercise (the “Anabolic Window”) is known to extend for many hours, numerous studies indicate that MPS potential is highest immediately after anaerobic exercise when blood flow to the muscles is at its peak.

The maximum rate of MPS then depends on the speed with which amino acids can be introduced into the bloodstream.

With success defined by slender margins in today's professional and high-level amateur sport, much effort and research has centred on achieving the rapid hyperacidaemia in the blood that will allow for the maximum MPS to occur. This is physiologically difficult to achieve and impossible using conventional protein sources because turning raw protein sources into the bioavailable amino acids craved by the muscles requires several time-consuming stages of digestion before they can be absorbed into the body. Once MPS has been initiated, all 20 proteinogenic amino acids are required for repairing and building muscle. This is where branch chain amino acid supplements fall short as a recovery supplement, although BCAAs have a role as an energy source and in reducing catabolism which is outside the scope of this document.

As discussed in this review, other strategies for maximising muscle recovery potential are either impractical or ineffective and a new approach is needed. Here we discuss a new protein source that better matches the biological demands of the muscles, i.e., to deliver a balance of all 20 essential and non-essential amino acids to the muscles within the first 30-60 minutes, allowing elite athletes to recover better and realise their full potential.

Hydrolysed whole salmon protein is a source of liquid protein derived from the Atlantic salmon that has been hydrolysed, using natural enzymes, into free amino acids and short chain polypeptides. In a direct comparison, over 96% of the hydrolysed salmon protein peptides are shorter than the smallest peptide in whey protein isolate.

Because the hydrolysed salmon protein's amino acids are already bioavailable, athletes can expect to see hyperacidaemia in the blood within 30 minutes after exercise, at the point at which blood flow and oxygen levels in the muscle are still elevated. This provides the optimum conditions for rapid amino acid uptake into the muscle tissue, something not possible through other supplements.

Further, since the amino acid profile of salmon protein is so close to that of human protein, the derived amino acid complex provides the closest match to the desired biological requirements of elite athletes looking to optimise recovery and compete at the highest level.

1. Introduction

The sports nutrition market has grown significantly in recent years and continued growth is forecast, until 2020 and beyond on current projections.

The range of nutritional products available has diversified, many new brands have been introduced and the user base has expanded beyond serious sports users into recreational, health and dietary usage as well.

As the market has grown and competition has increased, so have the nature and extent of product claims to the point where there is increasing potential for confusion and misinterpretation, especially in the suitability of product to address key physiological processes – and concerns that product claims lack scientific foundation have been raised by the British Dietetic Association and the British Medical Journal.

This review looks at the known science and a range of scientific and medical research into the processes which govern muscle recovery and growth. This is a subject area in which scientific and medical knowledge and product claims can be seen to diverge.

2. Objectives

The primary objectives of this review are: -

- To review published research on the scientific and medical factors governing muscle recovery and growth after the strenuous exercise associated with serious competitive sport
- To discuss the efficacy of available nutritional options from the perspectives of muscle recovery and growth
- To identify the optimal approach to recovery and the protein specifications required to meet the biological demands of the muscles.

An extensive literature search was undertaken from a wide range of recognised reference sources. This has been supplemented by discussions with experts in the field and internet searches.

3. The implications of hypertrophy in sport

At no time in competitive sport have the demands for enhanced performance and rapid recovery been greater. The physical intensity and competitive pressures of modern sport regularly push athletes to and even, beyond their physical limits. Allied to this, expanding sporting calendars are placing previously unseen demands on physical recovery in many sports and not just at the highest level. To cope with such intense pressures, increasingly sophisticated training methods, state-of-the-art sports equipment and intensive medical support have become, of necessity, essential components in the drive to improve performance levels.

Hypertrophy is the process which enables athletes to increase their physical strength and extend the limits of their performance. The same biological processes that drive hypertrophy also apply to recovery.

One method for improving physical capability in sport is to seek gains in muscle strength and explosiveness. There are two widely-accepted legal strategies available for achieving this. The first of these is by training muscle groups to fire to their best efficiency, termed neuromuscular adaptations, where the brain learns how better to coordinate the voluntary activation of muscle fibre, engaging the maximum number of motor units with each muscle contraction ^[1]. People less used to performing high intensity muscle movements are less likely to be able to access their maximum available muscle strength.

By repeatedly performing simple resistance exercises, the brain learns to activate the muscles more efficiently, with resulting significant early gains in strength and explosiveness. However, these initial gains soon plateau and a second approach is required as further strength and performance gains will only be achieved by actively increasing the strength or endurance of the skeletal muscles.

Our skeletal muscles are responsible for all our controlled movements as well as providing core stability, the two qualities essential for all physical performance. Skeletal muscle makes up nearly 40% of adult body weight and consists of close to 75% of the body's total protein ^[2]. For any sports that require power and explosive movement, the only route to improving strength after neuromuscular conditioning is to increase the force with which skeletal

muscles can contract. This is achieved through increasing the cross-sectional area and mass of the muscle, a measurement that is directly linked to higher strength.

Hypertrophy, the process of increasing muscle size, is therefore essential for strength gains and forms a key part of the training goals in many sports ^[3].

