MENNO HENSELMANS
Science to master your physique

PROTEIN

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Contents

➢ Lecture [optional]

Protein

Note: Unless otherwise referenced, all the basic biology and biochemistry comes from the Advanced Nutrition and Human Metabolism textbook by Gropper et al., a highly recommended book for those interested in extremely detailed biochemistry (but major overkill for anyone else).

What is protein?

The word protein comes from the Greek 'proteios', meaning 'of first rank'. Proteins are responsible for most functions throughout the entire human body. They are your body's 'worker bees'. Over 40% of body protein is found in skeletal muscle, over 25% is found in body organs, and the rest resides mostly in the skin and blood. Proteins are composed of amino acids, which the body needs to create its own variety of proteins and nitrogen-containing molecules that make life possible. Each body protein has unique characteristics and sequence patterns of the amino acids that comprise its structure. The following proteins are useful to know, as they'll also teach you some basic biology of the human body.

1. Structural elements

Several proteins have structural roles in the body. They are effectively part of the bricks, mortar and steel that is your body's house. These come in 2 primary forms:

1. Contractile proteins enable your muscles to contract. The key contractile proteins to know are actin, myosin and titin. We'll cover those in detail in the training modules.

2. Fibrous proteins provide support to your body. They include collagen, elastin and keratin. They're found in bone, teeth, skin, tendons, cartilage, blood vessels, hair and nails.

2. Enzymes

Enzymes are protein molecules, generally designated by the suffix -ase, that act as catalysts: they change the rate of reactions occurring in the body. For example, proteases are enzymes that speed up proteolysis, the breaking down of other proteins.

3. Hormones

Some proteins are hormones, which act as chemical messengers in the body. They are synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs, where they bind to protein receptors on membranes. Hormones generally regulate metabolic processes. For example, testosterone increases protein synthesis. Some hormones derive from cholesterol and are classified as steroid hormones, while others derive from one or more amino acids. Tyrosine, for example, is used along with the mineral iodine to synthesize the thyroid hormones that regulate our metabolism. Tyrosine is also used to synthesize the catecholamines, including dopamine, norepinephrine and epinephrine. The hormone melatonin that makes you sleepy at night is derived in the brain from the amino acid tryptophan.

4. Other roles

Proteins carry out many additional roles in the body, but they are generally not relevant for our purposes. For example, in cell membranes, proteins function in cell adhesion, and some others serve to transmit signals into and out of the cell. Many proteins, such as albumin, also function as transporters: they carry nutrients from the blood to other sites in the body. Moreover, proteins can serve as buffers in the body that help to regulate acid-base balance. A buffer is a compound that ameliorates a change in pH, the acidity level. The pH of the blood and other body tissues affects many biological processes. In addition to acid-base balance, proteins influence fluid balance through their presence in the blood and in cells. Proteins can help attract water to keep the water inside a particular area and contribute to osmotic pressure.

In addition to their use in the synthesis of body proteins, amino acids are used to synthesize nitrogen-containing compounds that are not proteins but nonetheless play important roles in the body.

1. Glutathione

Glutathione is a major anti-oxidant with the ability to scavenge free radicals (O_2 and OH), thereby protecting critical cell components against oxidation and inflammation.

2. Carnitine

Carnitine, another nitrogen-containing compound, is made from the amino acid lysine. It is found in most body tissues and is needed for the transport of fatty acids, especially long-chain fatty acids, across the inner mitochondrial membrane for oxidation (fat burning). Carnitine is also needed for ketone catabolism to produce energy.

Carnitine deficiency, though rare in anyone except vegetarians, results in impaired energy metabolism. Carnitine does not normally need to be supplemented to avoid deficiency, in contrast to what some supplement companies claim. In addition to being synthesized in the liver and kidneys, carnitine can be found in foods, especially meats such as beef and pork. Carnitine from food or supplements is absorbed in the proximal small intestine. Approximately 54% to 87% of carnitine intake is absorbed. Intestinal absorption of carnitine is thought to be saturated with intakes of about 2 g. Muscle represents the primary carnitine pool, although no carnitine is made there.

3. Creatine

Creatine is a key component of the energy compound creatine phosphate, also called phosphocreatine. Phosphocreatine functions like a storehouse for high-energy phosphate. Your muscles can break this down to produce energy to fuel their contractions. This process is sped up by creatine kinase.

About 95% of body creatine is in muscle, with the remaining 5% in organs such as the kidneys and brain. In tissues, creatine is found both in free form as creatine and in its phosphorylated form.

There is some creatine in food, primarily in meat and fish. The body can also produce some creatine from amino acids. To significantly increase muscle creatine stores, however, it needs to be supplemented. We'll discuss this in the course module on dietary supplements.

Creatine degrades with use into creatinine, which is excreted by the kidneys into your urine.

4. Carnosine

Carnosine is made in the body from the amino acids histidine and beta-alanine. In the body, carnosine is synthesized and found largely in skeletal and cardiac muscle, but it is also found in the brain, kidneys and stomach. Carnosine is found in foods, primarily meats, and may be digested into histidine and beta-alanine in the intestine or absorbed intact. Not all functions of carnosine have been identified, but some studies have shown that carnosine acts as both a buffer and an anti-oxidant within cells. It may also reduce calcium needs for muscle contractility.

5. Choline

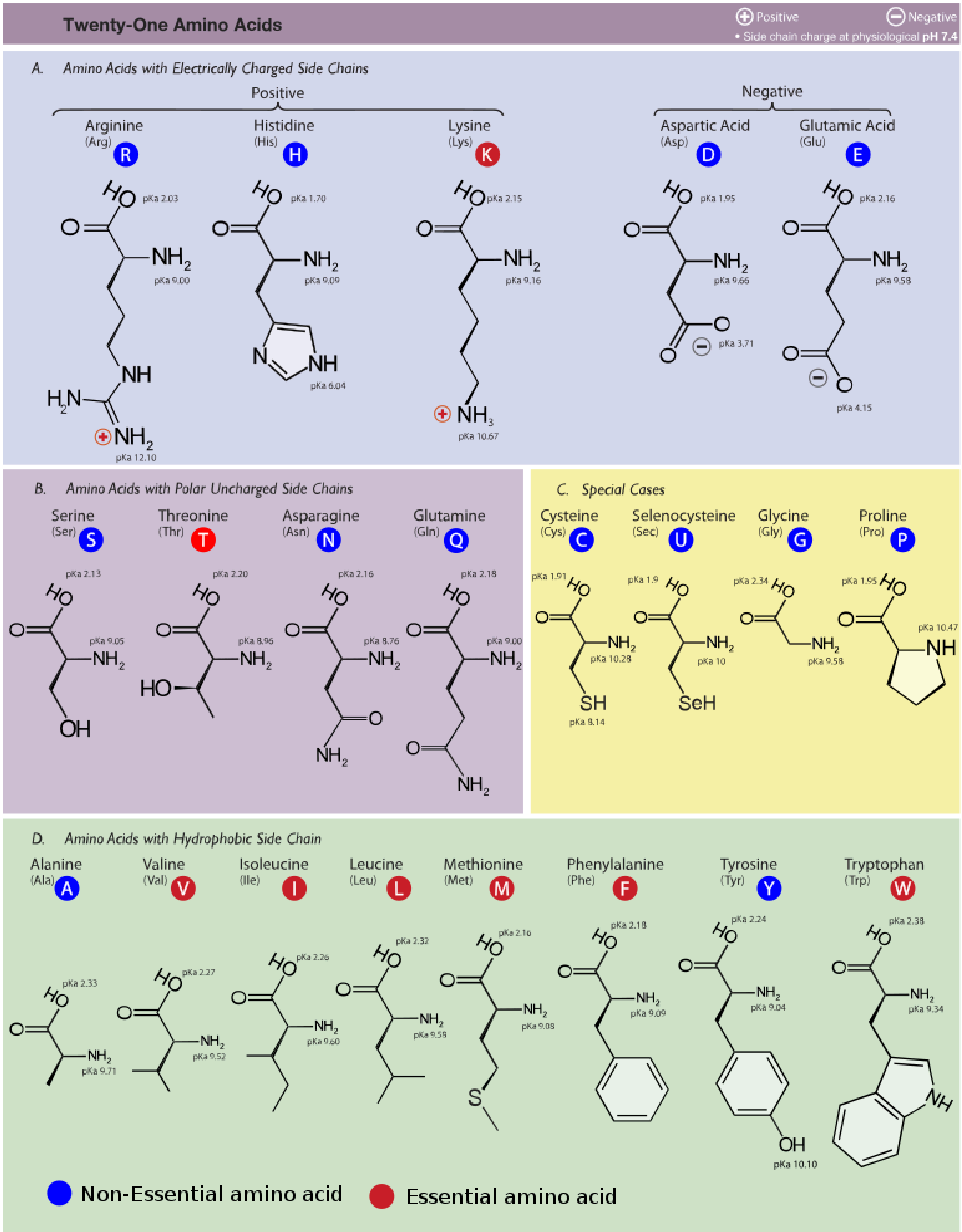
Choline is an essential amino acid-like nutrient. Our bodies can make some of it from the amino acid serine but not enough for optimal functioning, so we have to consume it in the form of free choline or a choline phospholipid, such as phosphatidylcholine (a lecithin), in our diet. Choline's important for our liver health and to synthesize the neurotransmitter acetylcholine. The Adequate Intake of choline is 425 mg for adult women and 550 mg for men per day. It's very easy to get that much choline in a high protein diet. Most animal protein sources are very rich in choline with hundreds of mg per serving. Organ meats and eggs are particularly rich in choline. Many plants also contain some choline, in particular soybeans and potatoes. Lecithin, which has phosphatidylcholine in it, is also added to many foods as an emulsifier. A Tolerable Upper Intake Level of 3.5 g of choline daily also has been set, though choline toxicity is exceedingly rare.

What are amino acids?

There are thousands of different proteins in the body and hundreds of natural amino acids. Yet our bodies use only 21 natural amino acids to synthesize proteins. Of these, 20 are in the standard genetic code. The 21st amino acid selenocysteine was only relatively recently discovered. Selenocysteine is created by a special process of translational recoding, it's not essential and it does not occur in free form in amino acid pools.

Essential and non-essential amino acids

An amino acid is categorized as essential if the body cannot create it fast enough to supply its demands, so you must obtain it from your diet. Of the 21 proteinogenic amino acids the body uses to create proteins, only 9 are nutritionally essential. However, identifying amino acids strictly as (non)essential is an inflexible classification. It allows no gradations in different physiological circumstances. For example, a dispensable amino acid may become indispensable if a certain organ fails to function properly. Therefore, a third category is commonly used: conditionally essential amino acids, also known as 'acquired indispensable amino acids.' The 6 amino acids commonly categorized as conditionally essential are arginine, cysteine, glycine, glutamine, proline, and tyrosine. For example, glutamine cannot be produced by the body at the rate it is needed during certain diseases and gut disorders. That leaves only the following 6 truly non-essential amino acids that the body can create itself: alanine, aspartic acid, asparagine, glutamic acid, serine and selenocysteine. The body can obtain the materials it needs to create non-essential amino acids from essential amino acids or by breaking down other proteins. The image on the following page lists all the 21 proteinogenic 'natural' amino acids found in the human genetic code.


Twenty-One Amino Acids
Positive
Negative
• Side chain charge at physiological pH 7.4
A. Amino Acids with Electrically Charged Side Chains
Positive
Negative
Arginine (Arg) R
Histidine (His) H
Lysine (Lys) K
Aspartic Acid (Asp) D
Glutamic Acid (Glu) E
B. Amino Acids with Polar Uncharged Side Chains
Serine (Ser) S
Threonine (Thr) T
Asparagine (Asn) N
Glutamine (Gln) Q
C. Special Cases
Cysteine (Cys) C
Selenocysteine (Sec) U
Glycine (Gly) G
Proline (Pro) P
D. Amino Acids with Hydrophobic Side Chain
Alanine (Ala) A
Valine (Val) V
Isoleucine (Ile) I
Leucine (Leu) L
Methionine (Met) M
Phenylalanine (Phe) F
Tyrosine (Tyr) Y
Tryptophan (Trp) W
Non-Essential amino acid
Essential amino acid

Protein metabolism

When the body needs an amino acid, it obtains it by breaking down a protein. Both dietary (exogenous) and the body's own internal (endogenous) proteins provide the body with amino acids for use. However, without just exogenous protein, the body can never be in a state of positive protein balance, as it will just be breaking down some of its own body proteins in one location to build proteins in another.

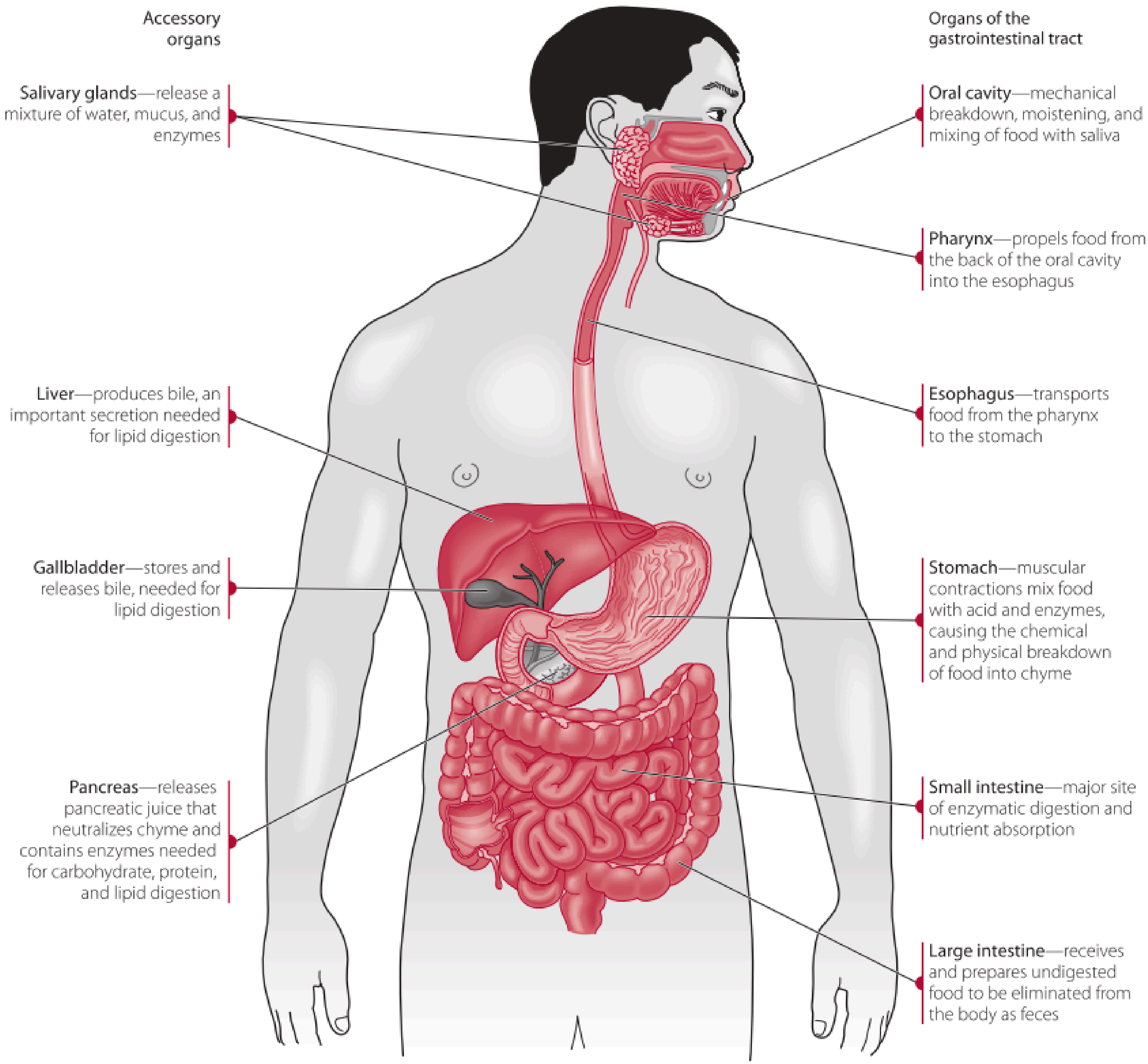
Protein in the diet can come from both plants and animals. Animal proteins are generally of higher quality than plant proteins, as we'll discuss in more detail later.

- Common animal protein sources include meat, poultry, fish, eggs and dairy products (except for butter, sour cream, and cream cheese).
- Common plant protein sources include grains, legumes and vegetables.

After you've ingested them, dietary proteins serve as sources of amino acids and additional nitrogen to create non-essential amino acids and nitrogen-containing compounds in the body.

Protein digestion

Amino acids are essential for the human body to function. To be in a state of positive protein balance, protein has to be consumed in the diet. Before it can be put to use in the body, protein, like any regular nutrient, first has to be digested and absorbed. The following graphics illustrate how the human body digests nutrients in general and protein specifically.



A primer on human digestion.

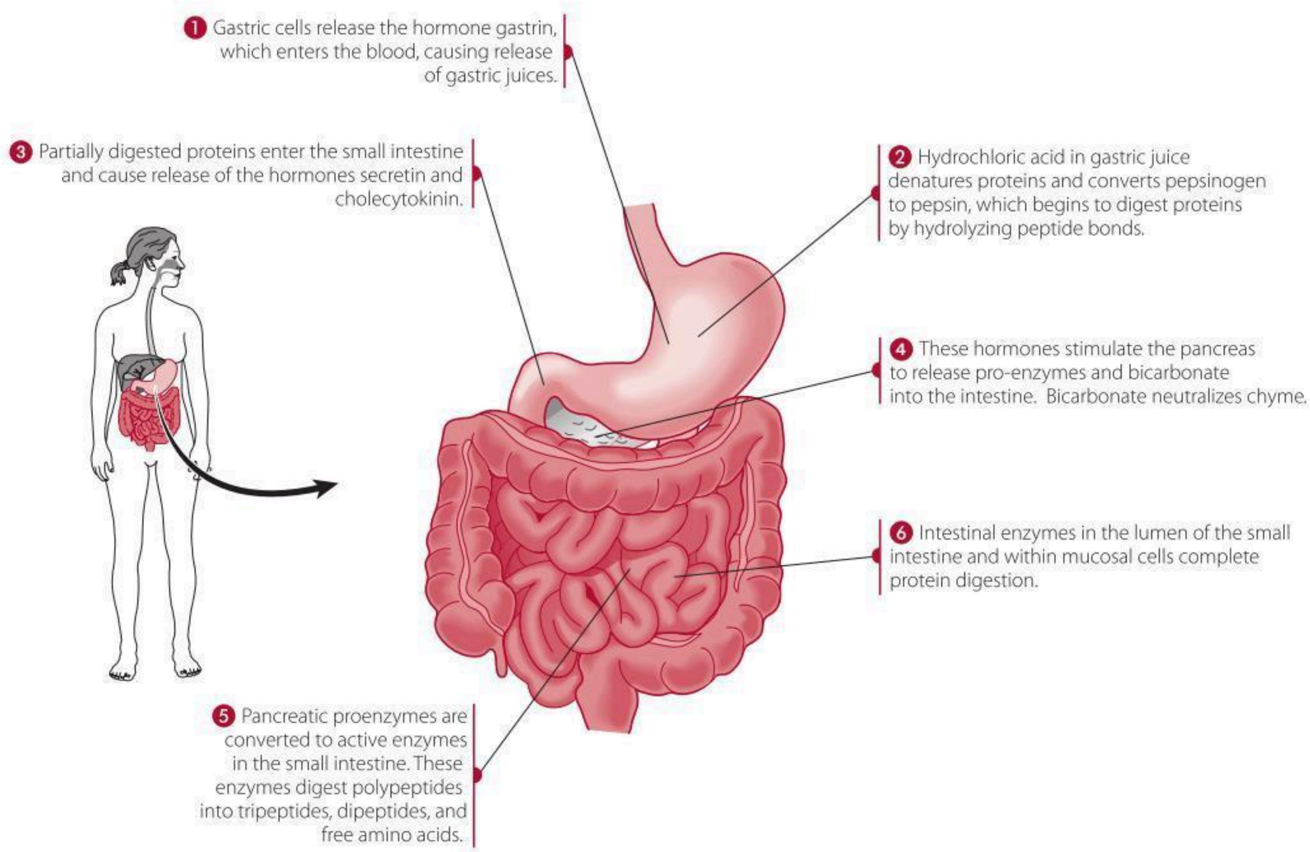


Figure 6.1 An overview of protein digestion.
Source: Derived from Beerman/McGuire, Nutritional Sciences, 1/e. © Cengage Learning.

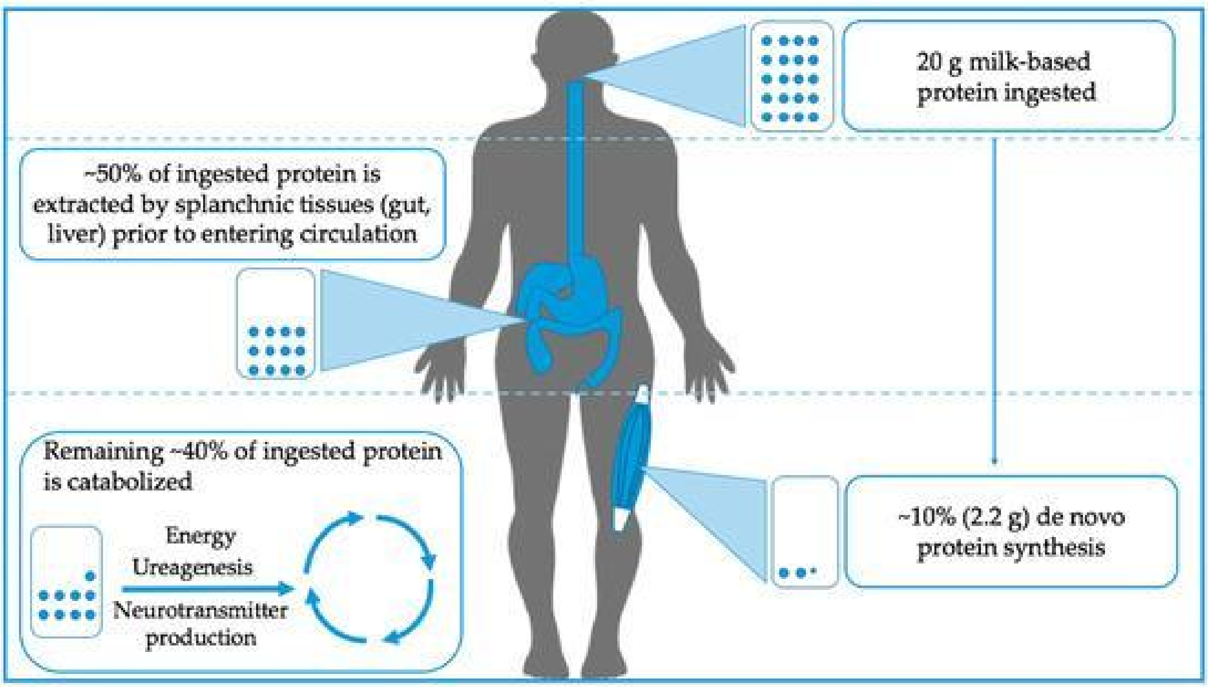
The end products of digestion are subsequently absorbed from the gastrointestinal tract, mostly from the small intestine, into the blood. The resulting amino acids are then transported in the blood to the liver for metabolism.

Amino acid metabolism

Before amino acids are taken up by a tissue in the body, such as muscle, they normally enter the 'amino acid pool'. This isn't a true pool somewhere in the body but rather a collective term for all the amino acids, both endogenous and exogenous in origin, that are floating around in the blood plasma or the liquid inside cells (cytosol). About 150 g of protein are in the amino acid pool of a regular person. The amino acid pool gives your body a temporary storage site of protein so that you're not required to continuously consume protein to avoid going into a net catabolic state. This is one of the reasons you don't have to consume protein every few hours to maximize muscle growth, as we'll detail later.

Anabolism: protein synthesis

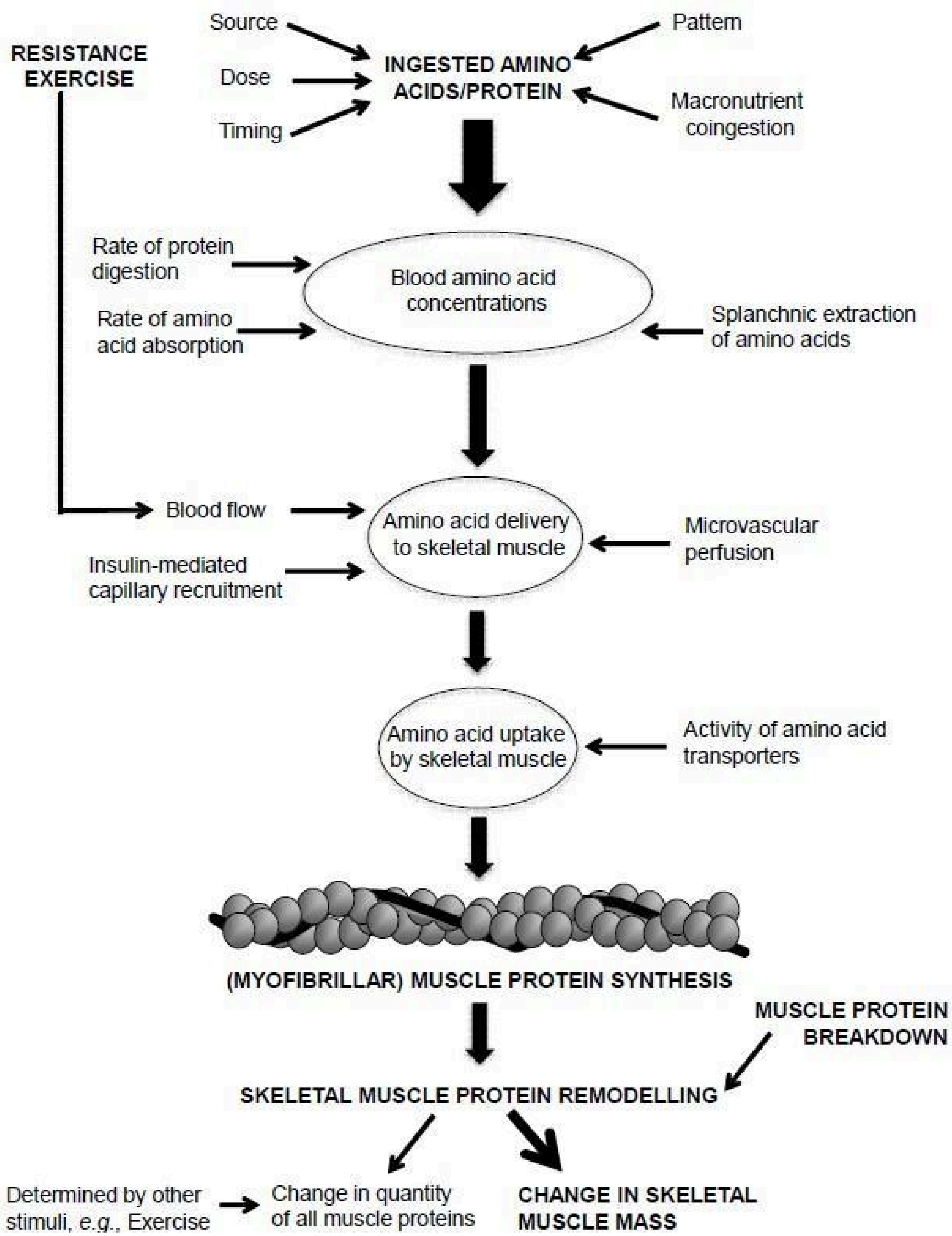
After being taken up from the amino acid pool, dietary protein is preferentially used for anabolism rather than catabolism: the body prefers to use protein for protein synthesis and carbohydrates and fats for oxidation to generate energy. This is much more efficient than oxidizing protein to generate energy. As a strength trainee, anabolism is obviously the desired use of amino acids, as it is required to build muscle tissue. However, in contrast to what many bodybuilders have in mind with their protein, most of the protein you eat is used for other purposes, as illustrated below. Even after a strength training session, less than 20% of the protein you consume is typically absorbed by muscle tissue [2]. Muscle is nice and all, but maintaining vital organ functions is a tad more important. Still, after practically every sizable meal, muscle protein synthesis increases for at least multiple hours.