4. Muscle equilibrium

Every cell and organ in our body, including our muscles, operate thanks to proteins. Proteins are large biological molecules made from specific arrangements of different amino acids. Each protein carries out specific tasks to aid the operation of cells in our body. A huge array of proteins perform many functions within living organisms and the largest proteins consist of over 30,000 individual amino acids ^[4], interacting with one another to form the specific shapes needed to operate as a biological machine.

Muscle protein is formed from several types of long filamentous proteins. The thick filaments consist of a protein called myosin, whilst the thin filaments contain actin, troponin and tropomyosin. In skeletal muscles, it is the arrangement of these filaments, plus their proximity to other body architecture (such as blood vessels and mitochondria) that divides them into either Type I and Type II muscle.

Type I muscle (slow twitch) is rich in blood vessels, is able to contract for long periods of time and is associated with endurance. Type II muscle (fast twitch) contracts with more force but does not have the endurance of Type I. Rapid recovery and strength gains to both types of muscle tissue are vital for athletes seeking to increase their physical performance.

Skeletal muscles are maintained through a constant state of muscle breakdown and muscle production, driven by key biological processes. Whilst this constant turnover is essential for the muscle to remain operational, the balance can be readily influenced by external factors such as nutrition, levels of activity, injury and ageing, all of which result in muscle loss or gain.

The rate that new muscle fibres are synthesised will usually keep pace with the breakdown of old, damaged muscle fibres. Because of this, carrying out

normal daily activities and eating an appropriate diet will keep muscle cross-section size relatively constant through most of normal adult life ^[5]^[6].

Whilst this constant turnover of muscle proteins usually maintains an equilibrium, it is also readily influenced by a variety of factors. For muscle size to remain the same, muscle protein synthesis (MPS) and muscle protein breakdown (MPB) need to be balanced. If MPS is promoted and exceeds MPB then a positive protein balance is achieved. This enables hypertrophy, an increase in the cross-sectional size and strength of the targeted muscles. Conversely, if MPB occurs at a faster rate than MPS, the result will be a negative protein balance and, ultimately, muscular atrophy – a decrease in muscle size and strength ^[7]^[8].

Poor nutrition, ageing, inactivity and injury are all factors that can result in a negative protein balance ^[9], whilst resistance exercise, physical exertion and an availability of essential amino acids in the blood at the right time will shift the equilibrium towards a positive protein balance and MPS. For that reason, modern training regimens centre on the behaviours that maximise MPS and minimise MPB.

Under normal physiological influence, skeletal muscle proteins regenerate with predictable regularity, with as many as 1–2% of proteins being broken down and re-synthesised daily ^[10]. This constant recycling of proteins provides ample opportunities to boost MPS. This makes hypertrophy readily achievable in the right conditions.

The most important factor in MPS is a molecule called mTOR, which acts as the master controller responsible for coordinating all new muscle growth. It is therefore imperative that any strategies aimed at achieving hypertrophy consider the conditions in which mTOR can best initiate MPS ^[11].

The simplest and most effective way to increase MPS is through optimising the diet. All human bodily proteins are constructed from combinations of 20 different amino acids, and the presence of an excess of amino acids in the blood alone (known as hyperacidaemia) is able to boost MPS. These necessary amino acids are detected by a nutrient-sensing molecule called Vps34 which in turn activates mTOR, the master controller of MPS. This process drastically swings the balance towards muscle generation ^[12]^[13].

In one study, providing a direct dose of amino acids into the blood led to an increase in the rates of MPS within half an hour, peaking after 90 minutes ^[14]. However, this amino acid-induced increase in the rate of MPS was not long-lived, with the results showing a return to normal levels of MPS after four hours ^[15].

A second strategy to increase MPS is to force muscles to recover from physical exertion by stimulating the molecular mechanisms responsible for muscle protein generation.

Muscle cells are designed primarily to contract and, during resistance exercise or competition, these contractions cause individual muscle cells and surrounding supportive cells to communicate with each through a variety of molecular signals ^[16]. One of these molecular signals is called 5' adenosine monophosphate-activated protein kinase (AMPK), and performs multiple functions within our cells, switching various molecules on and off so that some of the cell's processes can be controlled. During exercise, AMPK becomes greatly elevated, and these extra AMPK molecules suppress the muscle-building activities of mTOR ^[17]. Without mTOR working at full capacity, the normal rate of MPS drops, whilst MPB levels remain unchanged. For the duration of the exercise this results in a temporary negative protein balance ^[18] with resulting negative impacts on muscle size and strength.

Once the period of resistance exercise is over, the levels of AMPK drop back to normal and mTOR is able to reassert itself. It does so, and at a greatly elevated level, boosting the rate of MPS. At the same time MPB is also increased, leading to a period of rapid protein recycling that lasts several hours ^{[8] [19-21]}.

5. The vital importance of nutrition for enhanced muscle protein synthesis (MPS)

Achieving the optimum physiological conditions that promote fast recovery and maximum muscle gain requires combining muscle protein synthesis activation through sport with MPS activation from appropriate nutrition.

Individually, the act of consuming amino acids or performing intense physical exercise both lead to an increase in MPS. But numerous studies have shown that the combination of both strategies results in significant increases in MPS.