[Source]

Several hormones in the body, such as growth hormone, testosterone and insulin, are highly anabolic. They can increase protein synthesis and decrease protein oxidation. Insulin is of particular metabolic importance. It's secreted in response to a rise in blood glucose and some amino acids. Insulin primarily functions as a storage hormone. It stimulates cells to take up nutrients. For amino acids, it stimulates the movement of amino acid transporters to the cell membrane and increases the overall activity of amino acid transporters in the liver, muscle and other tissues. Insulin prepares your cells to store nutrients. Insulin aids protein synthesis by regulating protein translation along the ribosomes together with the amino acid leucine, which itself stimulates insulin secretion. Moreover, insulin antagonizes (counteracts) the activation of some enzymes responsible for amino acid oxidation ('burning'). Insulin thus benefits protein balance in multiple ways, but more insulin does not linearly lead to more muscle growth, as we'll discuss later.

After amino acids have been taken up by cells, they can be used for protein synthesis. In muscle cells this leads to muscle protein synthesis. Cell cores contain our genes, which contain our DNA. Our DNA contains the ‘blueprint’ of our bodies. These genes are transcribed into messenger (m)RNA. mRNA is essentially a temporary copy of our DNA that is then translated or ‘read’ by our ribosomes, which are our protein synthesis machinery. For muscle growth, muscle protein synthesis (MPS) is essential, in particular myofibrillar protein synthesis (myoMPS or MyoPS), which is the creation of new myofibrillar proteins, in particular actin and myosin, which are key proteins to make our muscles contract. The following diagrams illustrate how consuming protein stimulates muscle growth.

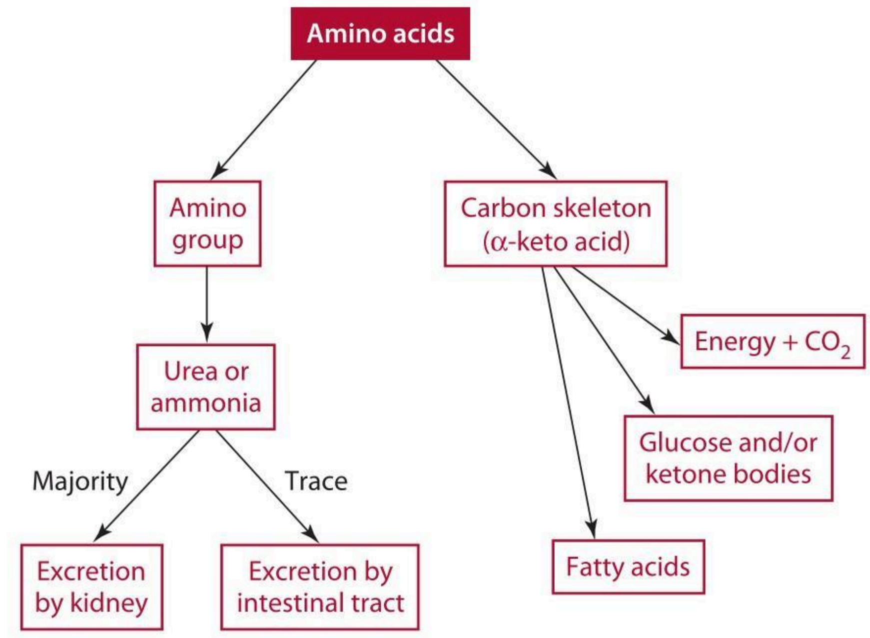


How the protein we consume stimulates muscle growth. [Source]

In short, muscle protein synthesis is vital for muscle growth and the sum of protein balance over time accurately reflects long-term muscle growth [2]. However, just measuring protein synthesis after 1 workout cannot accurately predict long-term muscle growth, especially not in untrained individuals [2]. You can't predict the effectiveness of several months of training from the results of your first workout of the program. It's like estimating how fast a house will be built based on how many bricks were laid down on the first day of construction. Acute protein synthesis only provides a snapshot in time of muscle growth, but the long-term accumulated protein balance over time closely tracks with muscle growth.

Catabolism

To look at the total accumulated protein balance over time, we need to consider not just muscle protein synthesis but also breakdown. However, muscle protein synthesis varies much more than protein breakdown in muscle tissue, so muscle protein synthesis is the primary driver of protein balance and muscle growth. Muscle protein breakdown normally only occurs when absolutely necessary, either to use the protein for something else, as illustrated below, or to remodel the tissue. Tissue remodeling requires a certain level of protein breakdown, much like adding a balcony to your house requires breaking down some walls. Amino acid oxidation occurs both when you consume too little or too many protein. If you consume too little, your body has to catabolize some of its own tissues, like your muscles, to get the protein it needs for more vital functions. If you consume too much, the body will typically oxidize the excess protein for energy.

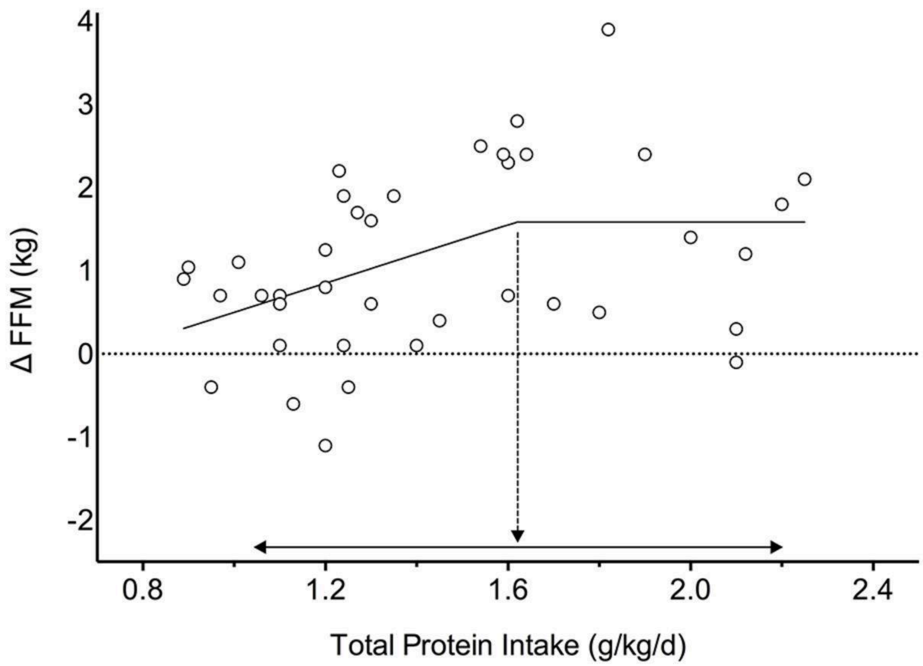


A summary of what catabolized amino acids can be converted to.

How much protein do you need?

Let's get into practical application. How much protein can the body use for protein synthesis to build muscle?

The latest meta-regression of the literature, co-authored by Menno, found that the break-off point of maximal muscle growth occurs at 1.6 g/kg (0.73 g/lb) protein per day. That's 160 g protein per day for a 100 kg (220.5 lb) trainee. Consuming more protein than that resulted in no benefits for strength development or muscle growth. Our results were closely replicated by a meta-analysis from Tagawa et al. (2022): they found a protein intake of 1.5 g/kg (0.68 g/lb) per day was enough to maximize strength development.



Our meta-analysis found that the benefits of protein intake topped off at 1.6 g/kg (0.73 g/lb) per day of total bodyweight for increases in fat-free mass ('muscle').

The 1.6 g/kg (0.73 g/lb) per day cut-off point is supported by dozens of studies, such as the following studies in which only protein intake varied between groups.

- Tarnopolsky et al. (1992) observed no differences in whole body protein synthesis or indexes of lean body mass in strength athletes consuming either 1.4 g/kg (0.64 g/lb) or 2.4 g/kg (1.1 g/lb) over a 2-week period. Protein oxidation did increase in the high protein group, indicating a nutrient overload.
- Walberg et al. (1988) found that 1.6 g/kg (0.73 g/lb) was sufficient to maintain positive nitrogen balance in cutting weightlifters over a 7-day period.
- Tarnopolsky et al. (1988) found that only 0.81 g/kg (0.37 g/lb) was required to maintain positive nitrogen balance in elite bodybuilders over a 10-day period. 1 g/kg (0.45 g/lb) was sufficient to maintain lean body mass in bodybuilders over a 2-week period. The researchers concluded that 1.2 g/kg (0.55 g/lb) was ideal for bodybuilders.
- Lemon et al. (1992) found no differences in muscle mass or strength gains in novice bodybuilders consuming either 1.34 g/kg (0.61 g/lb) or 2.62 g/kg (1.19 g/lb) over a 4-week period. Based on nitrogen balance data with a double standard deviation safety margin, the authors recommended 1.65 g/kg (0.75 g/lb).
- Hoffman et al. (2006) found no differences in body composition, strength or resting hormonal concentrations in strength athletes consuming either 1.7 g/kg (0.77 g/lb) or over 2 g/kg (0.91 g/lb) for 3 months.
- Roberts et al. (2017), co-authored by Menno, found that consuming 2.8 vs. 1.8 g/kg (1.27 vs. 0.82 g/lb) protein per day did not improve any measure of post-workout recovery.

Below is a full literature overview of studies that measured muscle mass, strength, or some measure thereof in groups with different protein intakes. These studies incorporated various groups that consumed a different protein intake without any other differences between the groups, like different macronutrient intakes or nutrient timing (as per research design). In this type of controlled setting, no benefits of more than 1.6 g/kg (0.73 g/lb) protein per day have ever been found in the over 40 studies that have been conducted. Many of the studies found that considerably lower intakes were already enough.

➢Literature overview

Protein intake for muscular development

Since protein intake is highly beneficial for our gains, we want to be extra sure we consume enough of it. It's possible that although research finds no benefits over 1.6 g/kg (0.73 g/lb) per day on average, there are a few special snowflakes with higher dietary protein requirements for maximum results. Business circles sometimes use a Three-Sigma rule to define performance: something has to be 3 standard deviations away from the mean to perform excellently. Based on Lemon et al.'s (1992) protein intake estimation in bodybuilders, adding 3 standard deviations to the mean protein requirement results in a recommendation of ~1.8 g/kg (0.82 g/lb) per day. This figure is also advised by a review paper by Phillips & Van Loon (2011). This is the recommended total daily protein intake from all sources expressed in relation to total bodyweight for optimal body recomposition and performance.

The one-gram-per-pound myth's origin

Why is it that some people say you need to consume 1 g/lb (2.2 g/kg) per day? Aside from the facts that there doesn't need to be any good reason for people to believe something that's untrue, that myths tend to perpetuate themselves via conformism and tradition, and that the fitness industry is flooded with myths, here are some plausible grounds for the 'confusion.'

- People copy the dietary practices of pro bodybuilders on anabolic-androgenic steroids, which enable them to assimilate far more protein than natural trainees can.
- The 'more is better' heuristic. There are so many studies showing protein is good for you; it's hard not to think more of it is even better.
- Supplement companies have an obvious financial incentive to make you want to believe you need more protein than you really do. Supplement companies fund many of the major fitness expos, they sponsor many pro bodybuilders and they have a stake in many of the popular fitness magazines. They even sponsor a lot of scientific research. There's not that much else to sell in fitness once you think about it, so if you follow the money, you often end up at a supplement company.
- People can't be bothered with decimals and just round up to the nearest convenient integer, which so happens to be an easy to remember 1.

Many people also don't realize that the 1 g/lb (2.2 g/kg) myth only exists in bro bodybuilding culture. During the first decades of research, the scientific community long refuted the idea that strength trainees require more protein than untrained individuals in the first place(!)

"But I train harder!"

If you think you need more than 0.82 g/lb (1.8 g/kg) because you think you train harder than the participants of all the above studies, think again. For example, Lemon et al. (1992) studied bodybuilders training 1.5 hours per day, 6 days per week and still found no improvement in gains above 0.61 g/lb (1.3 g/kg).

However, it is true that your protein intake logically depends on your training intensiveness. If you're not stimulating much protein turn-over and muscle growth with your training, you don't need as much protein for maximum muscle growth. If you don't train very hard, the protein intake could be decreased. Conversely, if someone is performing a high volume of both aerobic and anaerobic training, such as team-sport athletes, you may want to increase their protein intake. You can use the following table to assess how much additional protein is needed for athletes with extraordinarily high training volumes. Note that these recommendations are not additive. You always have to subtract the baseline protein requirement (RDA), which is ~0.9 g/kg (0.41 g/lb) per day, from all numbers you add up. So if you add an hour of moderate intensity jogging to daily strength training, you only have to add 0.1 g/kg (0.05 g/lb) per day. As a rule of thumb, concurrent strength-endurance athletes, such as team sports athletes training 10+ hours a week, may benefit from the conventional protein intake of 2.2 g/kg (1 g/lb) per day.

Table 1. Recommended Protein Intakes for Different Types and Volumes of Exercise

Exercise Type	Exercise Volume	Recommended Intake (g/kg/day)
Walking[21]	1 hr, 40% Vo_{2max}	unchanged
Moderate intensity jogging[21]	1 hr, 55–67% Vo_{2max}	1.0
Tour de France simulation[20]	~5 hr day, 70% Vo_{2max}	> 1.7
Habitual endurance (trained)[8,22]	≥10 hr/wk	0.94–1.67
Heavy resistance (novice)[15]	20–40 sets/day 70–85% 1RM	1.6–1.8
Habitual heavy resistance (trained)[22]	75 min/day	1.2

How protein needs change based on exercise type and training level according to [Fielding & Parkington (2002)](#).

“But I’m more advanced than these geeks!”

Another frequently heard objection is that people need more protein because they are more experienced than the studied populations. In reality, the opposite may be true. For example, Tarnopolsky et al. (1988) studied elite bodybuilders and found that less protein was needed than in novice bodybuilders. Several studies (Rennie & Tipton, 2000; Hartman, Moore & Phillips, 2006; Moore et al., 2007) have replicated the finding that the more experienced you are, the less protein you need. This makes perfect sense if you think about it. Protein synthesis and breakdown perform a balancing act for everybody at all times. Resistance training causes both breakdown and synthesis to increase, normally with a net favorable effect towards synthesis over time. As you progress in your training, the body becomes more efficient at stopping the breakdown of protein resulting from training: you experience less damage for a given workout. Since less muscle protein now needs to be rebuilt, this means less protein is subsequently needed for optimal growth.

Secondly, the more advanced you are, the less protein synthesis increases after training. As you become more muscular and you get closer to your genetic limit, progressively less muscle is built after training. This is very intuitive. The slower you can build muscle, the less protein is needed for optimal growth. It wouldn’t make any sense if the body needed more protein to build less muscle.

As such, since more advanced trainees experience both less protein breakdown as well as less protein synthesis, they need less protein than novice level trainees. However, since the extent of this adaptation has not been studied in a practical training setting and many studies already look at trained individuals, it is advisable that advanced trainees still consume 0.82 g/lb (1.8 g/kg) of protein per day.

What about when cutting? Or if you're already really lean?

Some researchers argue our protein requirements increase in energy deficit. While this may seem intuitively plausible, it does not logically follow. Protein requirements are a function of the demand to synthesize new proteins and replace oxidized proteins. In energy deficit, protein synthesis rates decrease, while protein oxidation rates largely stay the same. As such, protein needs could logically decrease in energy deficit, because your body isn't building as much protein as it is in energy surplus.

In untrained individuals, research shows increasing your protein intake in a deficit beyond maintenance protein requirements does not help preserve muscle mass and does not increase protein balance [2, 3, 4], even when doubling your protein intake at just 440 Calories a day. One particularly nice study is Hill et al. (2015), which looked at 3 different protein intakes at weight maintenance and during 2 weight loss phases for 5 weeks each. Protein content did not affect body composition and this effect was not different between weight maintenance and weight loss phases. Other research also found that the beneficial effects of protein intake on body composition occur independently of energy balance. How much muscle you lose is instead determined by things like the rate of weight loss, your training and your genetics. We also know that increasing your protein intake has little effect on muscle atrophy during disuse, like during bedrest. A 2020 meta-analysis by Hudson et al. confirmed there is no significant effect of protein intakes higher than the RDA on lean body mass with or without energy restriction in untrained individuals (in contrast to the authors' conclusions). Unfortunately, there were not enough data to make the same comparison in trained individuals.

It's possible albeit biologically uncommon that there is a double interaction effect between protein intake, energy balance and training status, i.e. protein requirements are higher for exercising individuals and the increase is greater in energy deficit. We have some research that tested this idea. In 2021, Gwin et al. estimated the protein requirements for maximum protein balance in strength-training young adults in 30% energy deficit. They found no increase in muscle protein synthesis (MPS) with 0.3 g/kg (0.14 g/lb) essential amino acids (EAAs) compared to 0.1 g/kg (0.05 g/lb) at rest or even after 16 sets of quad work, surprisingly. The lower amount of 0.1 g/kg (0.05 g/lb) corresponds with 8-10 g EAAs or 20 g of high-quality protein like whey or 0.3 g/kg (0.14 g/lb) high-quality protein, which is the same amount we know maximizes MPS in studies in energy balance or surplus. As such, the authors concluded: “mixed MPS appears to be optimally stimulated in young subjects after ingesting 8-10 g EAA, an amount delivered in 20-30 g of high-quality protein, *regardless of whether an individual is in a state of energy balance or moderate energy deficit*.” The authors estimated they had over 90% statistical power to detect an effect, if there had been one, and the diets were controlled for total energy intake, so these results strongly suggest protein requirements are the same when cutting or bulking. However, a big caveat is that there was no direct comparator group in energy balance in this same study.

There was also a twist: extra protein *did* increase total body protein balance, in contrast to MPS. Since we don't have good data on whole-body protein balance to compare to, it's unclear if this was the result of energy deficit or because in general the protein requirements for whole-body protein balance are higher than for muscular energy balance. It's possible that after essential and muscular protein requirements are met, the body can still use protein for things like extra skin, hair and nail growth, possibly even organ growth.

In 2023, Larsen et al. reported similar findings in postmenopausal women as Gwin et al. did in young adults. Even in a massive 60% energy deficit, the dose-response effect of 15 g vs. 35 g vs. 60 g whey protein on myofibrillar protein synthesis (myoMPS)

plateaued at 35 g. There was also a group in energy balance consuming 35 g whey protein in the fasted, fed and post-workout + fed states. This group did not significantly differ in myoMPS response to the 35 g whey group in massive energy deficit.
In 2024, Gwin et al. improved upon the earlier 2 study designs by comparing the effect of protein supplementation within the same individuals in energy balance vs in 30% energy deficit. A group of endurance-trained participants completed an endurance workout (treadmill walking with a backpack and submaximal bodyweight step-ups) once each in energy balance and in 30% energy deficit. Post-workout, one group consumed a high protein shake (56 g protein, mostly essential amino acids) and the other a moderate protein shake (34 g protein with added carbs to equate the calories). The extra protein intake increased quadriceps protein synthesis (MPS) in energy deficit but not in energy balance, suggesting energy deficit increases protein requirements. However, the effect of protein intake on whole-body protein synthesis and balance was not affected by energy balance. Moreover, only mixed MPS was measured, so given that it was an endurance workout, the extra protein synthesis may have been mitochondrial or sarcoplasmic instead of contractile muscle tissue. Despite these limitations, this study was the first somewhat direct evidence that energy deficit may increase protein requirements, at least for a single meal after a fasted endurance workout.

Longer-term research on the effect of energy balance on total daily protein requirements in strength training individuals is lacking, but many studies support that protein requirements in energy deficit are no higher than they are in other studies in energy balance or surplus.

- Walberg et al. (1988) found 1.6 g/kg (0.73 g/lb) protein per day was enough for cutting weightlifters to be in positive nitrogen balance.

- Helms et al. (2014) found no difference in strength or body composition changes in weightlifters after a 2-week diet with 40% energy deficit with a daily protein intake of 2.8 g/kg (1.27 g/lb) vs. 1.6 g/kg (0.73 g/lb).
- Pikosky et al. (2008) took a group of endurance trained subjects and had them consume either 0.9 or 1.8 g/kg (0.41 or 0.82 g/lb) of protein per day. They also added a thousand calories worth of training on top of their regular exercise. Of course, the nitrogen balance in the low protein group plummeted. However, the protein intake of 1.8 g/kg (0.82 g/lb) in the other group completely protected the subjects from muscle loss. Nitrogen balance, whole-body protein turnover and protein synthesis remained unchanged.
- Jose Antonio et al. (2015) studied strength trained subjects in a mild energy deficit. There was no effect of 3.4 vs. 2.3 g/kg (1.55 vs. 1.05 g/lb) protein on changes in lean body mass or strength.
- Prior research from Jose Antonio et al. (2014) in strength trained individuals had found the same thing: increasing protein from 1.9 to 4.4 g/kg (0.86 to 2 g/lb) did not affect body composition (including fat loss) or strength during the 8-week study. (These subjects weren't intended to be in a deficit, but body fat percentage went down in both groups.)
- Pasiakos et al. (2013) studied physically active individuals, mostly military personnel performing light strength training 3x per week, in a 40% deficit, for 31 days. There was no difference in fat or muscle losses between a group consuming 1.6 g/kg (0.73 g/lb) protein and a group consuming 2.4 g/kg (1.09 g/lb) protein per day. A strength of this study is that they also looked at muscle protein synthesis, anabolic signaling, gene expression, nitrogen balance and resting metabolic rate. None of the relevant measures were different between the 2 protein intakes. (There was a spurious difference in protein synthesis in response to a test meal with 20 g protein. This was somehow higher for the 1.6 g/kg (0.73 g/lb) group than the 2.4 g/kg (1.09 g/lb) group in energy balance but

reversed in energy deficit. The test meal had the same amount of protein in both groups, so it doesn't tell us anything about the effectiveness of the different protein intakes.)

- Verdijk et al. (2009) found no difference in fat loss, muscle growth, nitrogen balance or strength development between a group consuming 1.1 g/kg (0.5 g/lb) protein and a group adding 20 g casein to the diet. The subjects were elderly men who performed strength training 3x per week. They only trained their lower body though, which probably explains the very low protein intake. In case you think their age is a confounder, it is, but most research actually suggests the elderly have a higher protein requirement, if anything, due to anabolic resistance (i.e. less efficient protein metabolism, discussed in further detail in the course topic on strength training for the elderly).
- Campbell et al. (2015) found no difference in fat loss, strength development or muscle growth in subjects consuming 1 – 1.2 g/kg (0.45 – 0.55 g/lb) protein compared to more than 1.2 g/kg (0.55 g/lb). The subjects were overweight men and women that performed full-body strength training twice a week along with a weekly cardio session of 60 minutes. A notable strength of this study was that it had 117 subjects and it lasted 9 months. As you can see in the figure below, there was even a trend for better fat loss and more muscle growth in the group consuming 1 – 1.2 g/kg (0.45 – 0.55 g/lb) protein than the group consuming more than 1.2 g/kg (0.55 g/lb) protein.

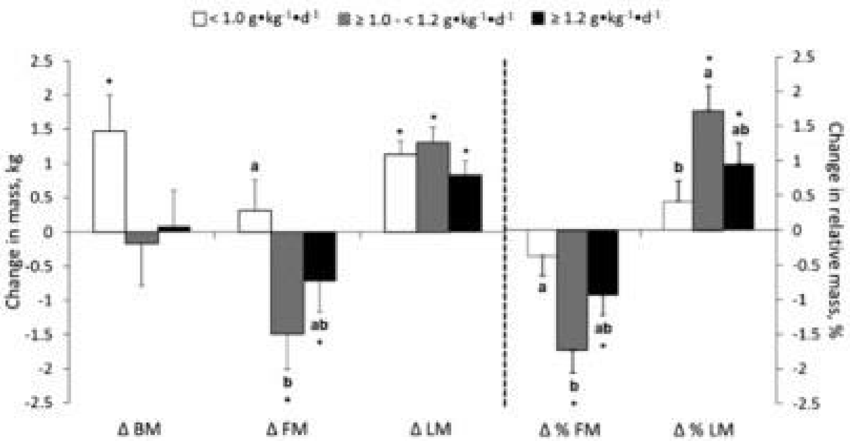


FIGURE 3 The changes in BM, FM, LM, %FM, and %LM throughout the 36-wk intervention among groups who consumed <1.0 ($n = 43$), ≥ 1.0 to <1.2 ($n = 29$), and ≥ 1.2 ($n = 45$) $g \cdot kg^{-1} \cdot d^{-1}$. Results are reported as means ± SEs. Analyses are adjusted for age, sex, preintervention variable values, and total carbohydrate and fat intakes during the intervention. *Different from zero, $P < 0.05$ (i.e., the change from before to after the intervention is significant). Values without a common letter are significantly different, $P < 0.05$. BM, body mass; FM, fat mass; LM, lean mass; LM:FM, ratio of LM to FM.

- Maltais et al. (2015) found no difference in lean body mass gains in strength training elderly subjects consuming 1.1, 1.3 or 2.1 g/kg (0.50, 0.59 or 0.95 g/lb) protein. They did 3 full-body strength training sessions per week for 4 months. The 2.1 g/kg (0.95 g/lb) protein group did lose fat though, while the 1.3 g/kg (0.59 g/lb) group didn't. This was probably in part because this group was consuming soy-based protein while the 2.1 g/kg (0.95 g/lb) group was consuming dairy. The 1.1 g/kg (0.50 g/lb) group actually lost only 200 grams less fat than the 2.1 g/kg (0.95 g/lb) group (0.9 kg / 1.98 lb vs. 1.1 kg / 2.42 lb fat), but their fat loss did not reach statistical significance.
- Campbell & Meckling (2015) surprisingly found that a protein intake of 0.9 g/kg / 1.98 lb (1.4 g/kg / 0.64 g/lb FFM) resulted in significantly *more* fat loss and better muscle retention than a protein intake of 1.2 g/kg / 0.55 g/lb (1.9 g/kg / 0.86 g/lb FFM) with no difference in strength development. The subjects were overweight women and they performed 3 full-body strength training sessions per week.
- Ofsteng et al. (2020) found no differences between a protein intake of 1 g/kg and 2 g/kg (0.45 g/lb and 0.91 g/lb) during a 10-day military exercise in extreme energy deficit (~77%) on performance losses (incl. 1RM bench and leg press

strength), body composition change and markers of muscle catabolism (incl. hormonal markers and CK).