This understanding of MPS allows strategies which boost MPS to be developed and the period immediately after exercise is when they have the most impact on aiding rapid recovery and making strength gains.

At this time, the increased turnover of protein following exercise leads directly towards the need for an inward transport of amino acids ^[8]. By ensuring an adequate supply of amino acids to fuel this growth potential, added to the ability of amino acid to stimulate MPS, it is possible to create the optimum conditions for MPS. Studies show the rate of MPS can be increased by up to 150% above normal levels when exercise and nutrition are combined^{[22] [23]}, while the rate of MPB remains normal. This strong shift in the MPS/MPB balance is instrumental in achieving hypertrophy ^[24].

6. The timing of nutrition

For athletes seeking to maximise the benefits of this drastically increased positive protein balance, timing is important. A study testing when amino acid intake should occur showed unequivocally that greater gains in strength and muscle size were seen when amino acid consumption was timed to coincide with resistance exercise rather than spaced over the day, other studies also indicate that nutrient timing can aid recovery ^[25-27].

Various studies have shown that levels of MPS rise rapidly after exercise or competition and, if amino acids are provided through consuming protein sources such as whey and soy, the rate of MPS peaks at around 3-4 hours^{[6][7][22]}. From this point it gradually declines, yet elevated MPS is still observable up to 48 hours after exercise ^[7].

Delays to appropriate amino acid intake can have a negative effect on MPS and therefore an impact on recovery and strength. If resistance exercise or high-intensity sport is carried out in a fasted state, whilst the rates of both MPS and MPB increase, the resulting protein balance remains approximately neutral or even negative ^[28]. In one study, those delaying nutrition for only two hours post exercise lost muscle mass as a result ^[29].

Any loss of muscle mass due to sub-optimal nutritional planning can impact on an athlete's ability to recover. In today's sporting environment, success at many levels can hang on the finest margins. This is why achieving maximum potential from the MPS window is essential.

Numerous studies indicate that MPS potential is highest immediately after exercise or competition, referring to it as "the window of anabolic potential" or "the 30 minute window". As this window opens immediately after exercise and wanes over time, it is imperative that readily available amino acids reach the blood as rapidly as possible. It is for this reason that protein consumption timing is so important for achieving maximum gains and plays such a big role in sports nutrition ^[29-32].

Following a short lag phase after exercise, the rates of MPS begin to spike, peaking after about three hours before steadily declining over the next 24-48 hours ^{[6] [33]}.

The height of this spike and, therefore, the maximum rate of MPS depends on the speed with which amino acids can be delivered ^{[6] [22] [34]}.

It has been suggested that the early peak in the window of anabolic recovery may be due to a combination of two factors; the reactivation of mTOR combined with the increased blood flow in the muscles, together creating the optimum recovery environment for the muscle cells.

During exercise, there is a dramatic increase in the blood flow to the muscles to provide oxygen and energy, with blood flow up to 64 times greater than normal ^{[34] [35]}. Following exercise, this blood flow remains elevated, gradually declining but remaining above resting level for around one hour. It is this increased blood volume that may aid recovery and, as such, offers the optimum environment for the hyperacidaemia required for maximum MPS. One study has reported that these conditions led to an increase in amino acid transport into the muscles of between 30-100% during this period ^[35].

7. The necessity of rapid hyperacidaemia

The potential for maximum MPS and therefore the greatest recovery and strength gains, declines quickly after competition and training. There is a

necessity to get amino acids into the muscles as quickly as possible in order to see maximum gains.

It is now unequivocal that immediate post-exercise amino acid provision is an effective nutrition-based strategy to enhance MPS above the rates observed with exercise or competition alone ^{[25] [34] [36]}. The importance of early post-exercise protein ingestion relates to the fact that exercise-mediated increases in rates of MPS are greatest immediately after exercise (~100–150% above basal rates) ^{[22] [23]}.

With success defined by slender margins in today's professional and high-level amateur sport, much effort and research has centred on achieving the rapid hyperacidaemia in the blood that will allow for the maximum MPS to occur. This is physiologically difficult to achieve. As a result, most studies report on experiments in which there are no replacement amino acids in the bloodstream within the first 30 minutes after exercise, the period of maximum potential ^{[22] [24] [37]}. This has been, simply, impossible using the protein sources available.

Consumption of protein represents the most easily accessible method for getting free amino acids into the bloodstream. Individuals typically acquire their protein through consumption of either high protein foodstuffs such as meat, poultry, fish and eggs, or via liquid consumption of protein sources such as milk, whey, casein and soy ^[37-43].

Turning these raw protein sources into the bioavailable amino acids craved by the muscles requires several time-consuming stages of digestion before they can be absorbed into the body via the jejunum, a part of the small intestine, ^[44] as discussed below.

To be absorbed, amino acids must cross from the digestive system into the blood stream via microscopic hair-like structures called villi which line the jejunum. Each villus has a good blood supply and serves to maximise the surface area of the intestine to allow for maximum absorption. The villi thus play a vital role in getting amino acids into the bloodstream quickly, with the limiting factor being the size of the molecules that can be absorbed and released.