- Pearson et al. (2021) found no difference in the effectiveness of a daily protein intake of 1.7 g/kg vs. 0.9 g/kg (0.77 g/lb vs. 0.41 g/lb) to maintain lean body mass or exercise performance in female sports athletes during a 2-week 40% energy deficit.

A different argument for increased protein requirements in energy deficit is that you're not consuming as many carbohydrates or fats, which are supposedly nitrogen sparing. However, carbs and fats don't spare protein to any considerable extent (and carbs aren't any more effective than fats) [2]. Only protein spares protein. The protein sparing idea seems to have come from a misinterpretation of the nitrogen balance literature showing more lean mass is lost in more severe caloric deficits. A simple explanation for that finding is that the more total mass you lose, the more lean mass you also lose. No surprises there.

What is nitrogen balance?

Nitrogen balance is the difference between nitrogen excreted from the body and nitrogen ingested in the diet, which mostly comes from protein sources. Nitrogen balance can be measured at the whole-body level by collecting or estimating intake and output (sweat, feces, urine) or in specific body parts by comparing arterial delivery with venous outflow. Arteries pump blood full of oxygen towards organs and muscles; veins carry oxygen-deprived blood back to the heart. *(Source)*

Another practical reason you often don't need more protein in energy deficit is that at lower energy intakes, high-quality protein sources like chicken breast typically make up a greater percentage of the total protein requirement, whereas more protein typically comes from other foods, such as grains, when bulking.

In conclusion, there is little empirically substantiated reason to think we need more than 1.8 g/kg (0.82 g/lb) of protein per day when cutting.

Biological sex

Women theoretically don't need as much protein as men, because they have an inherently more protein sparing metabolism that relies more on fat for fuel.

- Women oxidize less protein during exercise than men.
- Women also burn less protein while fasted or after meals than men.
- Due to their higher essential fat mass, women generally have less lean body mass than men of the same weight.

A meta-analysis found a trend that the protein requirement of women is almost exactly 10% lower than that of men, but the difference was not statistically significant.

When whole-body protein synthesis and breakdown are measured in strength training women on a training day, net balance peaks at 1.5 g/kg (0.68 g/lb) per day, indicating this is the required protein intake for maximum gains. The same method in male bodybuilders found a requirement of 1.7 g/kg (0.77g/lb) per day on rest days.

Research thus suggests an about 10% lower protein requirement for women compared to men. However, due to a lack of research directly examining the difference with men and a lack of research supporting less than 1.8 g/kg (0.82 g/lb) per day is enough even for women with a bodyweight below 60 kg, we still recommend 1.8 g/kg (0.82 g/lb) per day by default for female strength trainees looking to ensure optimal progress.

PEDs

Performance enhancing drugs (PEDs), in particular androgenic-anabolic steroids (AAS) most likely increase protein needs substantially. Some people argue that AAS decrease protein requirements because AAS increase nitrogen retention: they make your body's protein metabolism more efficient. However, muscle protein breakdown (MPB) rates vary severalfold less than muscle protein synthesis (MPS) rates and MPB doesn't decrease nearly as much during AAS usage, if it decrease at all, as MPS increases [2, 3, 4, 5, 6, 7]. Multiple studies found no significant decrease in muscle protein breakdown rates at all, whereas MPS tends to increase by around 50% on testosterone replacement therapy or low-dose (15 mg per day) AAS usage. If protein requirements scale directly with the increase in MPS, that would indicate protein requirements increase dramatically on AAS: 50% above 1.6 g/kg (0.73 g/lb) per day, so that's 2.4 g/kg (1.09 g/lb) per day, even on the lowest feasible dosages. On high-dose cycles, protein requirements may be higher, but anecdotally, few competitive male bodybuilders consume much more than that. A 2015 systematic review of the dietary practices of competitive bodybuilders reported average protein intakes ranging from 2.1 to 3.7 g/kg (0.95 to 1.68 g/lb) per day in enhanced bodybuilders. Without more concrete data to go by, 2.4 to 3.7 g/kg (1.09 to 1.68 g/lb) per day may be a wise range to go by, depending on the dosages used.

Muscle memory

Trainees that restart intensive training after a lay-off of multiple weeks to months may temporarily benefit from the traditional 2.2 g/kg (1 g/lb) per day as well due to muscle memory. Muscle memory will be discussed in more detail in the module on understanding muscle growth, but briefly, it allows someone that previously had a lot more muscle mass than they currently do to regain that muscle mass significantly more rapidly than it took to build it the first time. The optimal protein intake is primarily a factor of how high protein synthesis can be pushed, so cases such as these where we may see extraordinarily high rates of muscle growth may also require extraordinarily high protein intakes.

Should you set protein relative to bodyweight or lean body mass?

Theoretically, you would expect protein requirements to be influenced primarily by lean body mass and less so by fat mass. However, the vast majority of research we have uses bodyweight, so within the studied ranges of body fat percentage, using total bodyweight is evidently accurate enough. The little research we have that directly studied the effect of lean body mass on protein utilization hasn't found lean body mass differences between individuals to be a major determinant of protein requirements. In a study by Macnaughton et al. (2016), lean body mass did not influence the dose-response of protein intake for protein synthesis and anabolic signaling. Bartholomae & Johnston (2023) also found no correlation between nitrogen balance and fat-free mass. McAdam et al. (2022) did find that soldiers with higher estimated baseline fat-free mass (FFM) benefited more from whey protein supplementation than those with lower FFM, but they did not compare FFMI with total bodyweight. The reason for the unclear effect of FFM on protein requirements is likely that while muscle mass requires more protein than fat mass, organ mass is still the most protein consuming tissue in your body and most people have similar amounts of organ mass, given the same total bodyweight.

Plus, any greater predictive power from using lean body mass (LBM) rather than total body mass may be offset by the inaccuracy of estimating someone's body fat percentage (BF%). If your estimate of protein requirements if 5% more accurate when using LBM than when using total bodyweight yet your BF% estimate has 5% error, you're no better off.

However, in obese individuals, 1.8 g/kg (0.82 g/lb) per day is likely excessive. If you have a reasonably reliable estimate of someone's body fat percentage (discussed in the course's energy in the section on measuring progress), you can use the lower bound of Eric Helms et al.'s (2014) recommendations and prescribe 2.3 g/kg (1.05 g/lb) LBM per day if this results in a lower protein recommendation than 1.8 g/kg (0.82 g/lb) per day.

Take-home messages

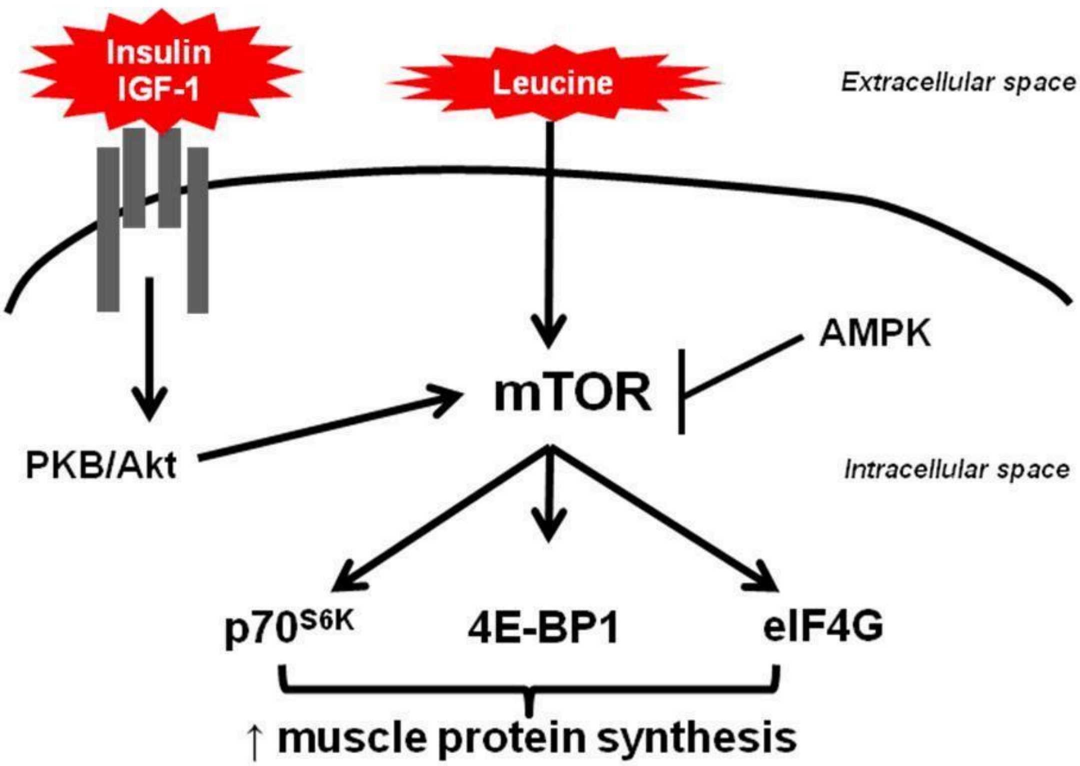
- There is generally no advantage to consuming more than 1.8 g/kg (0.82 g/lb) of protein per day, so this is a good minimum target to consume. This recommendation refers to total bodyweight and is irrespective of energy balance. It already includes a mark-up, since controlled research finds no more significant benefits after 1.6 g/kg (0.64 g/lb) per day.
- The only likely exceptions that need more protein are trainees with muscle memory, concurrent athletes, anabolic steroid users and vegans.
- The optimal protein intake theoretically decreases with training experience, because your body becomes more efficient at preventing protein breakdown during exercise and protein synthesis rates after the workout decrease along with muscle growth. Women and trainees that don't train very hard may require less protein, but in the absence of more data, 1.8 g/kg (0.82 g/lb) per day is still advisable to consume.

Protein quality

Total dietary protein intake is not all that matters. Ultimately, your body mainly cares about total amino acid intake. There's one amino acid that's particularly important to understand: leucine.

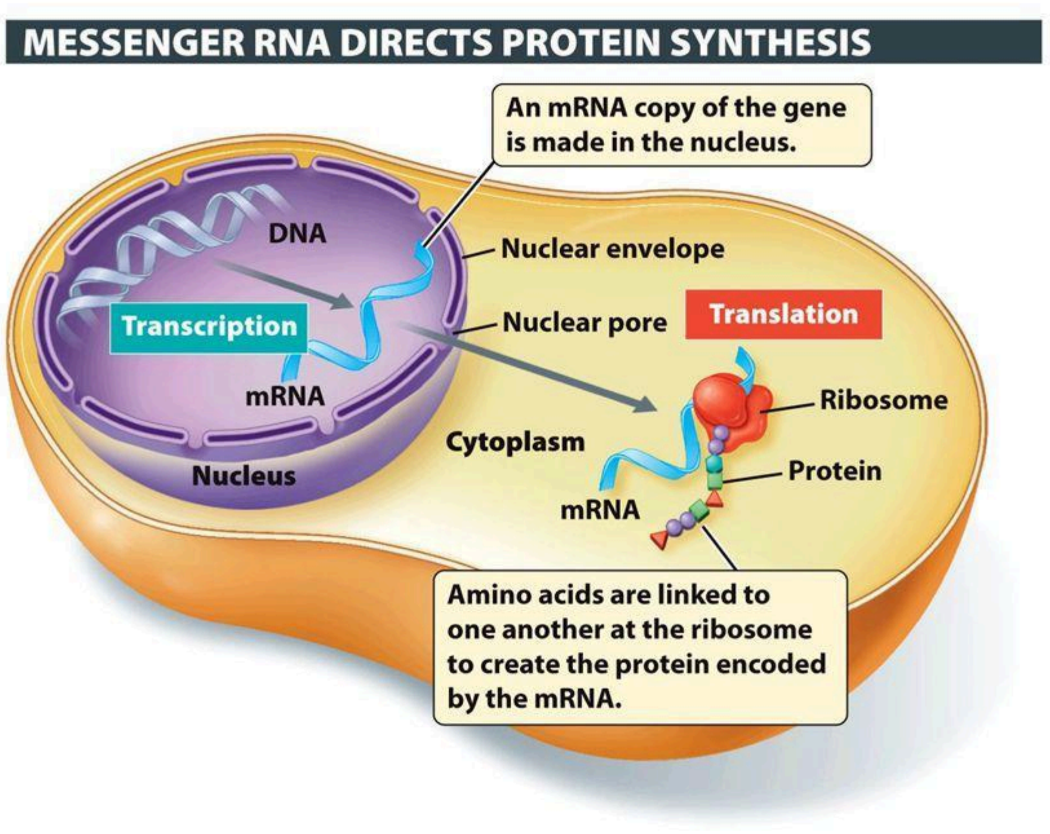
BCAAs

Leucine is an essential, branched chain amino acid (BCAA) that stimulates the mTOR enzyme that functions much like an activation switch of muscle protein synthesis. This knowledge has made leucine and BCAAs as a whole a highly popular supplement hypothesized to increase muscle growth.



Leucine is a key activator of the anabolic signaling cascade in muscle cells, in which mTOR plays a key role. Source

However, anabolic signaling or mTOR does not equate to muscle growth. It is merely the message sent to the nucleus, the cell core. In the nucleus, your DNA resides with the blueprint to create new proteins. This information from your DNA is transcribed to mRNA and sent to your ribosomes, where it is translated and there in the ribosome the actual production of new proteins takes place: see the illustration below.



Discover Biology, 5/e Figure 15.2

Leucine causes anabolic signaling (activating the mTOR-p70S6K cascade), but you need other amino acids, particularly the other 6 essential amino acids, to increase eIF2α and initiate protein translation to increase MPS.

Simply put, for maximal muscle protein synthesis, 2 things are important:

1. Optimizing the signal for muscle protein synthesis by leucine consumption.
2. Consuming enough amino acid building blocks to actually synthesize new proteins.

Consuming just BCAAs for MPS is like turning on a light switch when there's no power supply. You can flip the switch all you like, but you won't get any light (MPS) without electricity (other amino acids). Even increasing the leucine content of a 10 g EAA mix from 1.8 to 3.5 g does not affect post-workout protein balance despite increasing anabolic signaling (mTOR).

So BCAAs alone won't maximize muscle growth. If you consume BCAAs in isolation, they can even *decrease* muscle protein synthesis and turnover or at best just keep you in protein balance [2]. BCAAs don't stimulate muscle protein synthesis (MPS) or decrease muscle protein breakdown (MPB) in isolation. Previous research had inferred the stimulation of MPS from BCAAs by looking at muscle uptake of leucine. However, leucine will be oxidized instead of used for muscle protein synthesis when it's not accompanied by other amino acids.

To make BCAAs effective, you need to combine them with the other amino acids. You don't need that many BCAAs. In fact, the BCAA content of high-quality protein sources is high enough to make BCAA supplementation superfluous. It takes only about 10 grams of essential amino acids to make additional leucine supplementation redundant.

Even post-workout in elderly men, a scenario where theoretically you would see maximal benefits of leucine supplementation, adding leucine to just 0.16 g/kg (0.07 g/lb) whey protein (that's just 13 g for a man weighing 80 kg / 176 lb) failed to increase muscle protein synthesis or decrease protein breakdown. Similarly, in young strength trained men, increasing the leucine content of 2 post-workout servings of 20 g whey (in the form of native whey) from 2.2 to 2.7 g did not increase anabolic signaling or muscle protein synthesis. Muscle recovery speed was also unaffected.

In line with their effects on protein balance, a multitude of studies decisively show that consuming BCAAs on top of a sufficient dietary protein intake does not improve our gains in any way.

- Kephart et al. (2016) found that BCAA supplementation post-workout is no more effective than sugar at improving recovery after high-volume squatting exercise.
- Spillane et al. (2012) found that "when combined with heavy resistance training for 8 weeks, supplementation with 9 g/day of BCAA 30 min before and after exercise had no preferential effects on body composition and muscle performance."
- Aguiar et al. (2017) found that supplementing 3 g leucine post-workout for 8 weeks did not increase any measure of strength development or muscle growth (CSA).
- Mourier et al. (1997) compared several weight loss diet groups of elite wrestlers. BCAA supplementation on top of a sufficient protein intake had no effect on muscle retention, subcutaneous fat loss, aerobic performance, anaerobic performance or strength.
- De Andrade et al. (2020) found that "high-dose leucine supplementation did not enhance gains in muscle strength and mass after a 12-week resistance training

program in young resistance-trained males consuming adequate amounts of dietary protein.”

- Bagheri et al. (2021) found 9 g BCAA supplementation per day was no more effective than placebo to enhance muscle growth, strength development and muscle regulatory factors in postmenopausal women.
- Ooi et al. (2021) studied 132 Chinese adults on an energy deficit with either a low protein intake (14%) with placebo, a low protein intake with BCAAs (0.1 g/kg or 0.05 g/lb) or a high-protein diet. The BCAAs tended to reduce lean body mass losses in the low protein condition, but their results were still worse than just consuming a higher protein intake.
- Jacinto et al. (2021) found no effect of 6 g daily leucine supplementation on muscular recovery after strength training.
- Several other studies confirm that adding several grams of leucine to meals for several weeks does not affect muscle growth, muscle function or fat loss in the elderly [2, 3] or competitive canoeists and leucine generally does not prevent muscle loss even in cases of severe muscle wasting.

If you’ve read any study at all showing any positive effect of BCAA supplementation on top of a sufficient dietary protein intake, it was probably bunk research sponsored by the notorious BCAA selling supplement company Scivation.

In conclusion, to consume enough BCAAs, you don’t need to supplement them. Nature gave us a package deal with animal protein sources. Meat, dairy, eggs, fish and poultry all have enough BCAAs to make supplementation obsolete. As Van Dusseldorp et al. (2018) concluded: the benefits of BCAA supplementation already become “likely negligible” at a protein intake of just 1.2 g/kg (0.55 g/lb) per day. Additional leucine can still modulate the anabolic signaling of the meal, but not the total amount of actual muscle protein you build. You can generally assume that if you follow the recommended protein intakes from this course and you include at least one high-quality protein source with each meal (discussed below), you will automatically consume enough leucine and other BCAAs across the day. If you want to be meticulous, make sure you fill at least 50% of your protein requirement in each meal with a high-quality protein source.

Protein quality indices

BCAA content is not the only determinant of protein quality. Several other factors determine how useful certain proteins are to stimulate anabolism or prevent catabolism and ultimately make your muscles bigger. Let's review what determines protein quality [2]. Protein quality describes a protein source's ability to meet human requirements, in particular for growth but also all the other functions of proteins you learned in this module. For our purposes, protein quality is a good metric of how well a food can stimulate muscle growth. The chief 2 determinants of protein quality are:

1. Its amino acid composition: high-quality protein sources provide amino acids in a similar ratio as the body's requirements and contain all amino acids we need, in particular the essential amino acids. Your body doesn't really have a 'total dietary protein' requirement: it has a requirement for each amino acid. For example, even if you consume all the valine in the world and your total protein intake is easily adequate, insufficient leucine consumption still limits muscle growth.
2. Its digestibility: in addition to having the amino acids the body uses, they also need to be digestible for our bodies. If our body cannot digest the amino acids, it cannot absorb and metabolize them. As an example of poor digestibility, up to ~50% of raw eggs' protein is not absorbed or digested in the small intestine [2],

so you theoretically need about twice as much protein from raw eggs as from cooked eggs. Curiously though, the only study directly comparing raw vs. boiled eggs found no difference in their effect on anabolic signaling or myofibrillar protein synthesis.

For a long time, the most-used protein quality index was the Protein Digestibility-corrected Amino Acid Score (PDCAAS), which was a significant improvement over several previous measures of protein quality. However, it still had several limitations. For example, PDCAAS scores that are higher than 100% are truncated to 100% because the available protein in food is first limited by digestibility. This underestimates the protein quality of high-quality sources. Digestibility cannot exceed 100%; neither can PDCAAS. This leads to problems when calculating PDCAAS for mixed meals, where it's possible to have complementary amino acid sources that combine to create a high-quality protein. Moreover, it may be possible for high amounts of essential amino acids to compensate for a lack of non-essential amino acids.

The Digestible Indispensable Amino Acid Score (DIAAS) has been proposed as an improvement over the PDCAAS. A 2021 meta-analysis supports that protein sources with a higher DIAAS quality score result in more muscle protein synthesis, both at rest and after training, along with greater strength development, although the analysis could not detect a significant effect for changes in lean body mass, primarily due to conflicting evidence in the elderly. The DIAAS is the best measure of protein quality we currently have, and it's useful, but it's good to keep in mind the index is still an average estimate that may not fully represent the protein's utility for strength trainees on a high-protein diet. You also shouldn't read too much into the exact number, such as beef protein being a 90 but egg protein being a 95. While the numbers are different, there is likely no practical difference between the two. To see which protein sources are best at stimulating muscle growth, we need to turn to the empirical research.

Protein quality studies

The following table summarizes the literature on protein quality in relation to muscle growth. The following sections go into the conclusions from these data.

➢ Literature overview

Protein quality

The food matrix

The digestion and absorption of amino acids is not only affected by the structure of the protein, but also by its interaction with the food matrix in which the protein is consumed. There are various mechanisms by which components in dietary protein other than just the amino acid composition stimulate protein balance. As Hulmi et al. (2010) noted based on Kimball (2007): "via the PEPT-1 cotransporters' high capacity, low specificity rate of transport, and an apparent increased transport affinity for L-valine bound peptides [...] the bound form of an essential amino acid (EAA) may be more efficiently utilized than when delivered in its free-form." Due to the synergy between amino acids, pure EAA mixtures don't stimulate protein balance as well as complete amino acid profiles. For example, Katsanos et al. (2008) found that whey consumption stimulates greater muscle protein balance than only consuming its constituent EAAs.

Higher up in the matrix we also see a trend that the more whole the protein source, the better it is at stimulating protein balance.

- Research finds micellar casein and whole milk proteins are better at stimulating cumulative muscle protein balance over time than whey. Whole milk stimulates more protein balance than skimmed milk, even when the whole milk contains less protein than the skimmed milk. This is likely because of some beneficial effect of the food matrix in fatty milk, not to any direct beneficial effect of anything in the milk fat, because adding milk fat to casein does not increase myofibrillar protein synthesis. A 2021 meta-analysis by Huang et al. found milk protein stimulated slightly more muscle growth in the elderly than whey protein, but the between-group difference wasn't statistically significant (effect size 0.43 vs. 0.15).
- Blends of protein tend to promote greater protein balance than pure whey, even when the blend contains soy, which in isolation performs very poorly (discussed below).
- One level further up, we see that whole milk tends to stimulate greater protein synthesis than skimmed milk, indicating that substances other than just the protein in milk promote MPS.

As such, the evidence and evolutionary theory suggest that good old actual milk consumption, as opposed to processed protein powders, is the most reliable and effective way to stimulate protein synthesis and reduce protein breakdown. Nature provided you with a package deal of whey and casein in highly absorbable form: take advantage of it.

In contrast to milk, many plants have a food matrix that negatively affects protein quality. Plant foods contain many anti-nutrients as part of their evolutionary defense against being eaten.

These substances in the food matrix of plants make their protein difficult to use for the human body. Wheat and soy are particularly bad at stimulating muscle protein balance, even when they provide high amounts of essential amino acids. The isoflavones in soy may also antagonize mTOR and MPS.

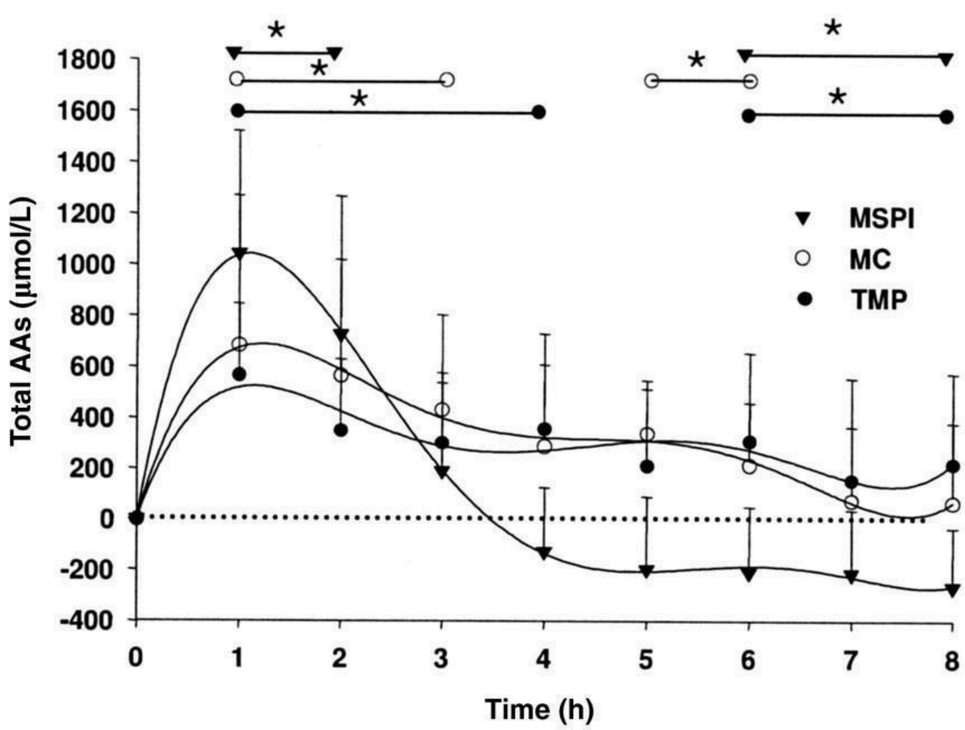
Fast vs. slow protein

Speed of absorption is often marketed by supplement companies as a major advantage of a protein source. You consume it and *BOOM*: all that protein is right there in your muscles, instantly. In reality, rapid absorption is not purely an advantage.

The 2 prototypes of slow and fast protein are the 2 major protein fractions of cow milk: casein and whey protein. Other than differing significantly in amino acid composition, whey protein is soluble in milk, so it remains in liquid form and passes quickly through your stomach. The resulting fast digestion earns it the distinction of a ‘fast protein.’ Caseins, on the other hand, are not milk soluble and will clot in your stomach. This delays the amino acid delivery to your intestines, hence them being known as a ‘slow protein.’

But is faster objectively better? Chan et al. (2019) modified a milk protein supplement to be absorbed faster without altering its amino acid composition. Compared to the native protein supplement, it was absorbed much faster and spiked leucine concentrations in the blood more quickly, but it did not increase muscle protein synthesis or anabolic signaling after a strength training workout in men. Similarly, [Weijzen et al. (2021)](#) found free amino acids were absorbed faster than the same amount of intact protein, but both resulted in similar protein synthesis rates.

Moreover, fast protein sources don't stimulate MPS for as long as slower proteins do. While there are significant differences in the concentration of amino acids in the blood over time (see illustration below), the total amount of net protein build-up is ultimately quite similar for whey and casein proteins. Most studies that suggested whey is superior to casein simply did not measure MPS for a long enough duration (< 6 h), which biased the results in favor of faster proteins.



Mean (±SD) changes from baseline in serum total amino acid (AA) concentrations in the subjects after the ingestion of ~23 g protein from total milk protein (TMP), micellar casein (MC) or milk soluble protein isolate (MSPI). [Source]

Third, as you learned in the course section on protein digestion and absorption, fast absorption can result in greater protein oxidation rates, particularly leucine oxidation. Whey protein in isolation actually seems to be digested too rapidly in humans to sustain postprandial amino acid requirements. This results in fewer amino acids actually being incorporated into muscle tissue compared to casein or whole milk protein.

Rapid digestion can also decrease the uptake of amino acids by your muscles. Hydrolyzed casein, marketed for its rapid and easy absorption, is in fact preferentially used by your gut cells, leaving less amino acids available for your muscles. (Note: One study in elderly individuals found the opposite, but this may have been related to the study period being 6 rather than 8 hours, thus biasing the results in favor of the faster protein. Also, elderly individuals may have more to gain from fast proteins in low quantities because of their anabolic resistance, which will be discussed in the course topic on strength training for the elderly.)

Fourth, while whey protein is superior to casein production in stimulating peak MPS, casein protein is more effective at reducing protein breakdown. In fact, whey protein doesn't prevent catabolism much at all. As a result, when looking at total muscle protein balance (not just MPS) over a period of 7 hours, casein results in greater net muscle protein balance. In long term studies, casein protein has also been shown to result in higher whole-body protein balance levels, greater strength development, more fat loss and more muscle growth than whey protein.

In conclusion, the idea that faster proteins, like hydrolyzed proteins and whey, are better for bodybuilding than slower proteins, like casein, is nothing but supplement company marketing. Slow release proteins actually result in better protein balance over time. However, the best of both worlds – high as well as long-lasting MPS and decreased protein breakdown – is attained when you mix a slow and a fast protein source, such as whey and casein. Basically, you’re back to milk and that’s indeed a general take-home message of protein quality: nothing beats whole foods, specifically poultry, meat, fish, dairy and eggs.

Vegetarian diets

Based on the above, it should be clear that vegetarian diets require special attention to protein quality. Literally all high-quality protein sources are animal foods. A 2024 meta-analysis found that plant protein sources improve athletic performance and specifically strength gains less than animal protein sources. Plants were similarly less effective at stimulating muscle protein synthesis, but this difference was not significant. Bartholomae & Johnston (2023) found that the recommended daily allowance (RDA) of protein does not support nitrogen balance in vegans. Some randomized controlled trials have also found that a given amount of dairy or beef protein stimulates considerably more acute MPS and long-term muscle growth than soy protein [1, 2, 3] although in most other studies the difference in muscle growth between soy and other protein supplements did not reach statistical significance. A 2021 meta-analysis by Lim et al. supports that on average, animal protein sources result in more lean body mass growth than plant protein sources (p = 0.06). While the median percentage gain in lean body mass did not significantly differ between protein sources, it was almost 5 times higher for animal protein sources than plant protein sources (1.5% vs. 0.3%). Another meta-analysis by Damaghi et al. (2021) found a 3-fold higher effect size of whey protein than soy protein for gains in lean body mass (0.9 vs 0.3 WMD). The between-group difference was not statistically significant, but whey protein significantly differed from control/placebo, whereas soy protein did not. Importantly, there isn't a single study showing any favorable effects of plant protein sources compared to animal protein sources, so the overall trend is in favor of animal protein sources.

General protein intake recommendations are based on the assumption that at least 50% of total protein intake comes from animal sources. When this is not the case, like for vegans, protein requirements increase in line with the lower protein quality of the diet. Non-full-vegan vegetarians are thus advised to rely on the animal foods they do eat for the bulk of their protein requirements. For example, lacto-ovo-vegetarians should consume dairy or eggs with each meal. Then there is no need to increase total protein intake.

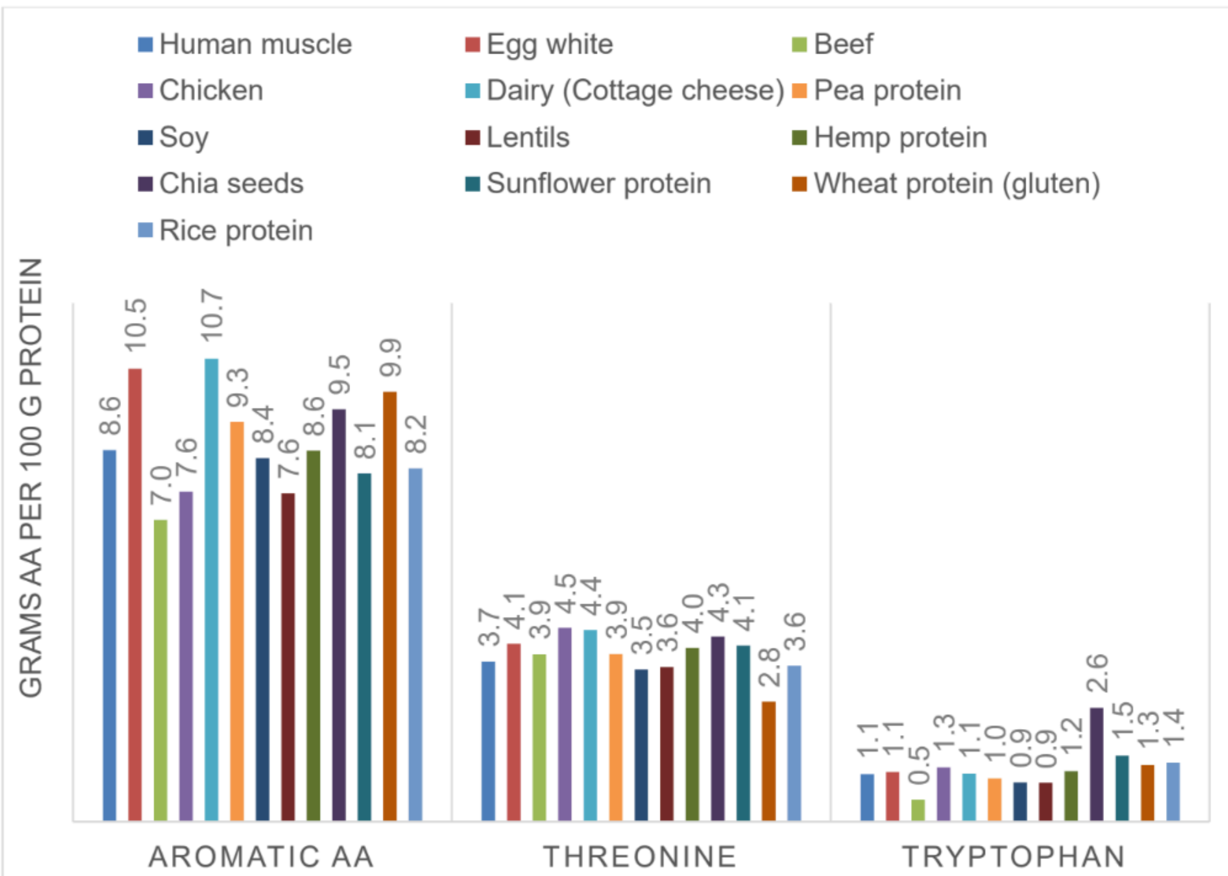
Diet name	Description
Flexitarian	Occasionally consumes animal flesh (meat, poultry) and fish, eggs, dairy
Pesco-vegetarian / pescatarian	Excludes animal flesh but does include fish
Lacto-ovo vegetarian	Excludes all flesh; includes diary and eggs only
Lacto vegetarian	Excludes all flesh and eggs; includes dairy only
Ovo vegetarian	Excludes all flesh and dairy; includes eggs only
Vegan	Excludes all animal products
Macrobiotic vegetarian	Variable dietary restrictions; includes wild meat/game and fish in some variations of the diet
Fruitarian	Includes fruit, nuts, seeds and some vegetables

Adapted from Phillips (2005).

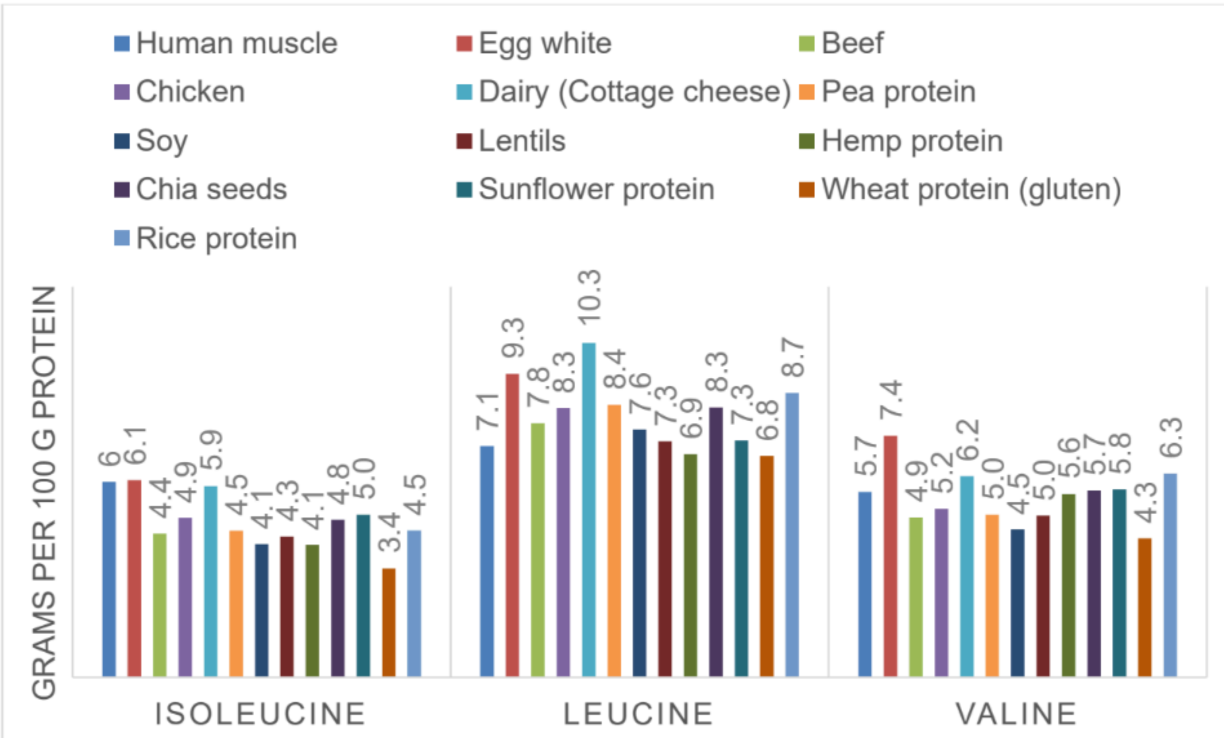
Amino acid availability

Fully vegan diets require further consideration. Remember that total protein requirements are based on a certain assumption of the amino acid distribution. Ultimately, your body does not care about your total protein intake, only whether it has enough of each individual amino acid for its needs. Since the body can create non-essential amino acids if enough essential amino acids (EAAs) are present, we should look at the EAA content of vegan protein sources.

Threonine, tryptophan and the aromatic amino acids phenylalanine and tyrosine are not a concern. They are found in plants in approximately the same proportion as in animal foods: see the graph below.



The main problem with vegan diets is often said to be their BCAA content. As you can see from the graph below, the BCAA content of plant protein sources is ~21% lower than that of animal protein sources. [For the most important BCAA, leucine, animal protein sources contain 8-11% compared to 6-8% for vegan protein sources].



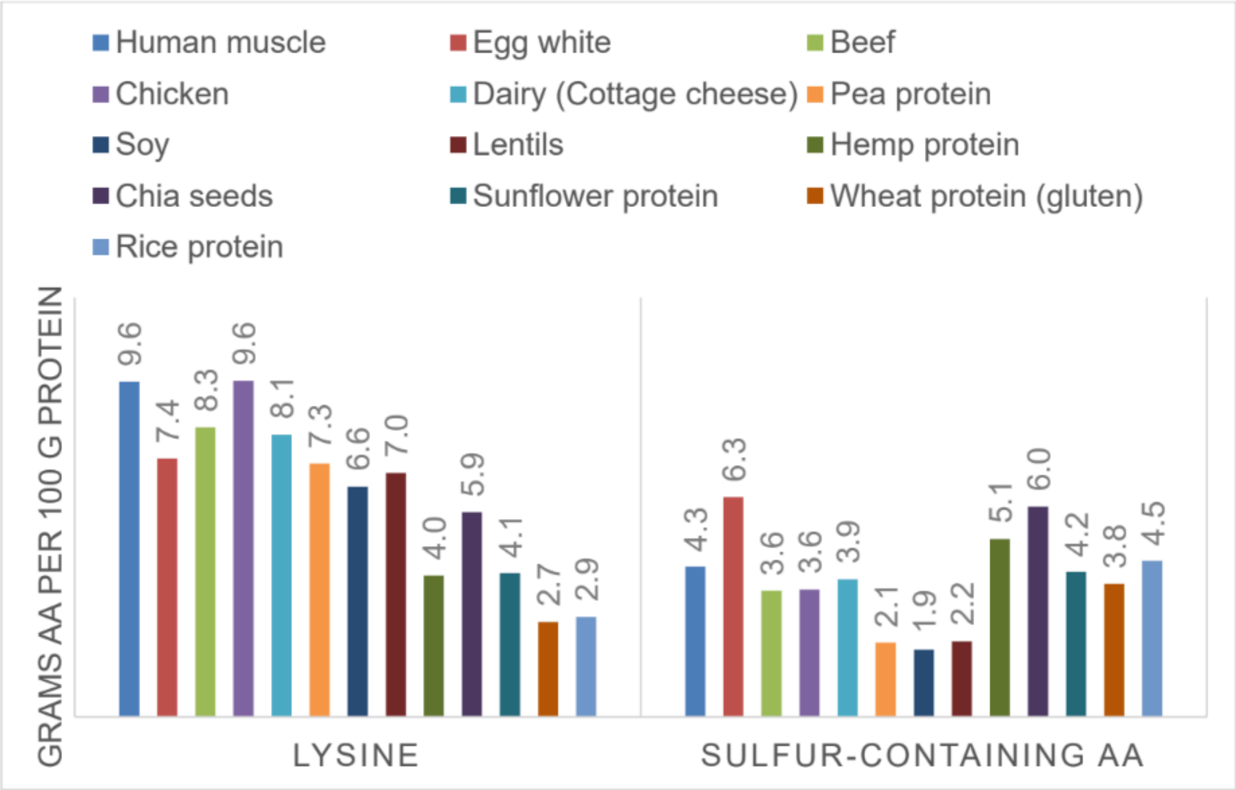
When looking at the sum of all essential amino acids (EAAs), plant protein sources contain ~16% fewer EAA per the same amount of total protein than animal protein sources as calculated by Young et al. (1994). This suggests BCAAs are indeed the most limiting EAAs in vegan diets and a 21% increase in protein intake in vegan diets should offset the difference with an omnivorous diet.

However, 21% additional protein may not cut it for all protein sources. Oikawa et al. (2020), corrected by Vorland (2020), found that increasing protein intake from 0.8 g/kg to 1.6 g/kg (0.36 to 0.73 g/lb) per day using potato protein did not significantly increase muscle anabolic signaling expression and only marginally increased myofibrillar protein synthesis with borderline statistical significance. Similarly, Lamb et al. (2020) found that 30 g protein from peanut powder did not significantly increase 24-hour integrated myofibrillar protein synthesis compared to a placebo(!) Moreover, the peanut protein only showed marginal trends to improve strength and muscle growth over the course of a 10-week training program in untrained, older adults. These findings suggest the commonly sufficient protein intake of 1.6 g/kg (0.73 g/lb) per day is likely not enough to maximize muscle growth in a vegan diet.

Lynch et al. (2020) found that supplementing 26 grams of soy protein isolate resulted in comparable increases in muscle mass and strength as 19 grams of whey protein isolate in untrained individuals on a 12-week strength training program. This would suggest you need roughly 37% more protein from soy than whey to get equivalent results.

On the other hand, equating for leucine content may not always be needed when the difference in leucine concentration is particularly large, such as with mycoprotein. Monteyne et al. (2020) found that a leucine-equated 70 grams of mycoprotein, containing 32 grams actual protein, resulted in greater post-workout muscle protein synthesis than 31 grams of milk protein, which contained 26 grams actual protein. These findings would suggest a 19% increase in protein intake is already more than enough for vegan protein supplements to rival animal protein supplements. Yet since the mycoprotein contained over *double* the calories of the milk protein, this is not the most fair comparison in practice when you have a limited number of calories you can eat. Another study by Monteyne et al. (2021) performed an isocaloric comparison of 1.8 g/kg (0.82 g/lb) per day of mostly mycoprotein (57%) or mostly animal protein (71%). Both diets were equally effective to stimulate myofibrillar protein synthesis. However, the subjects only trained their quads, so it's likely that both protein intakes were simply excessive and it remains to be seen if 1.8 g/kg (0.82 g/lb) protein per day from mycoprotein can maximize full-body muscle growth. Also, all of Monteyne et al.'s studies on vegan protein supplements had involvement of the manufacturer of that supplement, chiefly Marlow Foods, so they had a significant conflict of interests.

BCAAs aside, lysine and sulfur-containing amino acids, methionine and cysteine, can be even more difficult to find in large amounts in typical vegan foods. As you can see in the graph below, animal protein sources contain approximately the proportion of these amino acids also found in human muscle, but plant protein sources do not. Legumes, such as pea protein, soy products and lentils, contain nearly the amount of lysine that animal proteins do. Yet these foods are poor sources of sulfur-containing amino acids. In contrast, seeds and cereal grains contain a good amount of sulfur-containing amino acids, yet these food groups are lacking in lysine. The differences can be large, far larger than in BCAA contents. For example, you need *3 times* as much pea protein as egg white protein to get the same amount of sulfur-containing amino acids, or to get the same amount of lysine with rice protein instead of chicken.



Since no plant protein source has all EAAs in the ideal proportion for human needs, vegans should pay close attention to how they combine different foods groups in their meals to give each meal a high protein quality. Vegan diets that don't specifically pay attention to protein quality generally do not optimize protein quality. Simply increasing the total protein intake without regard for the source could necessitate a *3-fold* increase in protein intake to ensure you consume all EAAs in optimal quantities. That's an impractically large amount of protein to consume for digestive, financial, hedonic and caloric reasons.

Calculating each of your meals' amino acid profiles is a lot of work. As a guideline, the following proportions should result in a good amino acid profile of the diet: 50% protein from legumes (pea protein, soy products and lentils), 25% protein from seeds (hemp, chia and sunflower seeds) and 25% protein from grains (rice and wheat protein). Even if we assume such a diet composition, to get the same amount of all EAAs as 1.6 g/kg (0.73 g/lb) per day from a mixture of animal protein sources, a vegan diet still requires around 2.4 g/kg (1.09 g/lb) of protein per day based on our calculations of several vegan meal plans. Lysine is often the main problem in practice. With ~2 g daily lysine supplementation, 2.2 g/kg (1 g/lb) per day often suffices for vegans with a balanced diet.

Digestibility

The above protein intake comparisons based on amino acid ratios assume similar digestibility of the diet compared to an omnivorous diet. Equal digestibility of vegan and omnivorous diets is generally not the case because of the high amount of anti-nutrients in plants that interfere with the digestion and absorption of protein. Trypsin inhibitors, tannins and phytates reduce the digestibility of protein by up to 50% in various animals [2]. Human data on whole diets is surprisingly scarce. Digestibility of protein in traditional diets from developing countries such as India, Guatemala, and Brazil is only 54-78% compared to 88-94% in typical North American diets [3].

Based on average vegetarian protein quality (PCDAAS) scores and a meta-analysis of protein requirements to maintain nitrogen balance, vegetarians consuming less than 50% of protein from high-quality sources need an additional 25% protein to compensate for their lower average protein quality. Vegans would require greater compensation.

Based on the Digestible Indispensable Amino Acid Score, the FDA's replacement of the PCDAAS, digestibility of plants is only around 45% for nuts, 60% for rice and a highly variable 70% for types of beans, in comparison to nearly 100% digestibility of animal protein sources. The estimated DIAAS increases rapidly when combining complementary amino acid sources, however.

A 2019 study on triathletes found the average vegetarian diet had only 11% lower protein digestibility than that of omnivorous triathletes based on DIAAS protein quality scores. These were vegetarians, not vegans, and the omnivores included several people who ate so little meat they self-identified as vegetarian. Moreover, the omnivorous group consumed only 1.4 g/kg (0.64 g/lb) protein per day. So for a vegan to reach the protein quality of a strength athlete consuming at least 1.6 g/kg (0.73 g/lb) protein per day with a high-quality protein source in each meal, a considerably greater correction than 11% is likely needed.

A 2011 study computed an average DIAAS score of vegetarian women's diets of 80%. The women consumed 21% of their protein intake from animal foods.

With a conservative 30% reduction in protein digestibility of vegan diets, **protein needs of vegans rise to 2.2 / 0.7 = 3.1 g/kg (1.41 g/lb) per day with lysine supplementation or 3.4 g/kg (1.55 g/lb) per day without it**.

Traditional grain preparation techniques, including soaking or fermentation, can significantly reduce the presence of anti-nutrients and thereby improve protein digestibility. These will be discussed in greater detail in the topic on health science.

Even with the lysine supplementation, that's a problematically large amount of protein to consume in a vegan diet, especially in energy deficit. Most vegan protein sources are simply too caloric to obtain that amount of protein from without going over your planned calorie intake. Even if it's possible, due to the major emphasis on relatively high protein foods, the diet often becomes deficient in fat or micronutrients (see course module on micronutrition). Combined with the difficulty of meal planning to eat foods in the right proportions, virtually all of Menno's vegan clients resort to protein powder supplementation.

Based on the aforementioned protein quality research, rice and pea protein are preferable over the commonly used soy protein. Rice and pea protein also happen to have a highly complementary amino acid profile, so a blend of those is the preferred choice of protein supplement for vegans. An 80-20 blend of pea-rice results in a protein source with ~79% of all EAAs, including lysine, meaning a protein intake of 2.2 g/kg (1 g/lb) per day suffices (one gram per pound of total bodyweight protein per day). Van Der Heijden et al. (2024) found that 32 g protein from a similar plant protein blend (approx. 40% pea, 40% rice and 20% canola) stimulated just as much muscle protein synthesis (myoMPS) as whey protein after a heavy duty leg day (12 sets of quad work to failure) in strength-trained individuals.

An additional major benefit of this protein powder supplementation is that less further correction for digestibility is likely needed, as the processing of the protein powders removes most anti-nutrients. While not isocaloric comparisons, Lynch et al. (2020) and Monteyne et al. (2020) found that vegan protein powders are likely at least as effective as milk based protein powders when equated for leucine content.

For the pea-rice protein blend, the DIAAS is estimated to be as high as 0.94, basically as good as it gets for any vegan protein source. With a 6% correction for digestibility, **vegan protein requirements are 2.2 / 0.94 = 2.3 g/kg (1.05 g/lb) per day when relying heavily on supplementation of the 80-20 pea-rice blend**. For many vegans, this is by far the most convenient way to get their protein in.

While the pea-rice protein blend is theoretically highly digestible, it still causes digestive issues in many people, e.g. gas and bloating, but no more than what vegans with FODMAP issues often experience anyway.

For those who prefer finding complementary amino acid sources instead of supplementing protein, here are 2 tables to create meals with good complementary protein sources.

Vegetarian protein guidelines can be implemented on a per-meal basis just like for daily totals. For example, if you rely on a pea-rice protein blend for a meal that would have a protein target of 40 g with a high-quality protein source in there, with the pea-rice protein blend the meal's protein target would rise to 2.3 / 1.8 × 40 = 51 g.

Lysine-rich plant food	Grams lysine per 100 g food	Grams protein per 100 g food	Grams lysine per 100 g protein
Soy flour, defatted	3.1	51	6.1
Soybeans, raw	2.7	36	7.4
Lupins, raw	1.9	36	5.3
Peanut flour, defatted	1.9	52	3.6
Split peas, raw	1.8	23	7.7
Kidney beans, raw	1.7	25	6.9
Lentils, raw	1.7	25	7.0
Mung beans, raw	1.7	24	6.9
Broad beans (fava beans), raw	1.7	26	6.4

Sulfur-containing AA-rich plant food	Grams of SCAA per 100 g food	Grams protein per 100 g food	Grams SCAA per 100 g protein
Sesame flour, low-fat	2.7	50	5.3
Sunflower seed flour, defatted	2.0	48	4.1
Hemp seeds	1.6	32	5.1
Brazil nuts, dried	1.4	14	10.0
Chia seeds	1.0	17	6.0
Tahini	1.0	18	5.3
Walnuts	0.9	24	3.9
Wheat germ, crude	0.9	23	4.0
Oat bran, raw	0.9	17	5.3
Cashew nuts, raw	0.8	18	4.1
Oats	0.7	17	4.3
Flaxseeds	0.7	18	3.9
Teff, uncooked	0.7	13	5.0
Wild rice, raw	0.6	15	4.1
Quinoa	0.5	14	3.6
Millet four	0.5	11	4.7

Conclusion

The following table provides a guideline of protein quality categories. For best results, at least half of your protein requirement should come from high quality protein sources. Whole food animal proteins generally reign supreme as anabolic fuel for bodybuilders. If you do decide to supplement protein for whatever reason, a blend is best: whole milk protein or a mix of whey with 50-80% micellar casein.

For vegans, an 80-20% pea-rice protein blend is recommended to fill in a protein target of at least 2.3 g/kg (1.05 g/lb) per day. Without protein supplementation or consideration of complementary sources, vegans should aim for a total protein intake of at least 3.4 g/kg (1.55 g/lb) per day.

Top quality	Good quality	Medium quality	Low quality
(Whole) dairy	Pea-rice protein blend	Soy protein	Other plants
Meats	Whey protein in isolation	Pea protein	
Fish	Hydrolyzed protein	Hemp protein	
Boiled/baked eggs	Beef protein*	Rice protein	
Poultry			
Milk protein			
Casein protein			
Insects			

**: Beware some brands use collagen as the primary ingredient and sell it as beef protein.*

Effect of protein intake on fat mass

Higher protein intakes are not just useful to build more muscle and strength. Protein can also help you lose fat in 3 ways.

1. Satiety

First and foremost, protein is highly satiating up until the body's requirement. Because protein is essential and the body has no energy-efficient storage place for it, it appears that the body has a protein-specific appetite (protein leverage theory). Up until you've consumed enough protein to meet the body's requirements, your appetite stays elevated. As a result, people generally consume more calories on low-protein diets than on optimal protein intakes. High-protein diets thereby make diet adherence easier. However, people generally don't consume fewer calories on excessive protein intakes than on optimal protein intakes, so there's no need to consume more protein for satiety than you'd already consume for optimal body recomposition. We will discuss protein leverage theory in greater detail in the *ad libitum* dieting module.