The physical size of the villi and the structure of cells makes it impossible for large proteins to be absorbed directly and, for that reason, all protein sources

need to be reduced from large and complex to being just a few amino acids in length by the digestive system, a process known as proteolysis.

Proteolysis uses a variety of specially secreted digestive enzymes, each capable of breaking specific links between amino acids. For example, trypsin, one of the digestive enzymes involved, specifically breaks apart the links between polar amino acids. Long chains of amino acids can require many interactions with digestive enzymes before they are broken down into bioavailable single amino acids.

8. The digestive barrier to MPS

The length of time from protein consumption before the new amino acids can reach the bloodstream depends on a number of parameters, and the time taken has a direct influence on the potential for muscle protein synthesis.

Assuming lean meat consumption, the first step is for the body to begin to digest the food mechanically. Initial chewing increases the surface area of the food leaving it accessible by various digestive enzymes in the stomach in what is termed the cephalic phase. For solid protein sources such as meat, fish and some vegetables, this phase is vital. Consuming food causes the stomach to begin secreting an enzyme called pepsinogen which is then converted into pepsin by the low pH of the stomach. The churning action of the stomach and the pepsin begin the process of separating muscle tissue or other fibrous protein sources into long, complex chains of proteins called polypeptides.

A typical meal will require at least two hours of digestion before the consumed food is reduced to chyme, a semi-fluid mass of partially digested food. Once in this state, small portions of chyme will be passed at regular intervals of around 20 minutes into the duodenum, the first section of the small intestine ^[44]. Delivery of amino acids to the blood is still some time away when this stage is reached.

The time scales for liquid protein consumption are significantly shorter as, although requiring some digestion in the stomach, liquid protein sources pass into the duodenum faster as there is no need for mechanical digestion ^[45].

However, the maximal capacity of the small intestines is only 120ml, meaning any liquid in excess of this volume will not immediately begin the final processes of digestion ^[46]^[47].

Once in the duodenum, the chyme or liquid protein source must be further digested. Protein now consists of long chains of amino acids, and these must be broken down further before they can be released into the bloodstream. The arrival of chyme into the duodenum triggers the release of various digestive enzymes including intestinal gastrin, trypsin and chymotrypsin, all of which conspire to break the bonds between amino acids, turning the complex long-chain proteins into shorter and shorter polypeptides.

When these polypeptides are short enough, they pass into the jejunum, the next section of small intestine and the region from which the majority of protein is absorbed by the body.

The villi of the jejunum are equipped with inbuilt enzymes, embedded into their cell membranes. These work to further break down the peptides, converting them into free amino acids or very small peptides less than four amino acids in length. At this stage, the amino acids and peptides are ready for absorption.

The villi of the jejunum feature four types of transporter, one for each type of amino acid (acidic, basic, polar and non-polar amino acids). These transporters bind the amino acids and then change shape, depositing the amino acid inside the villi cells, before returning to their original position facing into the intestines. The free amino acids are then pumped out into the bloodstream.

In addition to these four transporter proteins, there is an additional transporter protein called PepT1 which is able to transport short peptides (two or three amino acids in length) into the cells where they are broken down into single amino acids.

This entire digestive process takes a significant portion of time. For solid foods such as meat and fish, the very first amino acids do not begin to enter the bloodstream until a minimum of two hours ^[48] after consumption, with some sources suggesting the process takes as long as four hours ^[49]. For proteins already in liquid form, the time for amino acid absorption is reduced because the need for mechanical digestion is removed, but the proteins

themselves still need to be digested. The most popular forms of liquid proteins, whey and casein protein, are derived from milk. A side-effect of being derived from milk is that these protein sources contain 5-7% milk fat alongside the protein. For many athletes, this fat content is undesirable, so manufacturers process the protein, using a technique called ultrafiltration. Here, any molecules smaller than 20,000Da* are disposed of as waste as a direct result of the filtration method.

[*Da = Dalton, a measure of weight that is used for determining the size of proteins. The hormone insulin, a small protein used in metabolising sugars, has a weight of 5,808Da, whilst Titin, a very large protein found in muscle has a weight of 3,906,488Da.]

This results in 'whey protein isolate' and, whilst possessing very low levels of fat, it comes with drawbacks. Removing all molecules smaller than 20,000Da means that all the small and most easily digestible protein is lost ^[50]. Amino acids have an average molecular weight of 110Da, which means that, after ultrafiltration, even the smallest size protein in a whey or casein protein isolate would be approximately 180 amino acids in length ^[51]. Yet, the largest peptide that can be absorbed by the villi of the jejunum is just three amino acids in size. This need for further digestion in the duodenum helps to explain why whey and casein protein ingestion can only result in an increased blood amino acid level after an average of one hour (most reports showing between 45 and 120 minutes) ^[52-55].

As previously discussed, the window for peak MPS occurs immediately after exercise, peaking between one and three hours with the MPS potential declining over 48 hours. The presence of significant amino acids in the blood during this window allows for high levels of MPS, boosting recovery and allowing for strength gains. Maximising the potential of the MPS window is therefore a priority for every athlete wishing to initiate rapid muscle recovery and thus achieve the largest possible strength and performance gains.

Solid foods consumed immediately post exercise require significant amounts of digestion. As such, amino acids will not be present in the blood until the window of maximum MPS potential is declining. Even when using digestible liquid proteins, such as whey, the valuable window of maximum MPS has begun to decline before the amino acids become bioavailable. Furthermore, the one hour period immediately post exercise when the muscles receive the most blood and are actively transporting new amino acids to aid muscle

recovery ^{[34][35]} is completely unreachable through conventional dietary means. This indicates that the full potential of the anabolic window cannot be reached by post exercise ingestion using conventional protein sources.

9. A comparison of alternative ways of reaching the window of anabolic potential

With traditional dietary intake unable to realise the full potential of muscle protein synthesis, alternative strategies must be considered by serious athletes to maximise recovery and strength gains following competition and training.

One option available to athletes is consuming protein before exercise. Here, studies have shown mixed results. Some studies showed that consumption of protein before exercise resulted in higher levels of MPS than when the same nutrients were eaten post exercise ^[56]. However other studies showed the opposite effect ^{[32][57]}. One study found that ingesting protein during exercise was also beneficial ^[58].

The mixed results seen in these studies could well be explained by amino acid availability declining quickly ^[59]. Protein ingested too early will have little benefit as amino acids do not remain circulating in the blood indefinitely, whilst those ingested too late will miss the window of maximum anabolic potential. The complexities of timing the digestion of a protein requiring digestion to result in the presence of free amino acids in the blood during the window of maximum anabolic potential is very complicated.

Compounding the pre-eating regimen conundrum is the fact that it can result in unwanted reactions such as increased nausea and decreased performance in training and competition ^[60].

A second option currently available to athletes is the consumption of branched-chain amino acids immediately after exercise to stimulate muscle growth. There are three such amino acids, leucine, isoleucine and valine and these are claimed by some to stimulate MPS.

Of the three branched-chain amino acids, it is only leucine which provides maximum stimulation for MPS, with the other branched-chain amino acids being essential for muscle growth only in the same way that all other amino

acids are essential. Leucine stimulates MPS by activating mTOR which, as previously mentioned, kick-starts MPS. Studies have shown that inadequate leucine results in poor stimulation of MPS whilst leucine alone can initiate it^[41] [61-63].

Once MPS has been initiated, all essential amino acids are required for building muscle, and the absence of any will impede muscle production and inhibit recovery. It is generally accepted that Liebig's Law of the Minimum applies – the principle that only by increasing the amount of the limiting nutrient can growth be improved^[73]. The presence of all 20 amino acids is desirable as the regenerating muscle tissues requires optimal body conditions and balanced metabolism^[64-67]. Absence of non-essential amino acids can impair these functions and reduce the window of anabolic potential^[67]. For this reason, branched-chain amino acids alone do not possess the ideal properties for maximising MPS in order to speed recovery and increase strength. It is possible to conclude that claims made for branched-chain amino acid nutritional products may be somewhat overstated. This view is supported by the recent study conducted at the Universities of Stirling, Birmingham and Exeter published in July 2017. Professor Kevin Tipton, Chair in Sport, Health and Exercise Sciences at the University of Stirling, said: "A sufficient amount of the full complement of amino acids is necessary for maximum muscle building, following exercise. Athletes interested in enhancing muscle growth with training should not rely on these BCAA supplements alone."^[74]

A third approach, the direct intravenous injection of amino acids is not appropriate and is not discussed.

10. The optimum dietary protein source

An ideal protein source to maximise hypertrophy will therefore consist of comparable essential amino acid levels to that of human muscle, with leucine levels near 100% of that found in human body protein, and inclusion of non-essential amino acids to support metabolism and promote homeostasis in the muscles. To compare two common protein sources, whey and soy, soy protein offers the closest match to whole body protein, but has lower levels of leucine. This could go some way to explaining the individual MPS profiles of

each protein. MPS following whey consumption was 18% higher than that of soy in one paper and the high leucine content of whey is thought to be the reason ^{[15] [18] [68-70]}.

Interestingly, whey has close to whole body protein levels of leucine, but it has low levels of phenylalanine + tyrosine, valine and histidine, all essential amino acids which could be part of the reason why some studies report a faster decline in MPS with whey than with other dietary proteins ^[26].

Understanding the biological factors behind maximum muscle protein synthesis allows us to identify the qualities required for an optimum protein source. One product, hydrolysed whole salmon protein, closely matches these parameters and may prove to be the best tool for maximising muscle protein synthesis.

Bearing in mind all we know about MPS, we can identify the qualities of the ideal protein source. It would consist of free amino acids and short-chained polypeptides, making it rapidly digestible allowing free amino acids to reach the bloodstream within the first 30 minutes of the anabolic window, the point at which blood flow to the muscles and maximum amino acid transport is at its maximum. This would allow the rate of MPS to achieve its full potential. Until recently, aside from the intravenous injection of amino acids, this has not been achievable. However, the arrival of enzymatically hydrolysed whole salmon protein to the sports nutrition market may address this problem with its profile suggesting it is capable of achieving rapid absorption - faster than whey, casein or any other whole protein source.