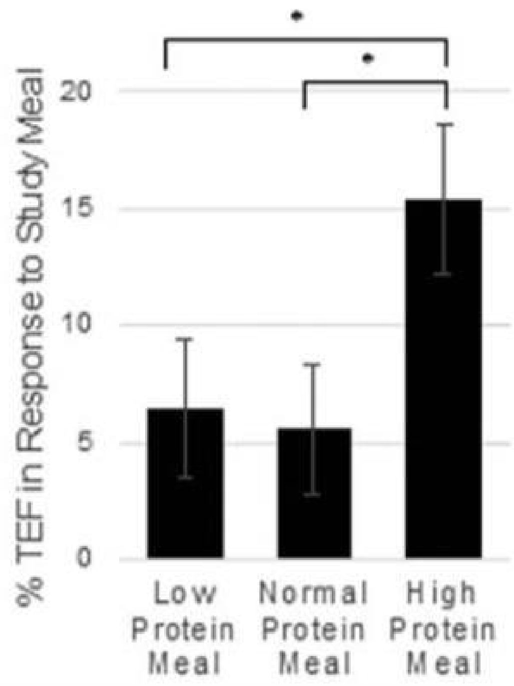
2. Anabolism

To the extent that protein helps you build muscle, this in turn also helps you lose fat. Muscle growth is an energy-intensive process. It increases energy expenditure. Protein also preserves your fat-free mass, which indirectly aids fat loss, because any fat-free mass that you lose is fat that you didn't lose. The more of your energy deficit is spent on burning muscle, the less fat you lose. (Economists would say that energy loss from the body's different compartments – notably muscle vs. fat – is a zero-sum game.)

3. TEF

Another potential benefit of higher protein intakes for fat loss is that protein has a high thermogenic effect (TEF). As discussed in the Energy module, protein generally has a higher thermic effect than carbohydrates and fats, at least when consumed in isolation in processed form.

Protein-induced thermogenesis is largely the result of protein oxidation, which is relatively inefficient for the body, and extra protein synthesis. Extra synthesis only occurs when there's anabolism, whereas protein oxidation primarily occurs when there's excess protein intake so that protein ends up being used as fuel. Under normal diet conditions with protein intakes around the level your body needs for protein synthesis, the effect of protein's thermogenesis on your total energy expenditure is small. While some research found a dose-response effect of higher protein intakes causing higher energy expenditures, other research found no increase in energy expenditure at all after higher protein meals vs. other isocaloric meals. See the image below for another study finding no linear dose-response effect of protein intake on the TEF.



[Source]

So how many calories are we talking about? Oliveira et al. (2020) provided some of the best data we have on the thermic effect of protein. They performed a carefully controlled metabolic ward study in which they compared a 15% protein intake to a **40%** protein intake with the same total energy intake in the same subjects. The 40% protein intake indeed increased energy expenditure by only 81 kcal a day. Whitehead et al. (1996) found a similar 71 kcal per day increase in energy expenditure with a 36% protein intake compared to a 15% protein intake. Mikkelsen et al. (2000) found a 3% increase in energy expenditure when going from an 11% to a 29% protein intake, amounting to 118 kcal per day. In the above studies that measured it [1, 2], protein balance was increased by the higher protein intakes, as the lower protein intakes were not high enough to maximize protein balance, so part of the thermic effect likely came from anabolism.

When protein contributes to anabolism, its calories are theoretically ‘free’. Protein intake is preferentially used for muscle protein synthesis rather than oxidation or conversion to fat. So as long as all protein is used for protein synthesis, protein's calories do not contribute to fat storage. This is illustrated well by a study by Peeters et al. (2023), in which up to 40 g pre-workout protein did not reduce fat oxidation rates during an hour-long cardio session.

So can we eat as much protein as we want and not count its calories? This was tested by metabolic ward research from Bray et al. [1, 2] with gold-standard measurement techniques. A metabolic ward is basically a science lab where oxygen and carbon dioxide consumption and production by your body are measured. Energy expenditure can be accurately measured. All meals in a metabolic ward are prepared for the subjects, so energy intake can also be tightly controlled. Basically, this research is as good as it gets. The researchers had the study participants consume 5% or 15% or 25% of energy as protein while massively overeating on calories by 40%.

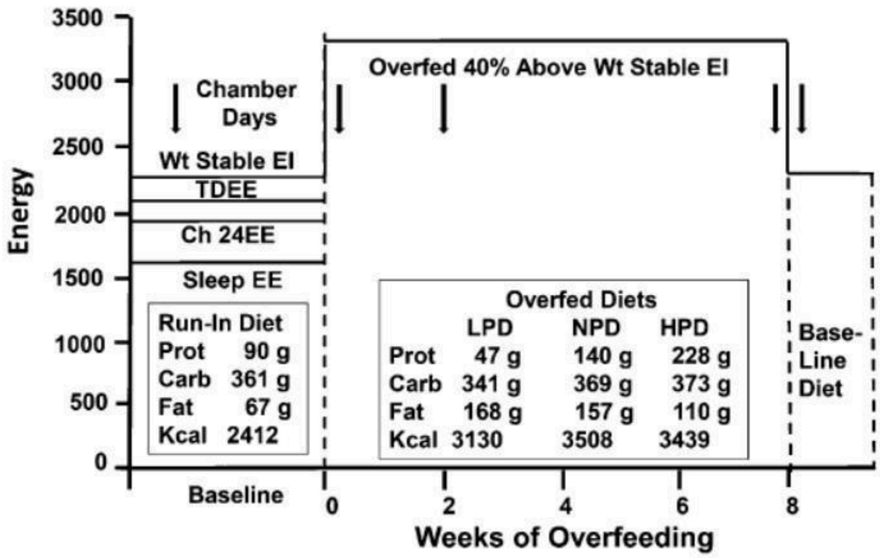


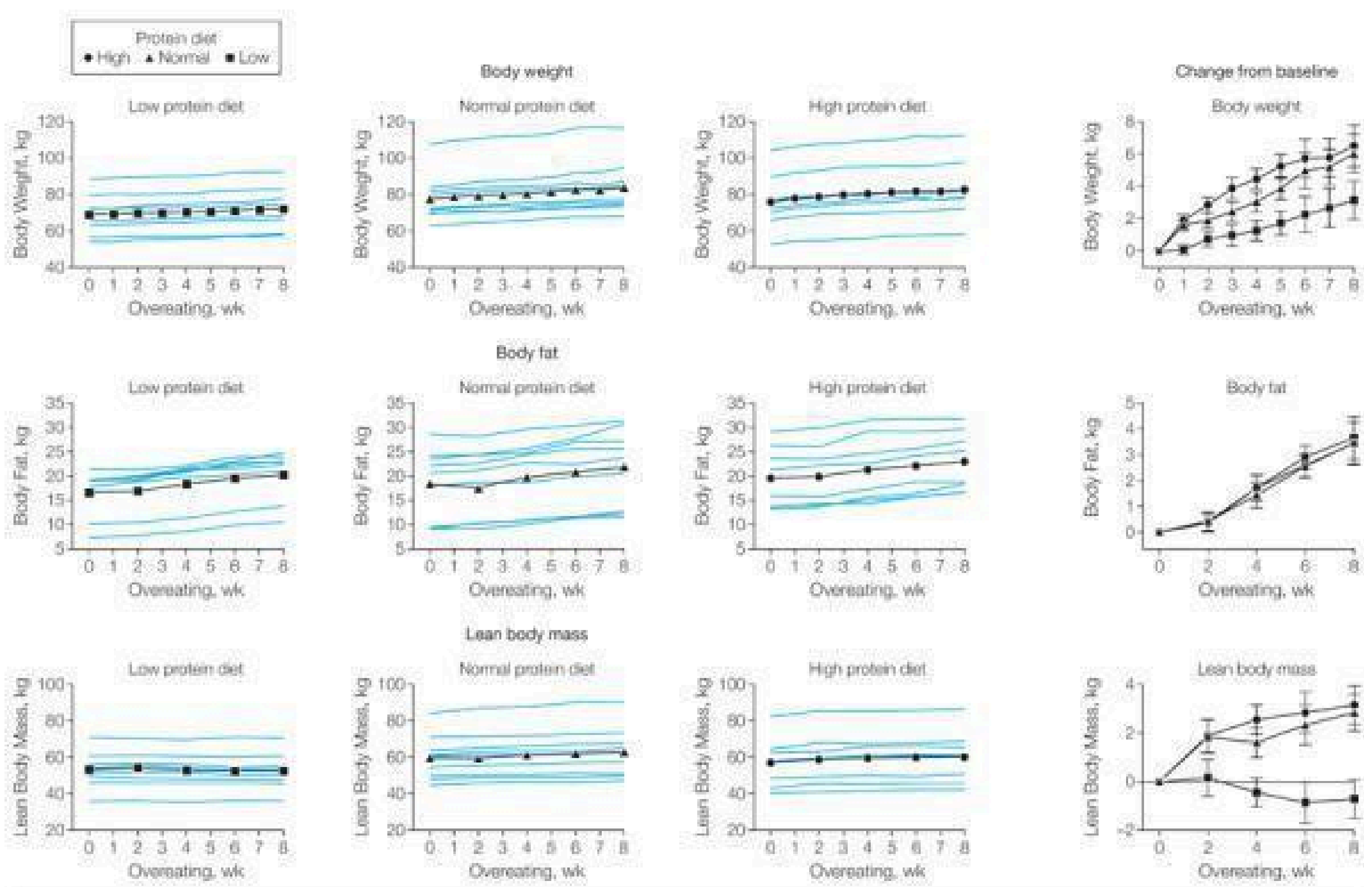
FIGURE 1 Experimental protocol. This figure shows the baseline period followed by the 8 wk of overfeeding and the timing of the experimental procedures. The top horizontal lines represent energy intake at baseline, during overfeeding, and after energy intake was returned to baseline levels. The boxes inside the baseline and overfeeding periods summarize the macronutrient intakes. The 5 vertical arrows indicate the timing of the 24EE assessment in the metabolic chamber. Carb, carbohydrate; EI, energy intake; HPD, high-protein diet; LPD, low-protein diet; NPD, normal-protein diet; Prot, protein; TDEE, total daily energy expenditure; Wt, weight; 24EE, 24-h energy expenditure.

The study design of Bray et al.'s second study for those interested in the details. The first study was similar in design.

In both of Bray's studies, there was no difference in the amount of fat gain between the groups. Even in a 40% energy surplus, there was no difference in fat gain between the woefully insufficient 5% protein intake (~0.7 g/kg or 0.32 g/lb per day) and the massively superfluous 25% protein intake (~3 g/kg or 1.36 g/lb per day). Fat gain was significantly predicted by energy intake, but there was no significant relation with protein intake.

Moreover, in both studies, the low protein group did not gain as much lean body mass as the medium and high protein groups, but there was no difference between the medium and high groups. The medium protein group even gained non-significantly less fat and more muscle than the high protein group in the second study. The exact same pattern held true for energy expenditure in both studies: low protein did not cut it, but there was no benefit to increasing protein above the medium intake.

You can see the body composition changes from both studies in the following figures, for those interested in the details.



The blue horizontal lines indicate individual participants. The error bars in the change from baseline graphs indicate 95% confidence intervals.

Body composition change over time in Bray et al.'s first study.

TABLE 3
Mass and energy expenditure of the brain, skeletal mass, adipose tissue, bone, and residual mass at baseline and after 8 wk using the multicompartment dual-energy X-ray absorptiometry model[1]

		8 wk			
Variable	Baseline	LPD	NPD	HPD	*P* value, diet effect from baseline
Brain, kg	1.53 ± 0.13	1.53 ± 0.13	1.54 ± 0.11	1.48 ± 0.12	0.18
Skeletal muscle, kg	30.9 ± 8.06	28.1 ± 7.1	34.2 ± 8.2	33.0 ± 10.8	0.0004 (HPD = NPD > LPD)
Bone, kg	2.56 ± 0.48	2.34 ± 0.38	2.73 ± 0.43	2.57 ± 0.58	0.34
Adipose tissue, kg	21.2 ± 7.33	21.1 ± 8.4	25.4 ± 9.4	27.2 ± 8.0	0.50
Residual mass, kg	18.1 ± 4.05	19.6 ± 10.4	19.5 ± 5.6	18.7 ± 4.55	0.64
Brain EE	367 ± 31.9	367 ± 31.4	369 ± 30.2	356 ± 29.0	0.18
Skeletal muscle EE	401 ± 103	365 ± 92.3	444 ± 105	429 ± 141	<0.0001 (HPD = NPD > LPD)
Bone EE	5.9 ± 1.09	5.39 ± 0.86	6.27 ± 1.00	5.94 ± 1.34	0.045
Adipose tissue EE	91.8 ± 47.2	106 ± 27.7	115 ± 42.4	122 ± 35.8	0.74
Residual mass EE	635 ± 206	685 ± 201	710 ± 151	785 ± 168	0.0006 (HPD = NPD > LPD)

[1]Values are means ± SDs. Comparison was done with the fit model platform adjusting for age, sex, and baseline value. *P* value was calculated from model of change from baseline by diet, age, sex, and baseline value with contrasts based on Tukey's honestly significant difference test. EE, energy expenditure; HPD, high-protein diet; LPD, low-protein diet; NPD, normal-protein diet.

The results of Bray et al.'s second study.

As a side note, the second study also showed that both nitrogen and protein balance overestimated muscle growth due to changes in the metabolism of residual mass (i.e. the skin, intestines, kidneys and liver). This suggests that the acute protein balance research may overestimate protein requirements.

In conclusion, the thermic differences of protein vs. other energy sources are too small in practice to change how much fat you gain when overeating. The carbon skeleton of dietary amino acids **can** be used by the body as a source of energy or even be used to produce fatty acids (de novo lipogenesis). Glucogenic amino acids, the vast majority, can be converted to glucose, which can in turn be converted to fat. The 4 ketogenic amino acids can be converted to ketone bodies. That said, the protein from your diet is rarely converted to fat in practice. Protein is usually oxidized and used as fuel. This frees up fat, so that more of the fat from your diet is stored as fat. Regardless of whether protein itself is directly converted to fat or if it simply contributes as a fuel source, the result is the same: protein intake from the diet can result in fat storage.

Protein intake also does significantly affect fat loss in practice on an isocaloric basis. A 2013 meta-analysis of the literature found that high-protein diets did not result in more fat loss than low-protein diets when both diets had the same number of calories.

Theoretically, there should be some benefit when going from very low or zero protein intakes to higher ones due to the anabolic benefits. There may also be some benefit when going from high to ludicrously high intakes due to the thermic effect of the protein oxidation. However, these effects are not practically relevant for most people. You should consume enough protein to optimize muscle growth and strength development, but consuming more than this is unlikely to materially affect your fat loss results.

Conclusion

High-protein diets can help you lose fat by promoting satiety, preserve muscle and increase energy expenditure. However, the gold-standard research shows that when you are already consuming enough protein within each meal and across the day (~1.8 g/kg or 0.82 g/lb per day), there is no metabolic, thermic or body composition benefit to increasing your protein intake further. Fat gain or loss is primarily dictated by energy balance, not by protein intake, since protein can be used as fuel by the body or even stored as fat.

Is excessive protein intake unhealthy?

Many older textbooks, doctors and mainstream media claim that high-protein diets are bad for your health. The most common concern is kidney damage. High protein intakes put higher demands on the kidneys, as they have to excrete more nitrogen and urea, especially in strength trainees who experience high protein turnover levels. In bloodwork, strength trainees on high-protein diets may show elevated creatinine, creatine kinase, blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and a correspondingly decreased estimated glomerular filtration rate (eGFR) [2, 3, 4, 5]. Similarly, in urine samples, strength trainees on high-protein diets may show elevated creatinine and urea nitrogen levels. These results often alarm doctors that don't understand exercise physiology, because these are normally markers of kidney and liver damage. However, in the case of strength trainees on high-protein diets, they can be normal, healthy adaptations. These markers just indicate that the liver and kidneys are busy processing more protein than normal, which makes perfect sense, as you're eating more protein and there's high protein turnover in your muscles. There is now strong research support that a high-protein diet is not bad for your kidneys [2, 3, 4]. A 2024 meta-analysis found that high protein intakes were associated with a *lower* incidence of chronic kidney disease, primarily when the protein came from plants or seafood. Even individuals with kidney problems on high protein intakes typically don't experience any problems in research, but anyone with a preexisting kidney condition should consult a doctor – one that's knowledgeable about exercise physiology – regarding the safety of high protein intakes for them.

You can verify lack of kidney damage by testing cystatin C. Cystatin C is a protein that is filtered out of the blood by our kidneys, so high levels indicate the kidneys are compromised and not doing their job. Unlike creatinine, the traditional marker for kidney function, cystatin C is not affected by muscle mass, physical activity or age.

Cystatin C is therefore a much better biomarker of kidney status than creatinine, especially in athletes, and is better suited to estimate glomerular filtration rate (GFR) [2]. You can test bilirubin levels to monitor your kidney health. Bilirubin levels can become elevated as a result of exercise, but it's generally not a large effect and any effect requires intensive exercise, so if you have very high levels, that can be cause for concern. If your creatinine levels are high but cystatin C and bilirubin levels are normal and you're overall healthy, your kidneys are probably fine.

You can verify lack of liver damage by testing gamma-glutamyl transferase (γGT or GGT): if this is not elevated like AST and ALT in a strength trainee on a high-protein diet, it typically means the other enzymes are just elevated because the liver's busy processing the high protein turnover, not because the liver is damaged.

Overall, even very high-protein diets appear to be perfectly safe in randomized controlled trials lasting up to a year. However, the very long-term health effects of high-protein diets have not actually been thoroughly investigated due to the difficulty of conducting long duration RCTs. As you learned in the research module, epidemiological studies are weak evidence in comparison to controlled studies. Some studies have found positive associations between higher protein intakes and healthy aging, whereas others suggest high-protein diets increase mortality. The latest 2020 meta-analysis of prospective cohort studies found no relation between total protein intake and all-cause mortality. A 2021 systematic review of randomized controlled trials also found no significant adverse health effects of high protein diets in elderly individuals. There's a trend for plant protein to be safer than animal protein in most research and the health effects of 'protein' likely depend in large part on the foods containing the protein, which means protein per se is likely not the issue. In the health science module, we'll go into the health science for each specific food group.

Protein timing

So far we've discussed protein intake in terms of a daily total: how much you need. But it is also important *when* you consume the protein. How should you distribute your protein over the day?

The leucine threshold

After the sections on BCAAs, you may have lost interest in leucine. However, there is more to leucine's story. Even if your total daily of leucine is sufficient, you could still not be optimizing your muscle growth because of the leucine threshold. The leucine threshold is a concept that has changed in definition over the years. Its original and most relevant definition for us is that there is a threshold, i.e. a minimum amount, of leucine required to start the protein synthesis process and cell growth. Below this threshold, the signal for muscle protein synthesis is not strong enough and little to nothing happens. So, if you consume a meal or snack that does not have enough leucine, that protein may not be used fully for protein synthesis. Multiple studies support that you need at least 3 meals with a protein intake above the leucine threshold to maximize total daily muscle protein balance [1, 2, 3, 4, 5, 6, 7]. For example, Mamerow et al. (2014) compared 2 protein distributions for total daily protein balance. The EVEN group consumed an even protein distribution at each meal: ~30 g at breakfast, 30 g at lunch and 30 g at dinner. The SKEW group consumed ~10 g at breakfast, 15 g at lunch and 65 g at dinner. Total daily protein was 1.2 g/kg (0.55 g/lb) per day (~90 g), which is generally enough for sedentary individuals. Despite this, the EVEN group had a ~25% higher daily average protein synthesis rate. The large 65 g protein meal was unable to compensate for the 2 smaller meals that seemingly did not stimulate protein synthesis enough. Since other research shows benefits of adding leucine to very small protein servings, it's likely that the leucine threshold was responsible for the greater protein synthesis in the EVEN group. In training individuals, Yasuda et al. (2020) found that a roughly even protein distribution across 3 meals led to a strong trend for greater lean tissue growth and strength development than having the same total daily macronutrient intakes with a breakfast with only 0.1 g/kg (0.05 g/lb) protein. The subjects were young, strength training men and the study lasted 12 weeks.

However, the leucine threshold is still not fully validated in research and may be a measurement artifact. It may be that human muscle protein synthesis responds to leucine intake in a simple dose-dependent manner (see figure below) without a true threshold. The leucine 'threshold' is sometimes arbitrarily defined as enough leucine to spike protein synthesis by 50% above baseline, so it may be that the increase in protein synthesis below that intake in other research just didn't reach statistical significance, even though it did occur (recall the course topic on statistics for what statistical significance means).

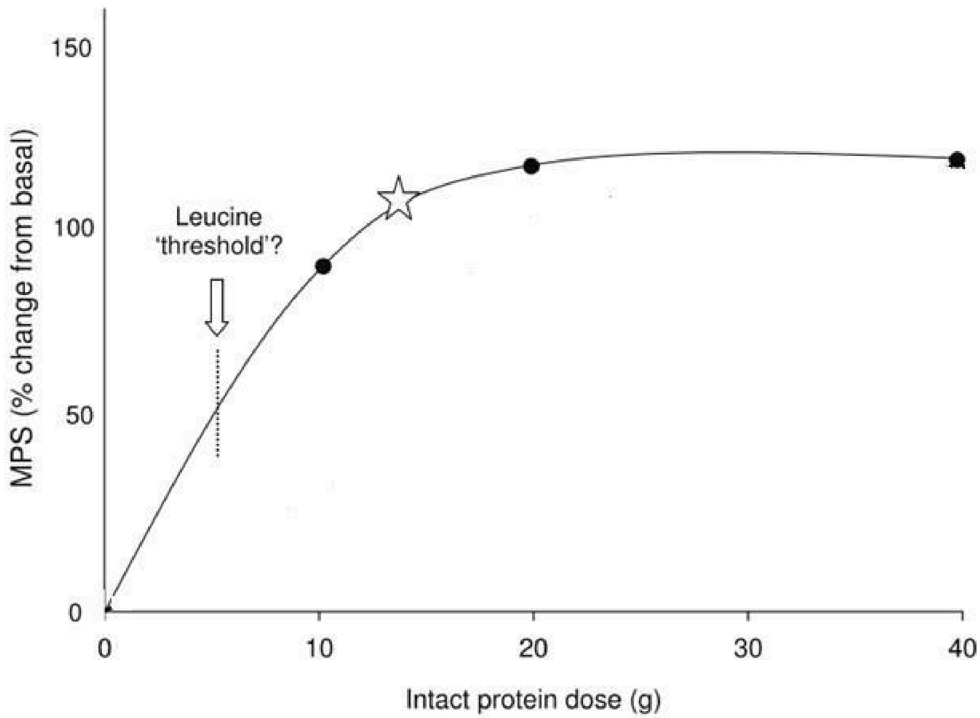


Figure modified from Breen & Phillips (2011)

The amount of leucine needed to trigger MPS is also very low. So low that any regular serving size of high-quality protein generally makes the leucine dose redundant. A 2023 systematic review of 38 studies found no significant relationship between leucine dose and MPS in young lifters consuming a meal around their workout. Multiple other studies have found that the protein distribution across the day does not affect protein balance or body composition changes [1, 2, 3, 4, 5, 6, 7, 8]. For example, Kim et al. (2015) found no difference in nitrogen balance or whole-body protein kinetics between an uneven 15-20-65% and an even 33-33-33% protein distribution across breakfast, lunch, and dinner, regardless of whether they consumed the RDA or double the RDA of protein. The participants were untrained elderly. Kim et al. (2018) replicated these findings in strength training elderly: an even protein distribution did not improve muscle growth, strength development or whole-body protein balance over a 15-20-65% protein distribution after 8 weeks.

In sum, the research on the leucine threshold is conflicting and is probably mainly relevant for elderly individuals with anabolic resistance (discussed in the course module on fitness for the elderly). However, for those intending to absolutely maximize muscle protein balance and growth, it may help to keep your protein intake in each meal above the leucine threshold. Concretely, consume a minimum of 0.3 g/kg (0.14 g/lb) protein in each meal, and include a high-quality protein source in each meal. For protein sources high in leucine, like egg protein and dairy, you can probably get away with just 0.24 g/kg (0.11 g/lb) protein.

The following table lists the exact amount of food you need to consume to hit the leucine threshold. As you can see, this is often not problematic in practice for any meal other than purely plant-based meals.

Food source	% leucine per 100 g protein	Amount of food needed to stimulate 50% MPS (0.5 g leucine)
Animal protein sources		
Beef	8 %	**35 g**
Chicken	7.5 %	**35 g**
Pork	8 %	**40 g**
Fish	8 %	**40 g**
Egg	9 %	**50 g**
Dairy		
Milk	8.5 %	**200 ml**
Yogurt	10 %	**90 g**
Cheese	11 %	**20 g**
Plant protein sources		
Tofu	8 %	**70 g**
Legumes	7-8 %	**40 g**
Nuts	6.5-7 %	**35 g**
Protein powders (ratio)		
Milk protein	8.5 %	**8 g**
Whey-casein-mix (1:1)	9.5 %	**6 g**
Whey-casein-mix (1:4)	9 %	**6 g**
Egg white protein	8.5 %	**7 g**
Pea-rice-mix (7:3)	7 %	**9 g**

Take-home message

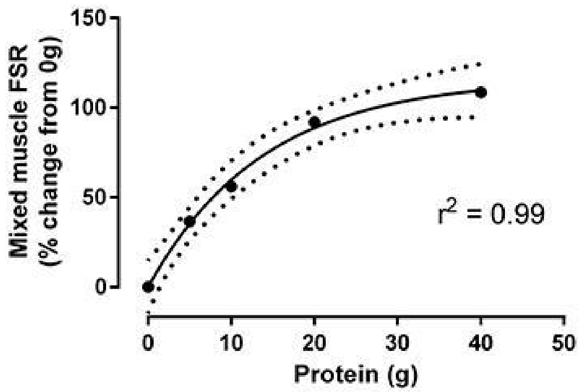
To maximize protein balance, it is advisable to consume at least 0.3 g/kg (0.14 g/lb) protein in each meal. If less than half of that comes from a high-quality protein source, foods with a complementary amino acid profile should be selected to ensure a leucine intake of at least 0.5 g and a complete amino acid profile for the meal as a whole.

Meal frequency

After a meal, protein synthesis normally increases. However, you can't just consume an enormous amount of protein and get extremely high levels of protein synthesis, force-feeding yourself to the Olympia. Your body maintains a maximum level of protein balance that it allows based on how much stimulus for muscle growth there is. From an evolutionary point of view, muscle growth is not always desirable, as it is an energy intensive process, so the body has adapted to only build muscle it really needs.

Physiologically, we see this regulation of muscle protein synthesis as the 'muscle-full effect': protein synthesis increases along with the entrance of amino acids into the blood after a meal (postprandial hyperaminoacidemia) only up to a point. After the muscle is 'full', muscle protein synthesis does not further increase even if you consume more protein and may over time even decrease despite persistent hyperaminoacidemia. This is why you can't get jacked simply by consuming a ton of protein: there needs to be an incentive for the body to build muscle. Protein intake is merely *permissive* of muscle growth: you also need a more potent stimulus for it, such as strength training.