Hydrolysed whole salmon protein is a source of liquid protein derived from the Atlantic salmon that has been pre-digested and hydrolysed into short chain polypeptides. Analysis of the product shows that 20% of the protein present is below 200Da in size indicating that these may be free amino acids ready for direct transport through the intestinal lumen. A further 20% of the protein is below 1,000Da, most likely consisting of short chain amino acids of the approximate size permitted for direct transport across the villi of the jejunum. The remaining peptides are also short, and therefore able to be rapidly digested by the enzymes of the duodenum and jejunum. This is in direct contrast to whey protein isolate which, due to the process of manufacturing, is invariably over 20,000Da in size taking vital minutes to digest. In a direct

comparison, over 96% of the hydrolysed salmon protein peptides are shorter than the smallest peptide in whey protein isolate.

Hydrolysed Salmon Protein - Molecular Weight Distribution

(Nofima BioLab 25/02/15)

Below 10,000 Dalton	87.9%
Below 6,000 Dalton	74.5%
Below 1,000 Dalton	35.0%

Figure 1: Low molecular size is vital to bioavailability

It should also be noted that the 100ml volume of this new protein source is less than the maximum volume of the small intestine, meaning that there is no lost time when useful protein remains outside the small intestine in the stomach where it cannot be absorbed. The physiology of the human digestive system means that when athletes consume large volumes of liquid protein, it takes several hours to process in full, meaning much of the protein completely misses the window of anabolic potential.

Because the hydrolysed salmon protein's amino acids are already bioavailable, athletes can expect to see increases of amino acid in the blood within 30 minutes after exercise, at the point at which blood flow and oxygen levels in the muscle are still elevated. This takes advantage of the optimum conditions for rapid amino acid uptake into the muscle tissue, something not possible through other supplements and previously only achieved via intravenous injection. By delivering amino acid sooner to the MPS window, a greater maximum MPS rate can be achieved. In addition, the salmon has close to human muscle levels of leucine, as well as an amino acid profile highly similar to human muscle. This high leucine content allows it to activate mTOR rapidly, driving the maximum response.

Whey protein is arguably the best commonly available protein for promoting hypertrophy. However, its slower absorption speed compared to hydrolysed salmon protein is just one disadvantage. Whey protein also has low levels of several essential amino acids and therefore it cannot sustain MPS ^{[52] [71] [72]}, a problem shared by branched-chain amino acid supplements. Hydrolysed salmon protein with a human-like essential amino acid profile, plus supporting non-essential amino acids, provides the optimum amino acid formulation to support MPS. This form of dietary protein is the closest match to the desired biological requirements of elite athletes looking to optimise recovery and compete at the highest level.

REFERENCES

- 1 R Maughan, M. G. *The biochemical basis of sports performance*. 2nd edition edn, (2010).
- 2 Matthews, D. *Modern Nutrition and Disease*. 9th edition edn, (1999).
- 3 Russell, B., Motlagh, D. & Ashley, W. W. Form follows function: how muscle shape is regulated by work. *J Appl Physiol* (1985) 88, 1127-1132 (2000).
- 4 Tskhovrebova, L. & Trinick, J. Titin: properties and family relationships. *Nat Rev Mol Cell Biol* 4, 679-689, doi:10.1038/nrm1198 (2003).
- 5 Hughes, S. M. & Schiaffino, S. Control of muscle fibre size: a crucial factor in ageing. *Acta Physiol Scand* 167, 307-312, doi:10.1046/j.1365-201x.1999.00622.x (1999).
- 6 Phillips, S. M. A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Med* 44 Suppl 1, S71-77, doi:10.1007/s40279-014-0152-3 (2014).
- 7 Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E. & Wolfe, R. R. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* 273, E99-107 (1997).
- 8 Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D. & Wolfe, R. R. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* 268, E514-520 (1995).
- 9 Cholewa, J. M. *et al.* Dietary proteins and amino acids in the control of the muscle mass during immobilization and aging: role of the MPS response. *Amino Acids* 49, 811-820, doi:10.1007/s00726-017-2390-9 (2017).
- 10 Welle, S., Thornton, C., Statt, M. & McHenry, B. Postprandial myofibrillar and whole body protein synthesis in young and old human subjects. *Am J Physiol* 267, E599-604 (1994).
- 11 Bodine, S. C. *et al.* Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3, 1014-1019, doi:10.1038/ncb1101-1014 (2001).
- 12 MacKenzie, M. G., Hamilton, D. L., Murray, J. T., Taylor, P. M. & Baar, K. mVps34 is activated following high-resistance contractions. *J Physiol* 587, 253-260, doi:10.1113/jphysiol.2008.159830 (2009).
- 13 Fujita, S. *et al.* Nutrient signalling in the regulation of human muscle protein synthesis. *J Physiol* 582, 813-823, doi:10.1113/jphysiol.2007.134593 (2007).
- 14 Bohe, J., Low, J. F., Wolfe, R. R. & Rennie, M. J. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* 532, 575-579 (2001).
- 15 Mitchell, C. J. *et al.* Consumption of Milk Protein or Whey Protein Results in a Similar Increase in Muscle Protein Synthesis in Middle Aged Men. *Nutrients* 7, 8685-8699, doi:10.3390/nu7105420 (2015).
- 16 Ambrosio, F. *et al.* The effect of muscle loading on skeletal muscle regenerative potential: an update of current research findings relating to aging and neuromuscular pathology. *Am J Phys Med Rehabil* 88, 145-155, doi:10.1097/PHM.0b013e3181951fc5 (2009).
- 17 Richter, E. A. & Ruderman, N. B. AMPK and the biochemistry of exercise: implications for human health and disease. *Biochem J* 418, 261-275, doi:10.1042/BJ20082055 (2009).
- 18 Dreyer, H. C. *et al.* Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol* 576, 613-624, doi:10.1113/jphysiol.2006.113175 (2006).

- 19 Chesley, A., MacDougall, J. D., Tarnopolsky, M. A., Atkinson, S. A. & Smith, K. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol* (1985) 73, 1383-1388 (1992).
- 20 MacDougall, J. D. *et al.* The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can J Appl Physiol* 20, 480-486 (1995).
- 21 Yarasheski, K. E., Zachwieja, J. J. & Bier, D. M. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol* 265, E210-214 (1993).
- 22 Churchward-Venne, T. A., Burd, N. A. & Phillips, S. M. Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism. *Nutr Metab (Lond)* 9, 40, doi:10.1186/1743-7075-9-40 (2012).
- 23 Kumar, V., Atherton, P., Smith, K. & Rennie, M. J. Human muscle protein synthesis and breakdown during and after exercise. *J Appl Physiol* (1985) 106, 2026-2039, doi:10.1152/jappphysiol.91481.2008 (2009).
- 24 Guimaraes-Ferreira, L. *et al.* Synergistic effects of resistance training and protein intake: practical aspects. *Nutrition* 30, 1097-1103, doi:10.1016/j.nut.2013.12.017 (2014).
- 25 Moore, D. R. *et al.* Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol* 587, 897-904, doi:10.1113/jphysiol.2008.164087 (2009).
- 26 Burd, N. A., Tang, J. E., Moore, D. R. & Phillips, S. M. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. *J Appl Physiol* (1985) 106, 1692-1701, doi:10.1152/jappphysiol.91351.2008 (2009).
- 27 Esmarck, B. *et al.* Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* 535, 301-311 (2001).
- 28 Rasmussen, B. B., Tipton, K. D., Miller, S. L., Wolf, S. E. & Wolfe, R. R. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol* (1985) 88, 386-392 (2000).
- 29 Weinert, D. J. Nutrition and muscle protein synthesis: a descriptive review. *J Can Chiropr Assoc* 53, 186-193 (2009).
- 30 Koopman, R., Saris, W. H., Wagenmakers, A. J. & van Loon, L. J. Nutritional interventions to promote post-exercise muscle protein synthesis. *Sports Med* 37, 895-906 (2007).
- 31 Drummond, M. J. & Rasmussen, B. B. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Curr Opin Clin Nutr Metab Care* 11, 222-226, doi:10.1097/MCO.0b013e3282fa17fb (2008).
- 32 Dreyer, H. C. *et al.* Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab* 294, E392-400, doi:10.1152/ajpendo.00582.2007 (2008).
- 33 Tang, J. E., Perco, J. G., Moore, D. R., Wilkinson, S. B. & Phillips, S. M. Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol* 294, R172-178, doi:10.1152/ajpregu.00636.2007 (2008).
- 34 Biolo, G., Tipton, K. D., Klein, S. & Wolfe, R. R. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 273, E122-129 (1997).
- 35 Bangsbo, J. & Hellsten, Y. Muscle blood flow and oxygen uptake in recovery from exercise. *Acta Physiol Scand* 162, 305-312, doi:10.1046/j.1365-201X.1998.0331e.x (1998).
- 36 Tipton, K. D., Ferrando, A. A., Phillips, S. M., Doyle, D., Jr. & Wolfe, R. R. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* 276, E628-634 (1999).