The muscle-full effect can occur quite quickly. Just ~20 g or 0.3 g/kg (0.14 g/lb) of high-quality protein, such as whey, is needed for sedentary individuals to virtually maximize acute muscle protein synthesis.



The muscle-full effect: muscle protein synthesis (MPS) generally plateaus after just ~20 g of high-quality protein, such as whey. [Source](#)

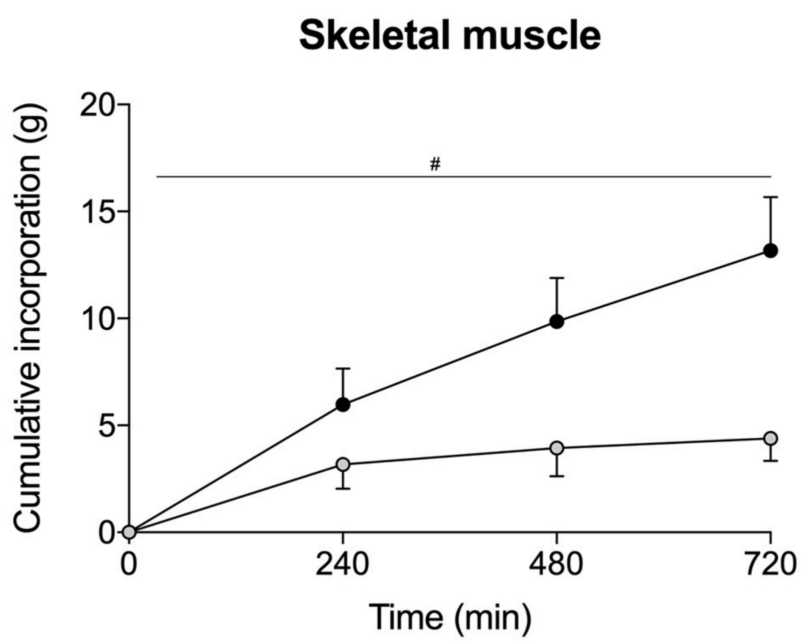
Post-workout, the anabolic ceiling increases, especially after high-volume full-body training: maximizing post-workout protein synthesis generally requires around 40 g or 0.5 g/kg (0.23 g/lb) of high-quality protein [2, 3].

Based on the muscle-full effect, you may expect that we need to consume protein every 3-4 hours to keep protein synthesis high across the day. One study seemingly supported this, finding that 20 g protein consumed every 3 hours is better than 8 × 10 g or 2 × 40 g. However, this study used whey protein and it only had a study period of 8 hours. Whey is digested very rapidly, thereby likely necessitating a higher meal frequency than most other protein sources and mixed meals with fat and fiber.

Other research finds that more slowly digestible meals stimulate MPS for far longer periods. The body can thereby use greater amounts of protein per meal. For example, 38 g milk protein concentrate can maximize MPS for at least 5 hours at rest. Kim et al. (2016, 2018) and Park et al. (2020) showed that in mixed meals, 70 grams of protein stimulated greater whole-body protein synthesis than 35-40 grams protein even at rest.

Moreover, prior fasting can also potentiate the cellular anabolic response to a meal, just like exercise. The body has evolved to be smart with its handling of protein, allowing for greater protein synthesis after periods of catabolism. This increased anabolic signaling by the muscle cells should lead to increased muscle protein synthesis (MPS), meaning the anabolic ceiling is likely higher after a fast than after you've consumed a meal just shortly before.

When we combine fasting, exercise and slowly digesting meals, it probably takes an enormous amount of protein to reach the muscle-full effect. Trommelen et al. (2023) found that 100 g milk protein can stimulate MPS for over 12 hours following a fasted whole-body workout: see the data below.



The total amount of protein incorporated into muscle after 25 g (grey dots) or 100 g milk protein (black dots) after a full-body workout in the fasted state. The initial anabolic response was not that different, but the larger protein serving resulted in far longer-lasting protein uptake and myofibrillar protein synthesis. Source

Based on how our body uses protein from our meals to build muscle, we should be able to stimulate maximal muscle growth with 3 or even just 2 meals a day, provided the following 2 conditions are met:

1. You consume mixed meals of largely whole foods, not just whey protein powder.
2. You roughly synchronize your protein intake with the body's capacity to increase MPS, i.e. you consume more protein post-workout, pre-bed and after long fasts.

The body can most likely use, for example, 0.6 g/kg (0.27 g/lb) protein post-workout and 0.5 g/kg (0.23 g/lb) protein in 2 other meals, amounting to 1.6 g/kg (0.73 g/lb) effective protein usage with just 3 meals a day.

Let's see what the research says about how many meals you need for maximum gains. The following table lists an overview of the literature on the effect of meal frequency on body composition change or energy expenditure (which would improve fat loss or indicate greater protein balance). All study designs equated total daily macronutrient intakes between groups. We will discuss the important studies on strength training individuals in the text below.

➢Literature overview

Meal frequency

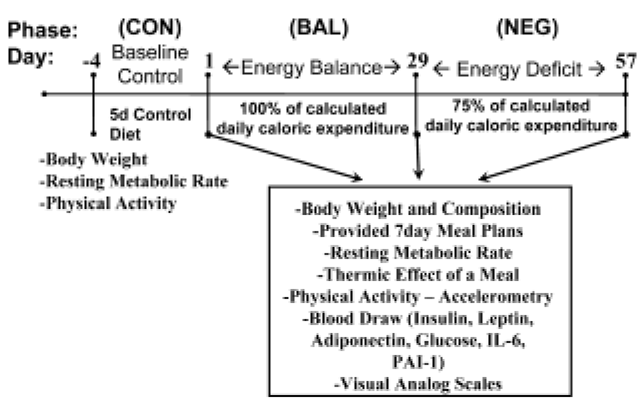
The Iwao (1996) boxer study showed improved muscle retention and a greater decrease in skinfold measurements during a crash diet with 6 compared to 2 meals a day. This study has several key limitations.

- The 1200 kcal liquid crash diet with only 61 g protein makes it questionable if the results can be extrapolated to less starvation like situations. Liquid meals may not sustain postprandial protein balance long enough for maximal protein balance across the day due to their unnaturally fast absorption speed. More substantial meals with fiber, unprocessed protein and fat make it far easier for the body to regulate protein balance.

- The subjects were boxers that performed their regular 'physical training and boxing exercises' for 2 hours a day. The 2-meal group ate only at 9 AM and 9 PM. Since boxing is extremely energy intensive and it's likely the exercise took place somewhere along the day, the thus fasted training condition for this type of exercise may well have been the reason for the greater muscle loss. Unfortunately, no details or measurements were provided for the physical training, though somehow the authors feel confident to state in the discussion that the training burned 800 kcal.

Even in these conditions, resting (!) energy expenditure did not differ between the groups either before or after the diet. However, there was a non-significant trend for greater energy expenditure in the 6-meal group after the diet, which is to be expected given the greater muscle retention.

Arciero et al. (2013) found improved body recomposition and thermogenesis on 6 compared to 3 meals a day. Their study design is summarized below.



TEF was higher in the 6 compared to the 3-meal group. This stands in contrast with an abundance of other research that finds meal frequency per se does not affect energy expenditure or fat loss [2, 3]. And since protein synthesis should increase energy expenditure, this indirectly also suggests meal frequency does not affect protein synthesis levels. The discrepancy between the overall literature and Arciero et al. is likely because the 6-meal group ate 3 additional meals that consisted almost purely of protein supplements from the study sponsor. As covered in the course topic on human metabolism, isolated protein has a high TEF, especially in comparison to the processed foods from the rest of the diet, which included “no-sugar-added applesauce and other American Heart Association approved cereals and granola bars”. As such, it’s not a major surprise that the TEF relative to caloric intake was higher for the protein snack in the 6-day group than the regular processed meal of the 3-day group. Also, the TEF was only measured at 1 time point and resting energy expenditure did not differ between groups, so the higher TEF value may also simply have been a biorhythm effect or a fluke without actually higher total thermogenesis across the day.

The higher TEF, if not an artefact, could explain the greater fat loss. The improved muscle growth and retention rates, however, must have a different reason. Just like in the boxer study, it may actually be that meal frequency *does* affect protein balance in the conditions of this study due to the highly processed and liquid nature of the meals. The fast absorption speed of the food may necessitate a high meal frequency to sustain postprandial anabolism, i.e. keep protein balance high throughout the day.

Alternatively, the 6-meal group had an additional high protein meal pre-bed. This may have improved circadian rhythm protein timing or fueled the anabolic window, as we’ll discuss later. This fits with the higher leptin levels found in the 6-meal group.

Also, it’s noteworthy that this study was sponsored by a protein supplement manufacturer.

Most other research finds no benefits of higher meal frequencies and The Meal Frequency Project from Norway found a detrimental effect. Finally, research with direct real-world relevance: experienced subjects performed controlled strength training while eating a high-protein diet, the study duration was relatively long (12 weeks) and the subjects tracked their macros while consuming either 3 or 6 meals a day. Surprisingly to many, the 3-meal group gained significantly more strength and muscle mass after controlling for confounding variables. The research wasn’t as well controlled as we’d like from scientific research and it’s hard to explain why 3 meals would lead to more anabolism than 6 meals. The diet data show no significant differences between groups, but in absolute terms, the 3-meal group had a higher energy intake. Still, this study strongly argues against the need for more than 3 meals a day for maximum muscle growth.

Another highly relevant study in elite rugby athletes found no effect of eating an average of 5.6 vs. 7.9 meals a day on muscle growth, although the diets in this study were evidently poorly controlled: the standard deviation of protein intake was a whopping 0.6 g/kg (0.27 g/lb) per day in one group.

Taguchi et al. (2020) again found no benefits of a meal frequency of 6 vs. 3 in bulking competitive rowers: both groups gained similar amounts of lean and fat mass throughout the 8-week study. This study was remarkably tightly controlled. Energy balance was set at a hefty surplus of 20 kcal/kg (9.1 kcal/lb), after calculating energy expenditure with accelerometers. The meals were provided to the subjects, resulting in excellent dietary adherence. The sample size of 10 was small, but all subjects completed both conditions for 8 weeks with a wash-out in between (randomized controlled cross-over). However, it should be noted these subjects were well-trained athletes, but their primary sport was rowing and they did not seem to do any structured weight lifting. Their body compositions were pretty good though (BW in kg close to height in cm minus 100 at 12% BF) and they gained a decent amount of lean body mass during the study, roughly half of total weight gain.

Another line of research also doesn't support the need for high meal frequencies. Between-meal protein supplementation is no more effective for strength trainees to gain lean body mass than with-meal supplementation. This was a meta-analysis with many studies supplementing protein on top of their habitual diet. Increasing their meal frequency with between-meal protein supplementation evidently wasn't beneficial for muscle growth. Since many people only consume 3 meals a day, these findings suggest there are no benefits to a higher meal frequency than this.

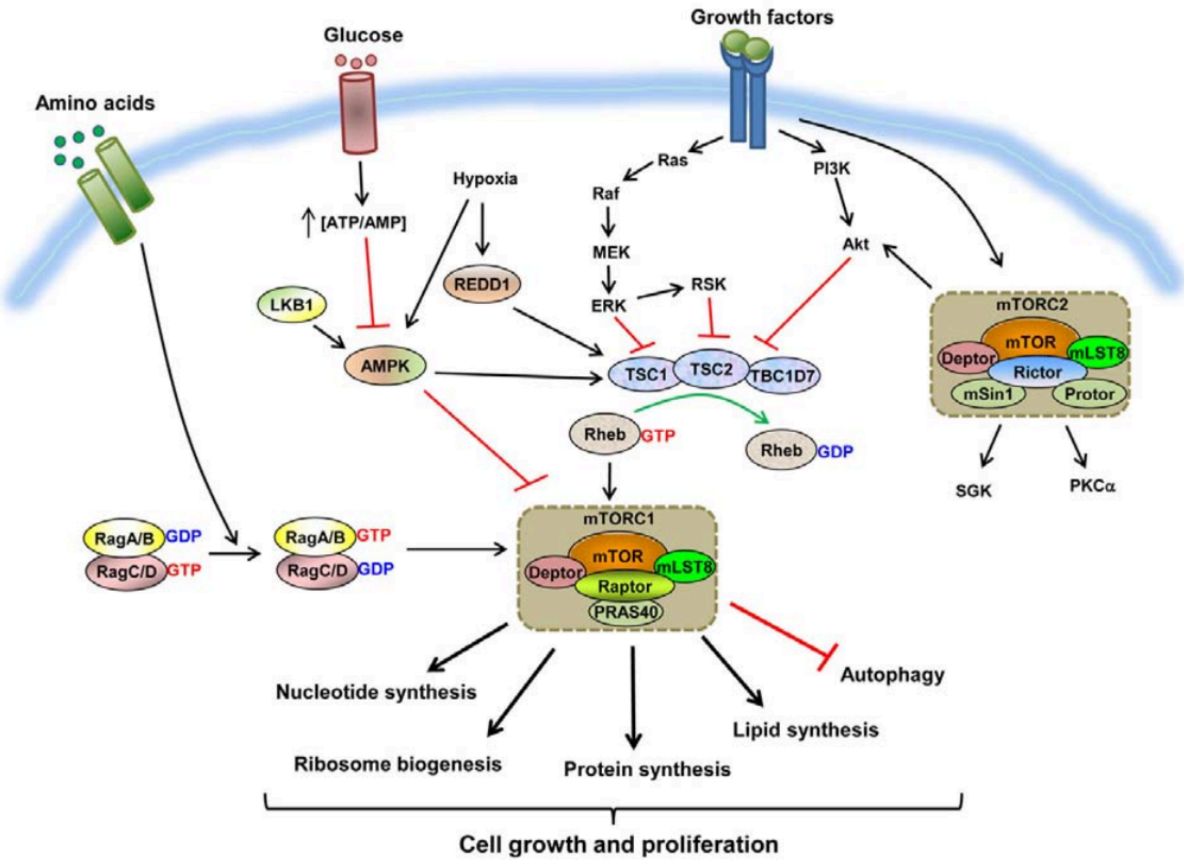
2 Meals a day probably won't cut it for maximum muscle growth though. If you recall the aforementioned study by Yasuda et al. (2020), it found that a roughly even protein distribution across 3 meals led to a strong trend for greater lean tissue growth and strength development than having the same total daily macronutrient intakes with a breakfast with only 0.1 g/kg protein. The subjects were young, strength training men and the study lasted 12 weeks. Since the evidence for the leucine threshold is shaky, it's plausible that the inferior gains in the group with almost all protein in meals 2 and 3 was because 2 meals simply can't maximize protein balance across the entire day in strength trainees.

Conclusion

Most research suggests that meal frequency per se does not affect energy expenditure, protein balance or body composition change and it thus does not matter over how many meals a day you distribute your macros. However, the limited research we have suggests that just 1 or 2 meals a day may not suffice for maximal muscular development in strength trainees. Additionally, The Meal Frequency Project shows that 3 meals a day may be superior to 6 meals a day, at least when bulking. As such, meal frequencies of 3-5 meals a day are recommended for optimal body recomposition.

Fasted training

During exercise, the mTOR protein integrates nutrient and exercise signals to determine how much muscle will be built. You can think of mTOR as a little computer that weighs the body's costs and benefits of turning on muscle protein synthesis. Positive inputs include exercise, the presence of amino acids, energy and anabolic hormones. Negative inputs include glucocorticoids (stress) and energy deficiency.



mTOR integrates anabolic signals to initiate protein synthesis for muscle growth.

[Source]

So when you are training in a fasted state, your body has a weaker signal for muscle growth. From an evolutionary point of view, it's not adaptive to ramp up protein synthesis greatly when there's not enough protein available to cover all bodily needs.

Indeed, after an overnight fast, consuming an essential amino acid mixture pre-workout results in greater protein synthesis than consuming it post-workout. Tipton et al. (2004) failed to replicate this finding with whey protein, but net amino acid uptake in the exercised muscle was 2.5 times higher when the participants consumed the whey pre- vs. post-workout. 4 of the 8 subjects in PRE had greater uptake over 5 hours than any of the subjects in POST. The lack of statistical significance between groups was likely due to insufficient statistical power: "Power calculations revealed that a statistically significant difference could be detected with two times the number of subjects."

A caveat that many fasted trainers will mention is that your body is good at autoregulating its protein balance over time and will compensate for fasting periods with greater elevations in protein synthesis during subsequent meals. If you train fasted and consume protein afterwards, your body has a super-compensatory increase in anabolic muscle signaling.

Anabolic muscle signaling is very interesting, but can the body fully compensate for the increased protein breakdown and the suppressed protein synthesis during fasted exercise? The acute protein balance during strength training with pre-workout protein intake is additive compared to rest, so it's questionable if protein balance after fasted training can fully catch up with that of fed training.

Esmarck et al. (2001) found that delaying post-workout protein consumption by 2 hours compared to immediately post-workout resulted in less muscle growth and strength development in elderly men, even though they had breakfast up to 90 minutes before the training session.

A similar study by Levenhagen et al. (2001) found that delaying post-workout protein consumption by 3 hours only led to an increase in whole-body protein balance of 12% compared to 300% when the protein was consumed immediately post-workout after endurance training. Endurance training may be more reliant on rapid replenishment of glycogen stores post-workout than strength training though.

Pre-workout protein consumption is likely also beneficial to minimize protein breakdown. Tipton's studies on the difference between pre- and post-workout protein consumption on protein synthesis assumed neutral nitrogen balance during fasted training. This is a simplification because it's methodologically difficult to measure protein breakdown, yet it is often incorrect. During fasted exercise, protein balance is often negative because protein breakdown rates increase and synthesis rates decrease for several reasons [2].

- Exercise results in muscle damage and muscle protein breakdown.
- Amino acids may be used as fuel (oxidation), to form glucose (gluconeogenesis) for glycogen resynthesis or to maintain acid-base regulation.
- Muscle anabolic signaling decreases. (See section above on mTOR.)

As such, strength training itself in a fasted state is acutely catabolic. The solution: protein consumption decreases muscle protein degradation during exercise. The anti-catabolic effect of protein consumption is 'only' about 50% though, as protein breakdown levels don't vary much compared to protein synthesis levels.

Interestingly, protein itself doesn’t directly decrease protein breakdown levels: insulin does. Protein is insulinogenic and you only need only a minor amount of insulin for maximal suppression of protein breakdown.

In short, pre-workout protein consumption can help to both increase protein synthesis and to decrease protein breakdown in your muscles, which should result in greater growth.

There are surprisingly few studies on the effects of fasted exercise, but the few we have support the theory that training fed is better than fasted for your gains, although the magnitude of difference is not major.

1. Tarpenning et al. (2001) found greater muscle growth in a group sipping on Gatorade (a carbohydrate rich sports drink) throughout their workouts than a group training fasted while sipping on a placebo. Type I and II muscle fiber areas increased only a few percent, not significantly, in the fasted training group, whereas the increases were over 20% in the Gatorade-sippers after the 12-week strength training program. The Gatorade drink increased insulin levels and reduced the cortisol spike during exercise. The elevation of cortisol during exercise was strongly negatively related with muscle growth, supporting the anabolic and anti-catabolic role of insulin during exercise. Both groups only fasted for 4 hours before their workouts and performed their training sessions in the afternoon, so this wasn’t even an overnight fast. Interestingly, total body composition and isokinetic strength changes did not differ between groups. In fact, body composition didn’t differ significantly from baseline at all after the study in either group. This goes to show it’s important to measure muscle growth specifically in a muscle and not just assess whole-body lean mass, especially when a study doesn’t control the participants’ diets.

2. Pihoker et al. (2018) also studied the effects of fasting on strength training with a 6-week study. They randomized strength-trained women into 3 groups.

- The control group trained performed whole-body strength training after an overnight fast.
- Group PRE consumed 16 g carbohydrate and 25 g protein before said workout.
- Group POST consumed the protein shake immediately after the workout.

Total macronutrient intakes were similar in all groups based on self-reported food logs. After the study, there were no significant differences between groups in body composition change or leg press strength development, suggesting that nutrient timing or in fact even protein consumption per se doesn't influence your gains. However, bench press strength increased more in the PRE and POST groups than the control group, suggesting that protein consumption was beneficial but its timing was irrelevant. And a closer look at the data paints a different picture. As you can see in the table below, the PRE group experienced the highest increase in lean mass (over 6 times higher than the control group), the largest decrease in body fat percentage and the highest increase in leg press and bench press strength. Importantly, the PRE group achieved a significantly higher training volume than the POST group, suggesting that fasted training impaired performance. Though it's odd that the control group also achieved a higher volume, it speaks for the benefits of the protein shake that the other groups made better gains despite training with less volume.

Table 1: Maximal strength change for lean mass (LM), body fat percentage (%fat), maximum leg press (LP1RM), bench press (BP1RM), and average training volume for each treatment group [Pre-workout (PRE), post-workout (POST), and control (CON) groups; Unadjusted mean ± SD].

Group	ΔLM (kg)	Δ%fat (%)	ΔLP1RM (kg)	ΔBP1RM (kg)	Average Training Volume (kgs)
PRE	0.96 ± 1.38	-1.06 ± 1.42	67.6 ± 30.5	5.6 ± 2.8*	82447.2 ± 18229.5
POST	0.64 ± 0.72	-0.70 ± 1.04	55.3 ± 23.5	4.8 ± 2.5*	63354.1 ± 14321.9+
CON	0.15 ± 1.35	-0.44 ± 0.91	62.9 ± 26.8	2.5 ± 1.8	86491.9 ± 21895.7

*indicates a change significantly greater than CON (p<0.05).

+indicates significantly lower than other groups (p<0.05).

It's not surprising most differences didn't reach statistical significance, because statistical power was low.

- The participants were previously strength-trained women and the training program had only 12 workouts in total throughout the mere 6-week study. The control group barely gained any lean mass at all.
- There was no control of the menstrual period. This is very relevant in a 6-week study, as the marginal between-group differences could be the result of menstrual cycle phase differences.
- Food logs were self-reported on just 3 days, there were no prescribed diets and none of the groups probably consumed an optimal amount of protein before and after their workouts.

It's not even reported if any of the gains the participants made were statistically significant from baseline.

3. A small study on bodybuilders during the month of Ramadan fasting by Trabelsi et al. (2013) found no difference in body composition change between fasted afternoon training and late-night fed training. However, neither condition was likely optimal for performance (not measured) or nutrient timing and neither group experienced any body

composition change at all during the month, so it's hard to say if fasted training impairs muscle growth based on this study.

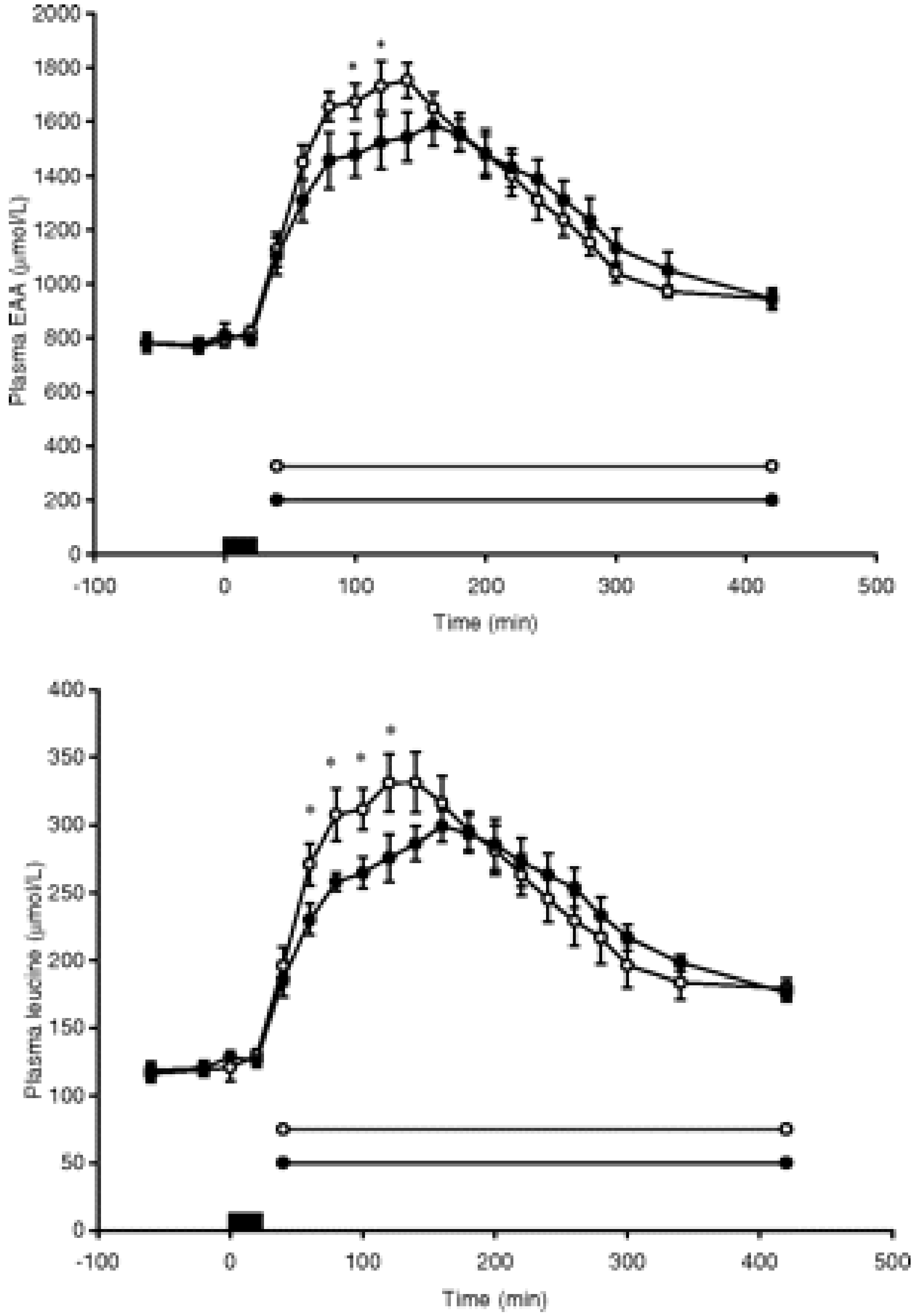
4. Another study on well-trained lifters during the month of Ramadan fasting by [Triki et al. (2023)] found that fasted training reduced squat and deadlift strength gains. One group trained between 4-6 PM before breaking their fast, whereas the other group trained after breaking the fast at 8-10 PM. Both groups consumed the same total macronutrient intakes, which seemed well-controlled. Bench press strength gains didn't significantly differ between groups, nor did quadriceps and biceps size or body composition. However, there was a trend ($p = 0.1$) for higher lean body mass values in the fed group and the fed group experienced a consistent increase over time in quad size compared to a decrease in the fasted group during the 3 measurements in Ramadan. Importantly, total work volume was equated between groups: both groups performed the same training program with the same number of sets, the same intensity (% of 1RM) and the same number of reps. This biased the results in favor of the fasting group, as fasted training could reduce performance otherwise. Indeed, the fasted group reported their workouts required more effort (higher workout RPEs). So the fasted group was training harder to get worse results.