- 37 Phillips, S. M., Hartman, J. W. & Wilkinson, S. B. Dietary protein to support anabolism with resistance exercise in young men. *J Am Coll Nutr* 24, 134S-139S (2005).
- 38 Symons, T. B., Sheffield-Moore, M., Wolfe, R. R. & Paddon-Jones, D. A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. *J Am Diet Assoc* 109, 1582-1586, doi:10.1016/j.jada.2009.06.369 (2009).
- 39 Moore, D. R. *et al.* Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 89, 161-168, doi:10.3945/ajcn.2008.26401 (2009).
- 40 Pennings, B. *et al.* Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am J Clin Nutr* 93, 997-1005, doi:10.3945/ajcn.110.008102 (2011).
- 41 Hartman, J. W. *et al.* Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *Am J Clin Nutr* 86, 373-381 (2007).
- 42 Schaafsma, G. The protein digestibility-corrected amino acid score. *J Nutr* 130, 1865S-1867S (2000).
- 43 Hoffman, J. R. & Falvo, M. J. Protein - Which is Best? *J Sports Sci Med* 3, 118-130 (2004).
- 44 Bowen, R. (Vivo pathophysiology, <http://www.vivo.colostate.edu/hbooks/pathphys/digestion/index.html>, 2015).
- 45 Conley, T. B. *et al.* Effect of food form on postprandial plasma amino acid concentrations in older adults. *Br J Nutr* 106, 203-207, doi:10.1017/S0007114511000419 (2011).
- 46 Schiller, C. *et al.* Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment Pharmacol Ther* 22, 971-979, doi:10.1111/j.1365-2036.2005.02683.x (2005).
- 47 Mudie, D. M. *et al.* Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. *Mol Pharm* 11, 3039-3047, doi:10.1021/mp500210c (2014).
- 48 Waikato. *The human digestive system*, 2011).
- 49 Nasset, E. S., Heald, F. P., Calloway, D. H., Margen, S. & Schneeman, P. Amino acids in human blood plasma after single meals of meat, oil, sucrose and whiskey. *J Nutr* 109, 621-630 (1979).
- 50 Archer, R. Whey products. (<http://www.nzic.org.nz/ChemProcesses/dairy/3G.pdf>).
- 51 Biro, J. C. Amino acid size, charge, hydrophathy indices and matrices for protein structure analysis. *Theor Biol Med Model* 3, 15, doi:10.1186/1742-4682-3-15 (2006).
- 52 Boirie, Y. *et al.* Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A* 94, 14930-14935 (1997).
- 53 Joy, J. M. *et al.* The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J* 12, 86, doi:10.1186/1475-2891-12-86 (2013).
- 54 Norton, L. E. *et al.* The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats. *J Nutr* 139, 1103-1109, doi:10.3945/jn.108.103853 (2009).
- 55 West, D. W. *et al.* Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *Am J Clin Nutr* 94, 795-803, doi:10.3945/ajcn.111.013722 (2011).
- 56 Tipton, K. D. *et al.* Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* 281, E197-206 (2001).

- 57 Tipton, K. D. *et al.* Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am J Physiol Endocrinol Metab* 292, E71-76, doi:10.1152/ajpendo.00166.2006 (2007).
- 58 Beelen, M. *et al.* Protein coingestion stimulates muscle protein synthesis during resistance-type exercise. *Am J Physiol Endocrinol Metab* 295, E70-77, doi:10.1152/ajpendo.00774.2007 (2008).
- 59 Pitkanen, H. T. *et al.* Free amino acid pool and muscle protein balance after resistance exercise. *Med Sci Sports Exerc* 35, 784-792, doi:10.1249/01.MSS.0000064934.51751.F9 (2003).
- 60 Gentle, H. L., Love, T. D., Howe, A. S. & Black, K. E. A randomised trial of pre-exercise meal composition on performance and muscle damage in well-trained basketball players. *J Int Soc Sports Nutr* 11, 33, doi:10.1186/1550-2783-11-33 (2014).
- 61 Koopman, R. *et al.* 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, E645-653, doi:10.1152/ajpendo.00413.2004 (2005).
- 62 Crozier, S. J., Kimball, S. R., Emmert, S. W., Anthony, J. C. & Jefferson, L. S. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J Nutr* 135, 376-382 (2005).
- 63 Wilkinson, D. J. *et al.* Effects of leucine and its metabolite beta-hydroxy-beta-methylbutyrate on human skeletal muscle protein metabolism. *J Physiol* 591, 2911-2923, doi:10.1113/jphysiol.2013.253203 (2013).
- 64 Young, V. R. Adult amino acid requirements: the case for a major revision in current recommendations. *J Nutr* 124, 1517S-1523S (1994).
- 65 Reeds, P. J. Dispensable and indispensable amino acids for humans. *J Nutr* 130, 1835S-1840S (2000).
- 66 Furst, P. & Stehle, P. What are the essential elements needed for the determination of amino acid requirements in humans? *J Nutr* 134, 1558S-1565S (2004).
- 67 Hou, Y., Yin, Y. & Wu, G. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. *Exp Biol Med (Maywood)* 240, 997-1007, doi:10.1177/1535370215587913 (2015).
- 68 Anthony, J. C., Anthony, T. G., Kimball, S. R., Vary, T. C. & Jefferson, L. S. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr* 130, 139-145 (2000).
- 69 Drummond, M. J., Dreyer, H. C., Fry, C. S., Glynn, E. L. & Rasmussen, B. B. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol (1985)* 106, 1374-1384, doi:10.1152/jappphysiol.91397.2008 (2009).
- 70 Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A. & Phillips, S. M. 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, 987-992, doi:10.1152/jappphysiol.00076.2009 (2009).
- 71 Beaufre, B. D., M. Boiree, Y. The 'Fast' and 'Slow' protein concept. *Proteins, peptides and amino acids in enteral nutrition* 3, 121-133 (2000).
- 72 Katsanos, C. S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A. & Wolfe, R. R. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* 291, E381-387, doi:10.1152/ajpendo.00488.2005 (2006).
- 73 Liebig's Law of the Minimum: wikipedia.org/wiki/Liebig%27s_law_of_the_minimum
- 74 Sarah R. Jackman, Oliver C. Witard, Andrew Philp, Gareth A. Wallis, Keith Baar, Kevin D. Tipton. Branched-Chain Amino Acid Ingestion Stimulates Muscle Myofibrillar Protein Synthesis following Resistance Exercise in Humans. *Frontiers in Physiology*, 2017; 8 DOI: 10.3389/fphys.2017.00390