We also have research on the effect of training fasted on subsequent recovery from the workout. Increased protein balance during fed compared to fasted training should result in greater recovery. Hoffman et al. (2010) found that strength and power athletes consuming protein pre- and post-workout had greater strength recovery and lower markers of muscle damage (creatine kinase) than a placebo group. The additional protein resulted in a higher total daily protein intake compared to the placebo group, but dietary protein intake was already 2 g/kg (0.91 g/lb) in both groups, so you wouldn't expect more total daily protein to make a difference and the benefits may have been due to the timing. Song-Gyu et al. (2018) also found that pre-workout BCAA consumption decreased muscle damage and soreness (DOMS) compared to a placebo. The effect trended to be greater for pre- than post-workout BCAA consumption. Depending on which marker you look at, other research finds little evidence of pre-workout protein's protective effect against muscle damage other than decreased elevations of creatine kinase, myoglobin and tumor necrosis factor alpha (TNFa), a marker of inflammation [4]. This is a notorious problem in research on recovery after strength training. We don't yet fully understand the relation between all biomarkers and actual strength recovery, which is arguably ultimately all that matters. Overall though, fasted exercise may hamper recovery.

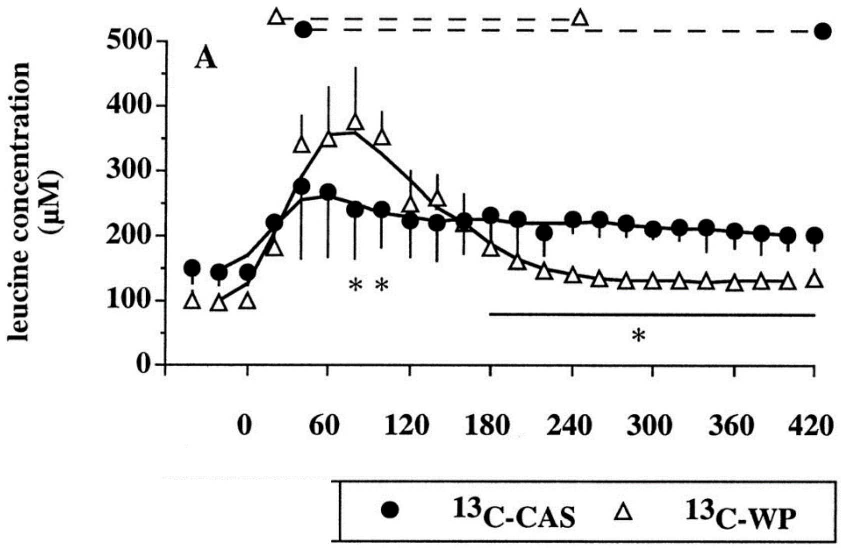
Conclusion & application

In conclusion, while the research is not very consistent, the trends in the data are in line with the theory that fasted exercise will increase protein breakdown, limit protein synthesis and impair exercise performance. Thus, training fasted will likely hinder muscle growth and strength development. Therefore, we want a high amount of amino acids in the blood (hyper-amino-acidemia) with correspondingly elevated insulin levels when exercising.

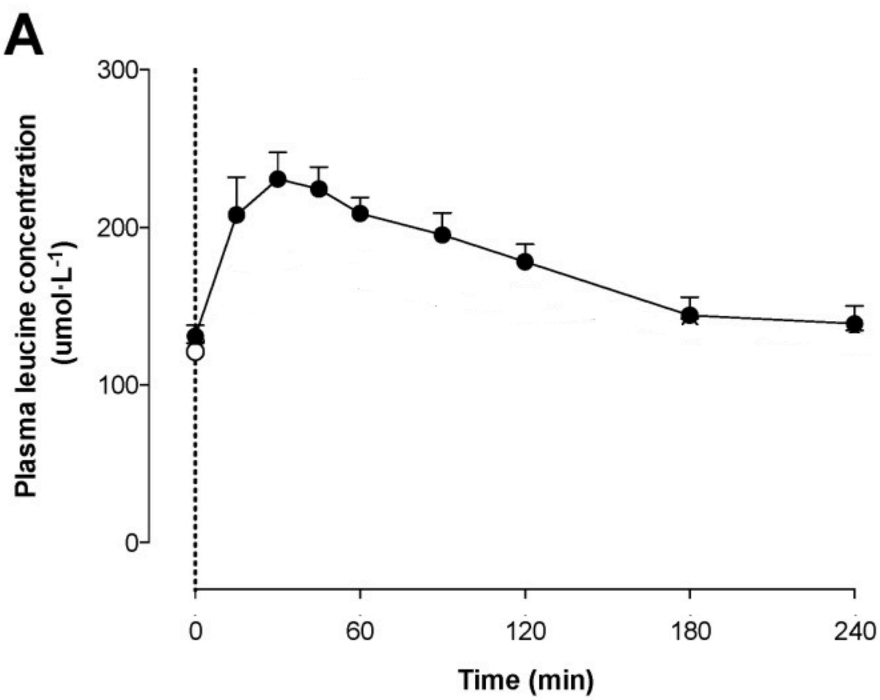
The graphs below provide reference points on how long amino acid levels stay elevated in the blood after different meals (postprandial hyperaminoacidemia). As you can see, postprandial hyperaminoacidemia may only last around 3 hours after the consumption of skimmed milk or a whey protein shake, but it lasts over 6 hours after consumption of a steak, casein protein or whole milk.



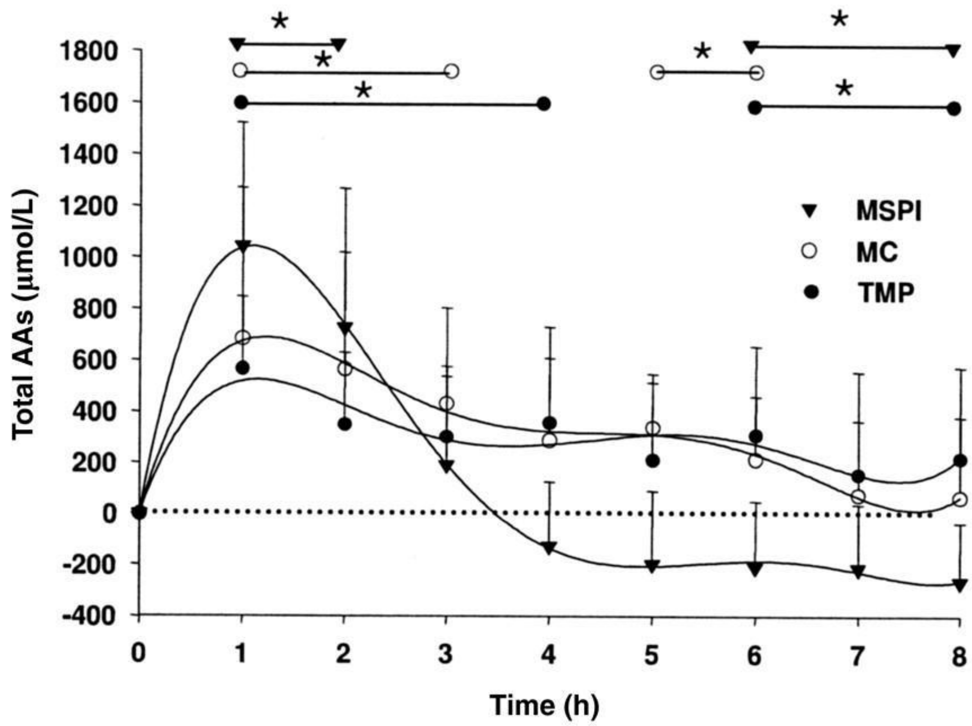
Blood concentrations of essential amino acids (above) and leucine (below) after consumption of a 120 g steak in elderly individuals. [Source]



Blood concentration of leucine after consumption of 30 g of whey or casein protein (matched for leucine content) in young, healthy individuals. [Source]



Blood concentration of leucine after 60 g of skimmed milk powder consumption (22 g protein) in healthy, young men. [Source]



Mean (±SD) changes from baseline in serum total amino acid (AA) concentrations in the subjects after the ingestion of ~23 g protein from total milk protein (TMP), micellar casein (MC) or milk soluble protein isolate (MSPI). [Source]

[Insulin levels can return to baseline much faster after a meal, generally within 3 hours, unless you're consuming very high carbohydrate meals] [[2], [3], [4]].

As a guideline, hard training individuals should consume at least 0.4 g/kg (0.18 g/lb) of protein and 10 grams of carbohydrate within 2 hours of starting their strength training sessions. Very large pre-workout meals may cover a longer period, but based on the data we have, it's not optimal to fast for more than 4 hours before a workout. Women generally don't suffer as much from fasted training as men due to their lower protein breakdown and oxidation during exercise (discussed in the course topic on gender differences), so they might get away with more leniency with their meal timings.

Fasted training for fat loss?

Intuitively, you may think fasted exercise increases fat loss. Fat oxidation levels ('fat burning') are generally higher when you train fasted, but some research finds that fasted cardio doesn't increase fat oxidation rates during exercise and you achieve higher fat oxidation rates post-exercise if you consumed protein pre-workout. This may be the result of the body relying more on fat as fuel and less on protein.

Regardless, if you studied the course module on human metabolism, you should understand that fat oxidation rates don't necessarily correspond with body fat loss over time. Cumulative energy balance is the deciding factor of total energy loss. For fasted exercise to improve fat loss, it needs to either increase energy expenditure or improve nutrient partitioning compared to fed exercise. Neither is the case.

Energy expenditure is *lower* during fasted exercise. Pre-workout protein consumption increases energy expenditure during and post-exercise in the form of increased excess post-exercise oxygen consumption (EPOC) compared to placebo, even post-workout nutrition is provided in both situations and when total daily macronutrient intake is controlled for [3]. The increase in energy expenditure may be the result of the greater protein balance, since muscle growth is an energy intensive process.

As for nutrient partitioning when training fasted, here too fasted exercise is probably suboptimal for the same reasons fasted strength training is suboptimal for muscle growth. Even diabetics have better glucose and triglyceride control during dinner when it is consumed pre-workout instead of post-workout. This is interesting, since insulin sensitivity is greater post-workout than pre-workout.

Since fasted cardio doesn't improve energy expenditure or nutrient partitioning, fasted cardio or strength training doesn't lead to more fat loss than fed cardio, given the same energy intake [2].

In conclusion, fasted exercise is generally suboptimal for energy expenditure, performance and muscle growth. Some people may not like training on a full stomach or complain they don't have time to prepare a meal before their workout. These are generally limitations of imagination rather than time, as you can prepare many meals that are easy to digest and require minimal preparation time, such as a small piece of fruit and some dairy or smoked poultry.

The anabolic window & post-workout nutrition

As we discussed earlier, when nutrient timing is the same in both scenarios, the research is quite clear there are no benefits to increasing your protein intake above 1.6 g/kg (0.73 g/lb) per day in most if not all circumstances. However, there are several studies that do find benefits of protein intakes over 1.6 g/kg (0.73 g/lb) per day when the protein is consumed at specific times ('protein timing'). Usually, the additional protein is provided as pre- and/or post-workout protein shakes in these studies. Since protein intake itself is well-researched not to have additional effects above this point, it's likely the protein timing resulted in the additional strength development and muscle growth in these studies.

➢ Literature overview

Effect of protein intake with nutrient timing

A particularly important study on the distinction between protein intake and nutrient timing is Hulmi et al. (2015). In this study, novice level male trainees consumed a post-workout drink consisting of either carbohydrates, protein or both. What makes this study so special is that the carbohydrate group actually had the highest total protein and energy intake (see table below). So the 2 protein groups had the benefit of protein timing, but the carbohydrate group had the benefit of more overall protein and energy.

The protein timing group with only 1.5 g/kg (0.68 g/lb) total protein gained more fat-free mass per kilogram bodyweight than the carbohydrate group with 1.7 g/kg (0.77 g/lb) total protein. And both protein timing groups lost more total fat mass than the carbohydrate group. Strength development was similar in both groups. Thus, based on this study, the timing of protein can impact your results, even more so than additional protein intake after a certain point. Interestingly, these were only novice level trainees.

Table 3
Characteristics of subjects after the habituation before the actual 12 -week RT interventions started and average daily dietary intakes from four-day diary during the second four-week training block

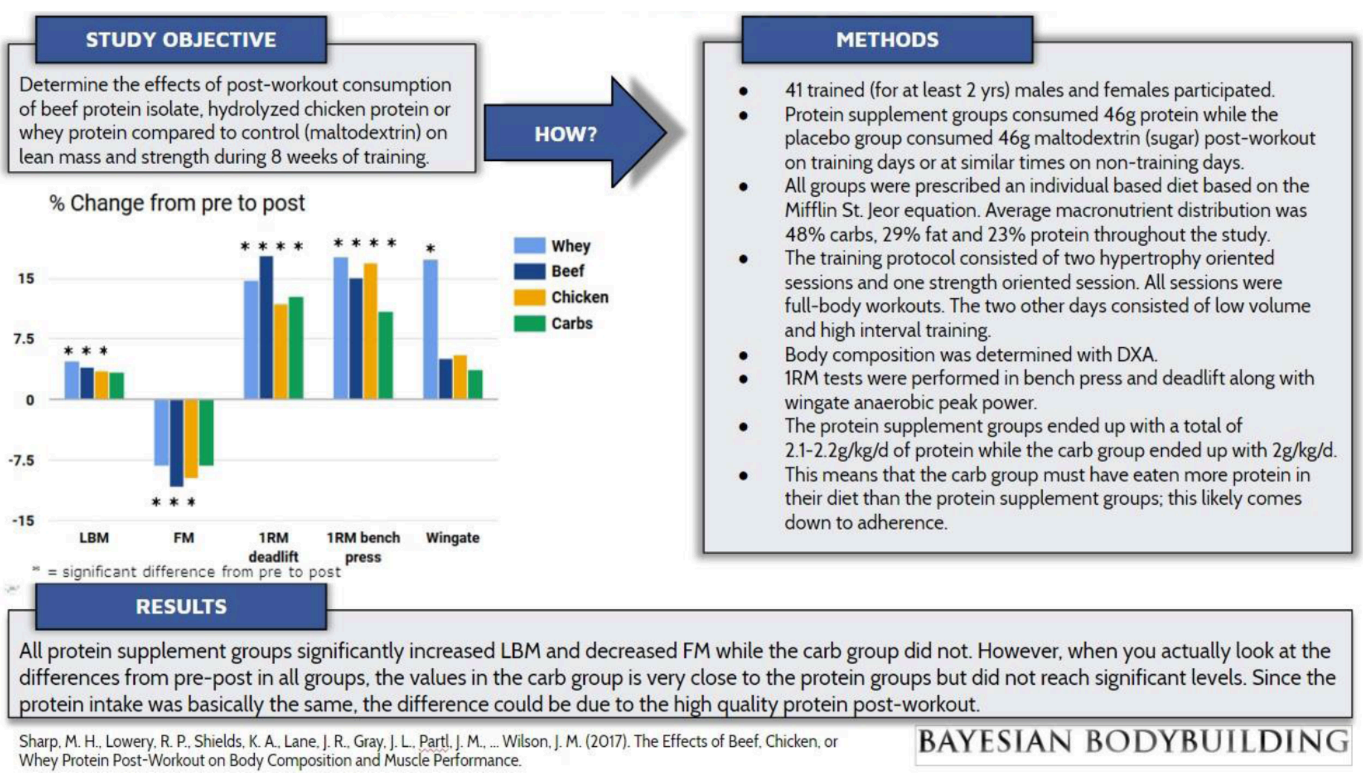
	CHO (*n* = 21)	**Protein (*n* = 22)**	**Protein + CHO (*n* = 25)**	**All (*n* = 68)**
Age (y)	36.4 ± 4.2	31.4 ± 1.4	36.2 ± 1.2	34.7 ± 1.4
Height (m)	1.79 ± 0.02	1.81 ± 0.02	1.80 ± 0.02	1.80 ± 0.01
Weight (kg)	81.4 ± 2.5	83.8 ± 2.4	85.1 ± 2.3	83.6 ± 1.4
Energy (kJ/kg/day)	146.5 ± 8.4	124.0 ± 10.3	122.5 ± 8.9	129.4 ± 5.5
Protein (g/kg)	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Protein (%)	20.0 ± 0.6	21.2 ± 1.1	20.2 ± 1.1	20.5 ± 0.6
Fat (g/kg)	1.4 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
CHO (g/kg)	3.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	3.2 ± 0.2
HS (n)	10	13	14	37
SP (n)	11	9	11	31

Data are means ± SE. There were no significant differences between the groups. FFM = fat-free mass, CHO = carbohydrates. HS = hypertrophic-strength training and SP = strength-and power training. The nutrition results also include the supplement that was ingested for 1 or 2 days during the four day diary recording.

Technical note: while the differences in macros between groups did not reach statistical significance, this is almost certainly a case of insufficient statistical power and the result of using percentage protein intake instead of protein g/kg/d. There was still a statistical trend for greater energy intake in the carbohydrate group and you can see that the protein intake in g/kg was 2-3 full standard deviations higher in the carbohydrate group.

A similar case happened in Mori & Tokuda (2018). Over the course of a 24-week strength training program, one group of previously untrained women supplemented whey protein post-workout, while the other group did not. However, total daily protein and energy intake didn't end up significantly different. Still, the post-workout whey group gained more muscle and strength. The improved nutrient timing must thus have been the reason for the greater gains.

[Sharp et al. (2017)](#) also found evidence that consuming protein shortly after a workout improves muscle growth and strength development in trained individuals even when they were already consuming enough protein. In this study, several protein supplemented groups achieved slightly more muscle growth and strength development than a post-workout carbs group even though the difference in total protein intake was only ~2.15 vs. 2 g/kg (~0.9 vs. 1 g/lb) per day. See the infographic below for further study details if you're interested.



The period of heightened responsiveness to protein after your workouts is known as the post-exercise window of anabolic opportunity, or just the anabolic window. After exercise, your muscles become more sensitive to amino acids, like a dry sponge. The muscle-full ceiling increases and your muscles can achieve higher levels of protein synthesis. Importantly, the anabolic window is not inherently a period of higher protein synthesis levels in the body. Rather, it's a period of delayed muscle-fullness, which means there is a greater potential for protein synthesis. To fuel this potential muscle growth, we need to consume protein within the anabolic window. Since energy intake and certain nutrients can work synergistically with protein to increase protein balance, at least over the long-term, it might be beneficial to also target more total energy intake with the anabolic window.

While most supplement companies have focused on the immediate post-workout period, the anabolic window typically lasts over 24 hours. In untrained individuals, the anabolic window can last over 72 hours after (endurance) training. Based on the training frequency literature, which we'll discuss in its own module, the anabolic window may even last a full week when training volume is high enough. See the graphs below for real-life examples of the anabolic window.

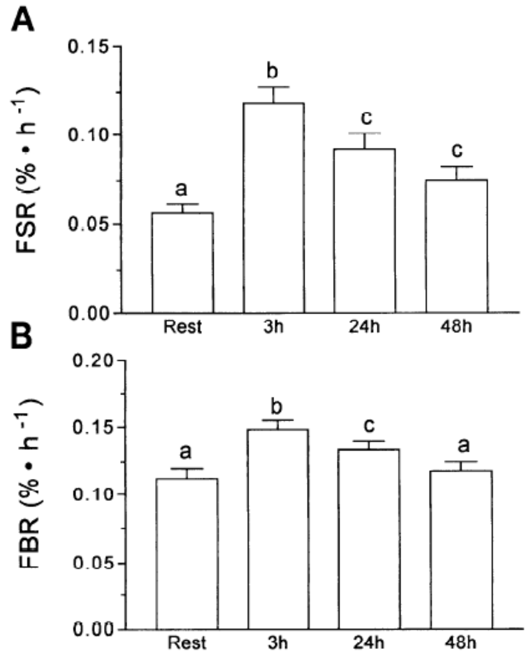
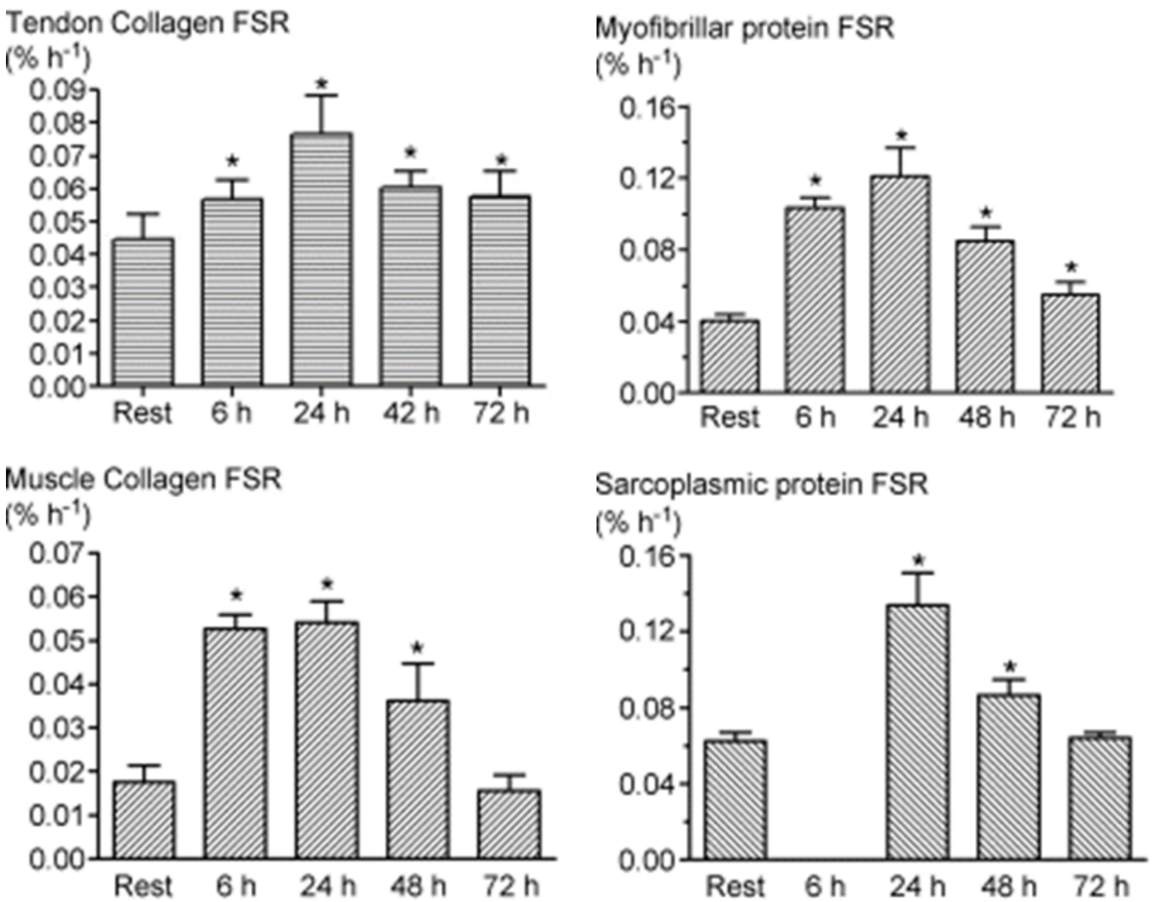


Fig. 4. Mixed muscle protein fractional synthesis rate (FSR, *A*) and fractional breakdown rate (FBR, *B*) at rest and after exercise bout. Means with different letters are statistically different ($P < 0.05$). A main effect for time was found for FBR ($P < 0.01$). Values are means ± SE ($n = 8$). Rest, *day 1*; 3h, 3 h postexercise; 24h, 24 h postexercise; 48h, 48 h postexercise.

Quadriceps protein synthesis (top) and breakdown (bottom) rates over time after 8 sets of high-intensity leg extensions in untrained individuals. (Source)



The time course of different muscle protein fraction synthesis levels after bicycle ergometer endurance training. Myofibrillar MPS was still significantly elevated above pre-workout/rested levels 72 hours after the training session. (Source)

Since the anabolic window lasts for days after a strength training workout in untrained individuals, there is probably not much need for precise protein timing. In untrained individuals, you get the same increase in muscle protein synthesis whether you consume protein 1 hour or 3 hours after your workout. If at least 3 meals a day are consumed spread across the day with at least 0.4 g/kg (0.18 g/lb) protein each, the exact timing of the meals and their protein intakes is likely not important.

Trained vs. untrained

As the body adapts to strength training and post-workout muscle damage decreases, protein synthesis rates decrease correspondingly within a matter of weeks. In addition, the adaptation process to strength training becomes more specific. A strength training session generally still poses considerable cardiovascular stress. Workouts require both endurance and strength training adaptations. As such, your muscles create various proteins: not just contractile proteins (myofibrillary protein synthesis) but also proteins in your mitochondria, your cells' energy production centers (mitochondrial protein synthesis), in the sarcoplasm of the cell (sarcoplasmic protein synthesis) and in collagen. Over the first few months of training, the rates of non-myofibrillar protein synthesis decrease significantly in particular and the duration of total muscle protein synthesis decreases.

Damas et al. (2015) compiled the data we have on the time-course of muscle protein synthesis post-workout in trained vs. untrained individuals in the following graph. Whereas the anabolic window lasted for days in untrained individuals, peak MPS occurred within the first 10 hours after a workout in trained individuals with little further elevation after 24 hours.

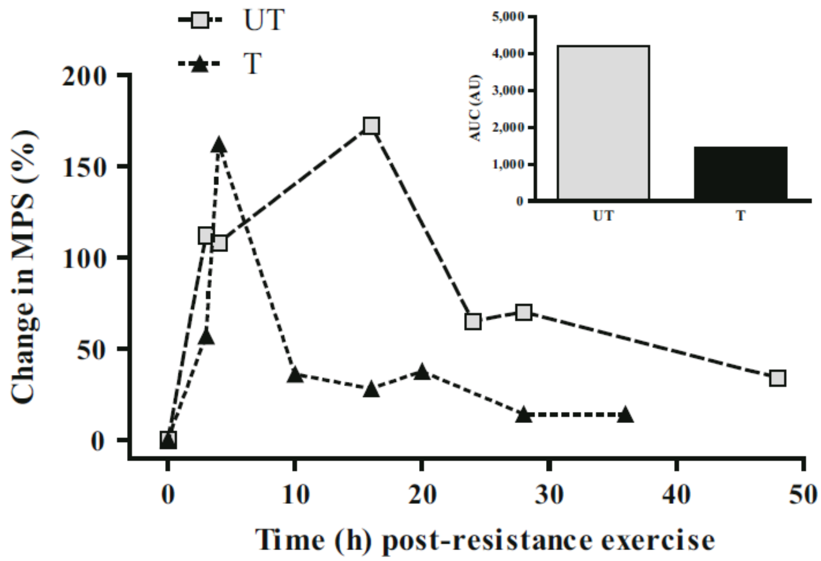
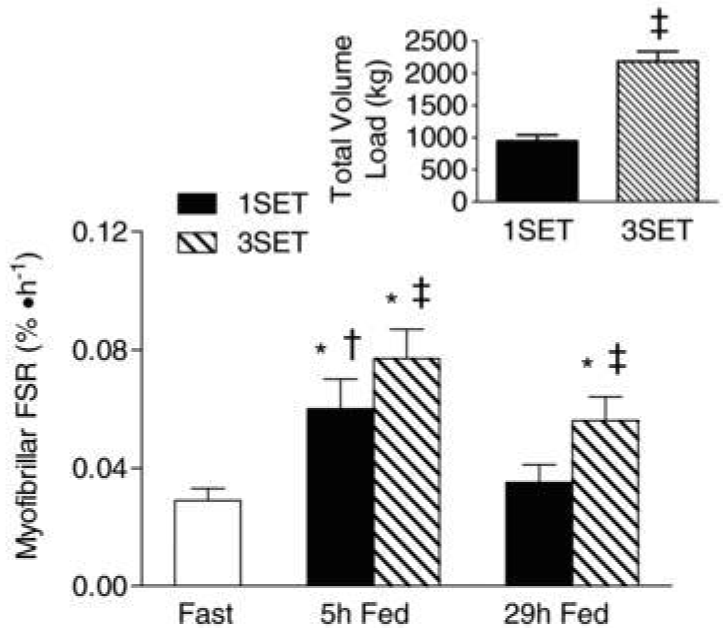
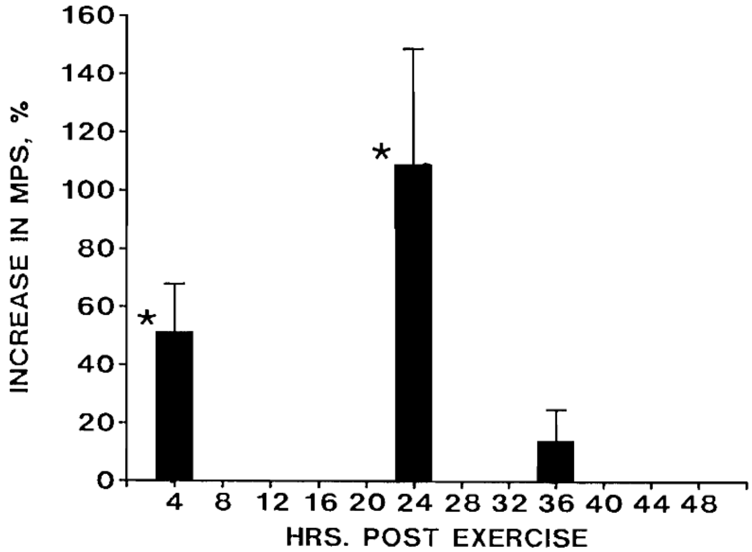


Fig. 1 Time course of the increase in mixed muscle protein synthesis (MPS) following a bout of resistance exercise in the untrained (UT) and resistance-trained (T) states. The data were compiled from Tang et al. [4], Kim et al. [28], Yarasheski et al. [3, 29], Roy et al. [38], Phillips et al. [8] and MacDougall et al. [7]. *Inset* area under the curve (AUC) for the percentage change in MPS from the UT and T curves, expressed in arbitrary units (AU)

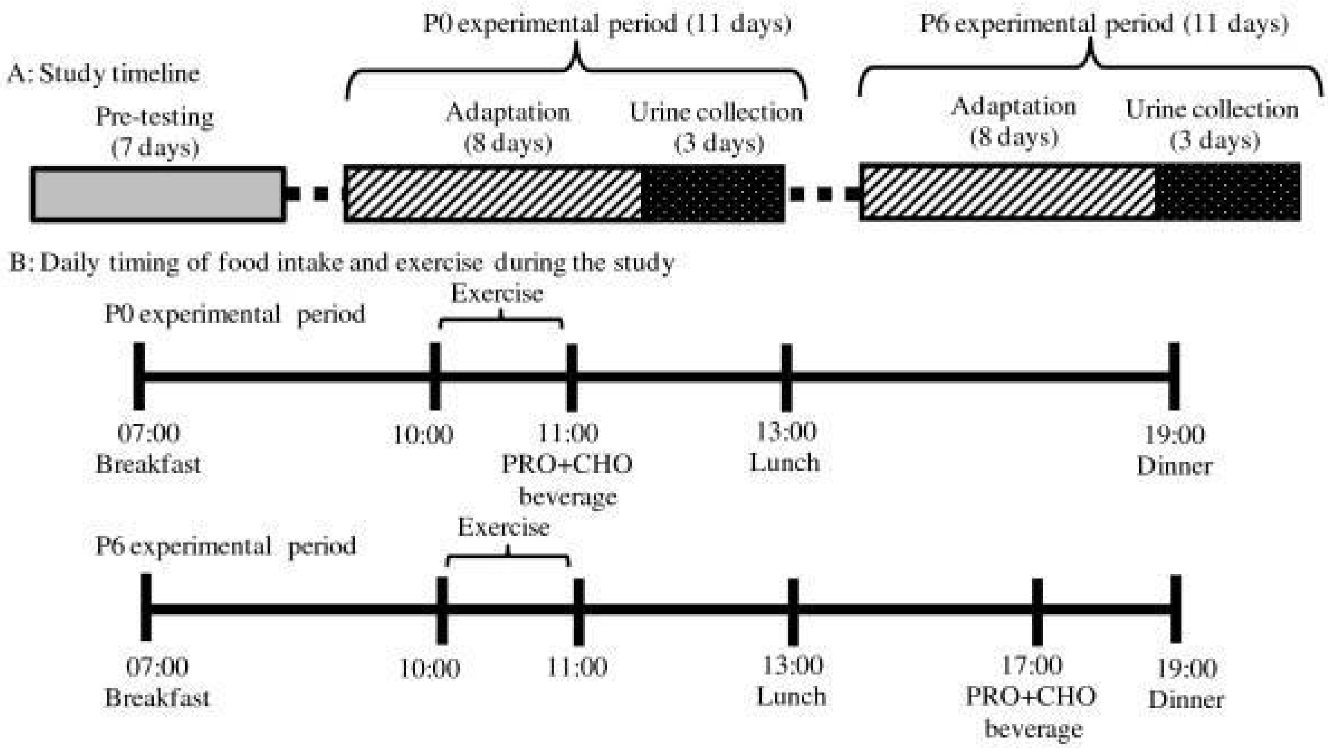
Importantly, performing a higher training volume can prolong the duration of the anabolic window. Myofibrillar protein synthesis levels may still be elevated 29 hours after a strength training workout in trained individuals if they performed sufficient volume, but peak MPS levels occur much sooner after the training session in trained individuals (see graph below).



Still, some studies find even high-volume workouts don't stimulate an anabolic window longer than about 2 days, although peak MPS seemed to occur later than in Damas's research. In recreational bodybuilders, biceps MPS levels had returned to within 14% of baseline (resting) levels within 36 hours even after 12 sets of biceps exercises to failure at 80% of 1RM (see graph below).



The shortened anabolic window in trained individuals makes protein timing much more important. In line with this, [Mori (2014)](#) showed that bodybuilders but not untrained individuals achieved higher nitrogen balance when sandwiching their workouts closely with protein consumptions. Immediate post-workout protein consumption was more beneficial than waiting 2 hours for lunch, even though breakfast was consumed only 6 hours before lunch.



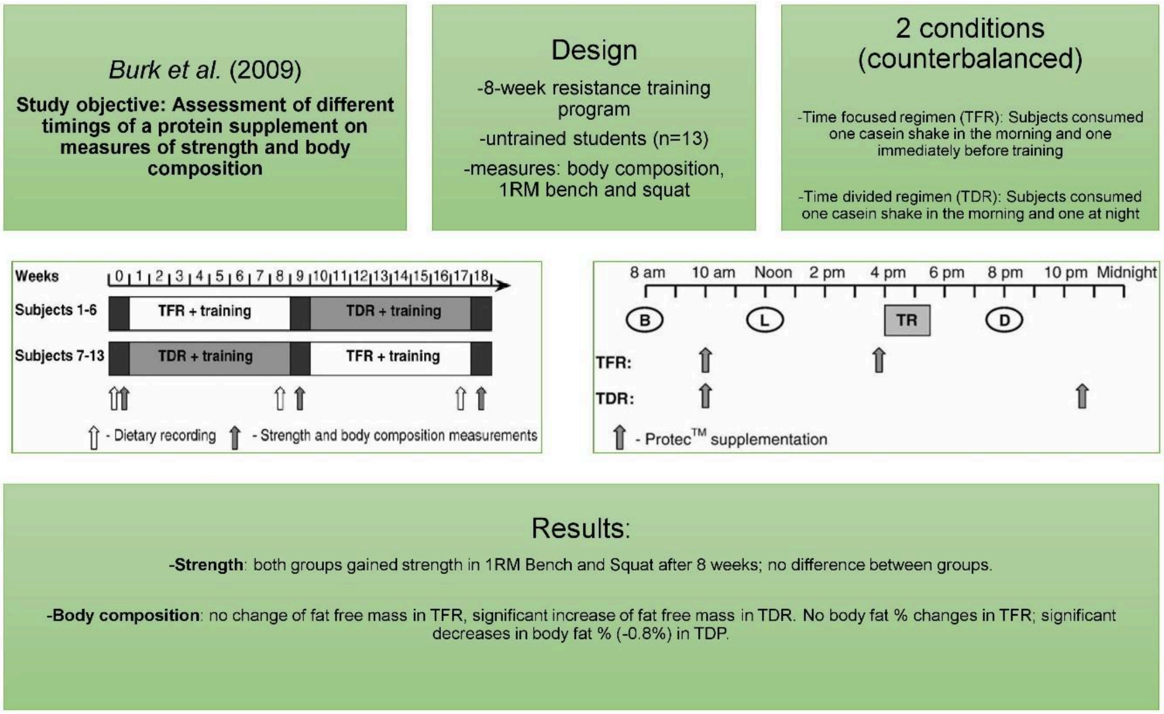
The research design from [Mori (2014)]: trained bodybuilders experienced higher nitrogen balance, a measure of protein balance and muscle growth, in the P0 condition than the P6 condition.

If you train first thing in the morning on Monday, Wednesday and Friday, you will probably experience much of your net positive protein balance during those 3 days of the week and it's particularly important to consume at least 1.8 g/kg (0.82 g/lb) protein per day on those days.

Protein synching

We can take the idea of post-workout nutrition a step further and apply it to the rest of the anabolic window: by synchronizing our protein target with the potential for muscle protein synthesis, we should be able to maximize MPS. If you train later in the day, your protein requirements will be higher in the period of the day after the workout than before the workout, as you'll be in the anabolic window in the later part of the day. Several studies find positive effects of increasing protein intake in the post-workout part of the day compared to the pre-workout part of the day.

Burk et al. (2009) used a long-term cross-over design to compare 2 groups of men performing serious resistance training and eating a diet containing more than sufficient protein. The total daily macronutrient intakes and workouts were the same in both groups: the only difference between the groups was the timing of a protein supplement containing 70 grams of protein. One group received it 8 hours after awakening, closely before their workouts, and the other group received it 90 minutes before going to sleep. The group receiving the protein later in the day gained significantly more muscle mass than the group receiving it at midday and lost fat to boot. Pre-bed protein intake was thus more beneficial than pre-workout protein intake. See the infographic below for more details if you're interested.



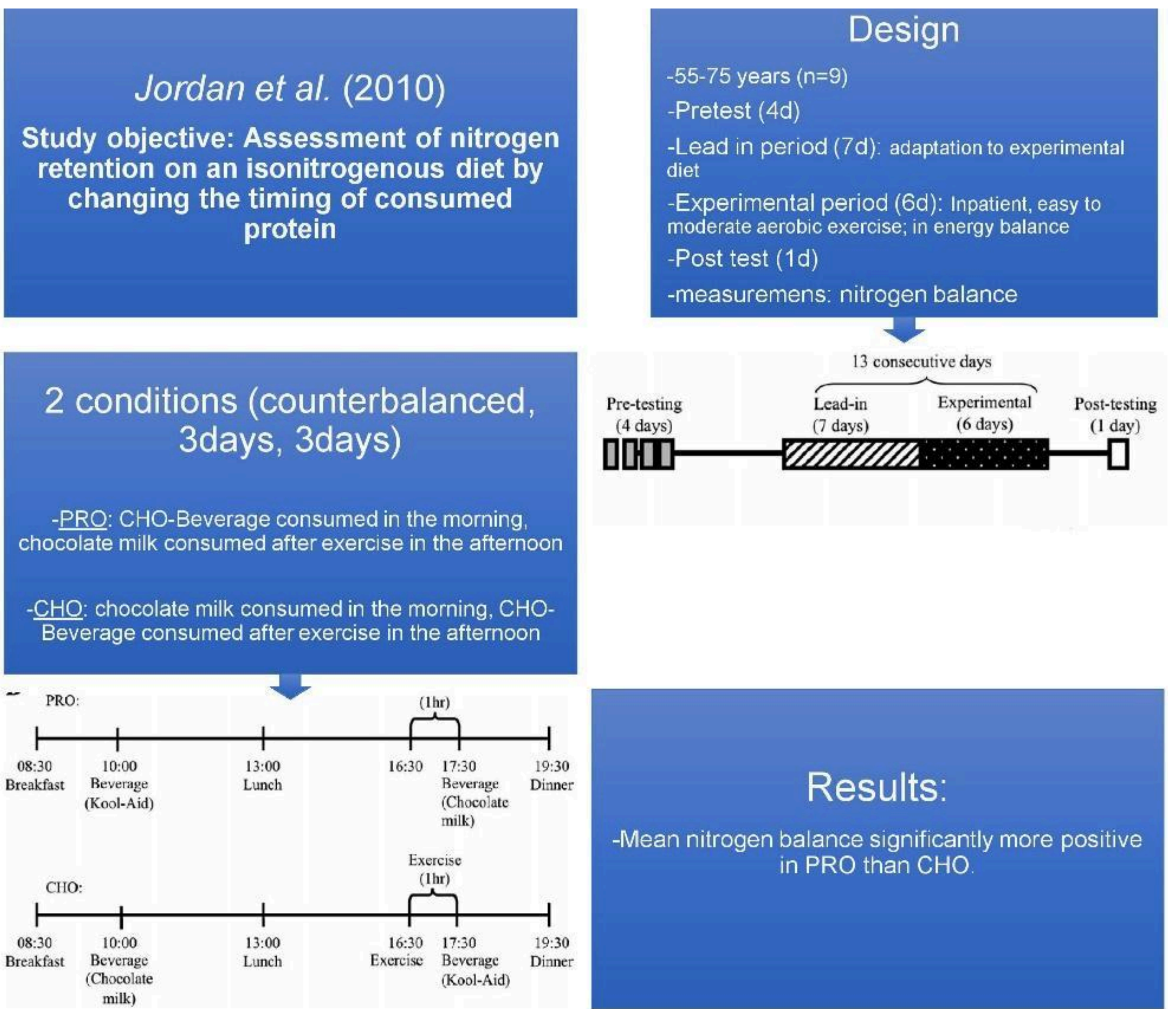
[Keim et al. (1997)](#) used a long-term cross-over design to compare two groups on a weight loss diet in a metabolic ward where they could control all food intake. The only difference between the groups was when they consumed most of the food. One group consumed 70% of their total energy intake in the AM and the other group consumed 70% of their total energy intake in the PM. Both groups got up before 08:00 h. Both groups performed the same exercise program:

- 30-54 minutes of treadmill or cycling cardio at 65 – 70% of $\dot{V}O_2max$ in between 09:00 – 11:30 h 5x per week
- a whole-body strength training circuit also in between 09:00 – 11:30 h 3x per week
- outdoor walking at 4.8 – 6.4 km/h in between 13:30 and 14:30 h every day

The PM group had better muscle retention during the diet and a higher reduction in body fat percentage than the AM group. See the infographic below if you're interested in more details.

That's 2 well controlled studies in favor of eating much of your daily protein after instead of before your workout. However, these studies cannot tell us if it was the energy or just the protein timing that caused the positive effects.

Enter [Jordan et al. (2010)](). These white-coat-wearing amigos compared 2 groups receiving a carbohydrate and a protein beverage at breakfast and post-workout about 10 hours after waking up. They measured nitrogen balance, a measure of daily protein balance and lean tissue growth. One group received the carbs at breakfast and the protein later in the day and the other group received the carbs later in the day and the protein at breakfast. The group receiving the protein later in the day had a significantly more positive nitrogen balance than the group receiving the protein at breakfast. Because the study authors controlled for carb and therefore energy timing, only the protein timing could have caused this difference. The most remarkable thing about this study is that the protein beverage only contained 15 grams of protein in a diet containing more than sufficient protein and still they found a significant effect. See the infographic below if you're interested in more details.



Roy et al. (2002) found that consuming a protein-carb beverage post-workout resulted in higher nitrogen balance than consuming it at breakfast. The participants in this study were endurance trained women performing aerobic exercise. Total daily energy and protein intake were the same in both groups.

Several other studies found no effect of protein timing and we can largely explain why based on the training status of the subjects and the timing of their protein intake in relation to their workouts.

- In Joy et al. (2018), strength-trained men experienced similar body recomposition and strength development when supplementing 35 g casein early in the day at least 3 hours before their workout or later in the day before going to bed. Protein intake in the night-time group was already 1.6 g/kg (0.73 g/lb) per day before supplementation, so further supplementation may have been redundant. There was no control group, so we can't say if the additional protein did anything in the first place, regardless of its timing. Dietary nutrient timing and workout timing were uncontrolled. The study also didn't perform a power analysis and had only 13 subjects, meaning the study was likely underpowered to detect any potential small advantage of protein timing in trained individuals.
- In Branco et al. (2020), 30 g whey protein supplementation had similar effects on body recomposition and strength development when consumed directly post-workout in the morning or later in the afternoon. The participants were untrained post-menopausal women, so the additional protein intake fell in the anabolic window for both groups and it makes sense that further timing within the anabolic window was irrelevant.
- Holwerda et al. (2018) found consuming 21 g protein post-exercise and pre-bed did not increase strength development, muscle growth or myofibrillar protein synthesis in elderly men, despite the total protein intake only being 1.2 g/kg

(0.55 g/lb) per day in the placebo group compared to 1.4 g/kg (0.64 g/lb) per day in the supplement group. The seemingly low protein requirements may be related to the low workload of the training program: the elderly participants performed strength training was 3x per week without taking any sets near muscle failure. Also, the diets were not controlled, so it's possible the placebo group was also consuming sufficient protein post-exercise and pre-bed.

- Antonio et al. (2017) found no difference in effect of adding 54 g of casein in the morning or pre-bed. Total protein intakes were already ~1.8 g/kg (0.82 g/lb) per day before the supplementation, so it makes sense that additional protein intake did not have any benefits, regardless of timing. In fact, neither group experienced any body recomposition or strength development in the first place, so we can't compare their gains.
- Chen et al. (2022) found no significant difference in gains between supplementing 25 g whey protein before bedtime or in the morning in untrained men over a 6-week training program. Whey is a fast-absorbing protein that cannot fuel overnight anabolism and untrained individuals have very long anabolic windows, so these results make sense.
- Lak et al. (2024) compared consuming a 25 g protein shake right before and after training compared to consuming one shake 3 h before the workout and the other 3 h after the workout. Total protein (2 g/kg or 0.91 g/lb per day) and energy intake did not differ significantly between the 2 groups. The participants were 31 strength-trained men. After 8 weeks, the difference in protein timing had no effect on the men's gains in strength (on the leg press, chest press, bodyweight rows and training volume) or muscle mass (measured by InBody scans).

In conclusion, trained individuals generally have an anabolic window that peaks within 24 hours. To maximize MPS, they need to consume adequate protein during this period. Specifically, trained individuals should generally consume a disproportionate amount of their daily protein requirement in the period between the workout and bedtime. It's likely advisable to have at least 2 meals during this period, or else to have an early meal the next day.

If you train in the evening, especially if you only have 1 post-workout meal, you should stop thinking in terms of calendar days with your macros. Instead, think in terms of anabolic windows across the week. Each workout triggers an anabolic window: specifically, every time you stimulate a muscle group, you create a 'window potential' for that muscle group. This means that, when your anabolic window is long enough, if you train in the evening, you want to increase your protein intake not just post-workout but also the next morning. So just grouping your nutrition plan into 'training days' and 'rest days' may not always suffice, although it's arguably advanced enough for many recreational trainees. Ideally, you want to think in terms of periods in- and outside the anabolic window, regardless of which days these periods overlap with.

Energy balance

Another factor that may explain why some studies find more effect of protein timing than others is energy balance. The importance of post-exercise protein intake seems to be higher when bulking than when cutting. Minor et al. (2012) found that post-exercise protein supplementation more favorably altered nitrogen balance in energy surplus than in energy deficit, compared to consuming the same amount of protein earlier in the day. When bulking, you can achieve higher levels of MPS, so timing becomes more important to fuel around-the-clock anabolism. Muscle retention is comparatively easy to realize.

Mixed vs. myofibrillar protein synthesis

A limitation of the research we have is that we currently only have data showing that the anabolic window of total muscle protein synthesis rates decreases in length. Myofibrillar protein synthesis rates are more directly relevant for muscle growth than sarcoplasmic or mitochondrial MPS, but we don't have enough data on the time course of pure myofibrillar protein synthesis levels. It fits the theory of muscle growth, however, that the creation of other proteins, like sarcoplasmic and mitochondrial protein synthesis, decreases before the duration of myofibrillar protein synthesis decreases after training. Strength training provides an endurance training effect for novice trainees, but endurance adaptations soon become redundant for pure strength training, long before the rate of muscle growth significantly diminishes. In any case, for the purpose of nutrient timing it doesn't matter which protein fractions are elevated and which aren't: maximum MPS is the goal to facilitate recovery and maximum training progression, regardless of which sub factions this MPS occurs in. We want to fuel all adaptations to our training. Plus, if total MPS has returned to baseline, that should logically imply myoMPS has also returned to baseline, as myoMPS is a component of total MPS.

Meal frequency vs. workout nutrition

Remember the boxer study from the meal frequency topic? Assuming the boxers trained somewhere midday and they were quite advanced, the superior results of the 6-meal group over the 2-meal group may well have been the result of workout nutrition, since they sandwiched their workouts better between meals.

Pre-bed protein

Even without strength training, an argument could be made to consume an extra high protein intake during the latter part of the day [2]. It may help maximize MPS and the night hours may be an inherently anabolic time compared to the daytime, as the body is recovering and producing most of its growth hormone and testosterone.

Alves et al. (2014) found that consuming a high-protein dinner led to better fat-free mass retention with equal fat loss compared to a macro-matched diet with the high protein intake at lunch.

Buckner et al. (2017) found that sedentary individuals who consumed more protein at night had more muscle mass and strength than individuals who consumed the same amount of protein in the afternoon.

In contrast to these findings, remember that multiple studies in exercising individuals found no effect of protein timing pre-bed vs. earlier in the day (see protein synching section). Especially on a rest day, you don't need a large amount of protein in the later part of the day.

Concretely, if you consume over 1.6 g/kg (0.73 g/lb) protein per day and spread that out roughly equally across the day in 3-4 meals, that should generally come close to maximizing MPS. As a contrary example, consuming only 0.2 g/kg (0.09 g/lb) in the last 2 out of 4 meals would probably be suboptimal. Preliminary evidence suggests you need to consume 40 g of protein to maximize nighttime MPS compared to just 20 g at daytime. If you train in the morning or if you consumed a large meal shortly before the last meal of the day, you may not require as much protein. If you already synchronize your protein intake with your anabolic windows, you should generally already allocate extra protein to the last meal of the day, but it may be beneficial to have the last meal of the day be a bit larger still, around 40 g or 0.6 g/kg (0.27 g/lb) protein.

Pre-sleep protein consumption does not compromise the muscle protein synthetic response to protein ingested the following morning, so there's no downside to ensuring you consume a protein serving shortly before bedtime.

In case you're worried about the absorption of food consumed close to bedtime, "protein ingested immediately before sleep is effectively digested and absorbed, thereby stimulating muscle protein synthesis and improving whole-body protein balance during post-exercise overnight recovery" with the result of promoting muscle growth.

Also, protein consumed pre-bed does not seem to impair overnight fat burning [2].

Sleep quality is typically unaffected as well by pre-bed protein consumption, as we'll discuss in the topic on sleep.

Protein timing application

Traditional pre- and post-workout nutrition guidelines were based on a supplement industry fueled idea of a 1-hour anabolic window around your workouts. In reality, the anabolic window is the full process of muscle growth that occurs after a training session. It is formally a delay in the muscle-full effect that creates a window of opportunity for increased protein synthesis. This window can last over 72 hours, but it quite quickly decreases with training experience so that the most major elevation in protein synthesis lasts fewer than 24 hours. As such, to stimulate maximal protein balance and muscle growth, you can synchronize your protein and energy intake with the anabolic window and protein timing becomes more important for advanced trainees. Your workouts should be surrounded by 2 meals with no more than 5 hours in between them and together those meals should have at least 0.8 g/kg (0.36 g/lb) protein, based on the research in bodybuilders from Mori and Cribb & Hayes. In the period between the workout and bedtime, you should consume at least 0.9 g/kg (0.41 g/lb) protein, generally over half your daily requirements. Your last meal of the day may benefit from a higher protein intake in particular, especially if you have a long fast afterwards. Do not restrict your thinking merely into training vs. rest days, however, as the anabolic window can easily span across calendar days. Think in terms of anabolic windows in the week. Protein synthesis is affected by the time since the last workout, not the position of Earth in relation to the sun.

A useful way to design nutritional programs with anabolic window nutrient timing is to think of your energy and protein intakes on a weekly level. In the anabolic windows, in particular the meals in the period in between your workouts and bedtime, we can achieve considerably higher rates of MPS than outside the anabolic window. In novice level trainees, the anabolic windows are multiple days long, so nutrient timing is likely irrelevant, but in more well-trained individuals, we may be able to improve nutrient partitioning by roughly synchronizing our protein intake with your muscle-full ceiling during the anabolic window: consume more protein when MPS can be elevated more.

As a guideline, since the muscle-full ceiling goes from roughly 20 to 40 g of protein in the anabolic window, but there are sharp diminishing returns, meals within the peak of the anabolic window should have a protein intake up to twice as high as meals outside of the anabolic window. Meals outside the anabolic window generally needn't be larger than 0.4 g/kg (0.18 g/lb), whereas those within it may benefit from going up to 0.6 g/kg (0.27 g/lb) or even 0.8 g/kg (0.36 g/lb) if it's the last meal of the day and we really want to maximize the duration of MPS stimulation and MPB suppression. Here are a few examples of protein timing for trained individuals based on the above principles. Energy intake can scale linearly with protein intake, although error margins up to 20% for the energy intake per meal are most likely perfectly fine.

Example of nutrient timing with a 3-meal set-up for a trained individual

13:00 h: lunch with 0.5 g/kg (0.23 g/lb) protein and 30% of energy intake
15:00-16:00 h: strength training session
18:00 h: dinner with 0.6 g/kg (0.27 g/lb) protein and 35% of energy intake
23:00 h: pre-bed meal with 0.7 g/kg (0.32 g/lb) protein and 35% of energy intake
24:00 h: bedtime

Example of nutrient timing with a 4-meal set-up for a trained individual
23:00 h - 08:00 h: sleep
08:30 h: meal 1 with 0.3 g/kg (0.14 g/lb) protein and 20% of energy intake
13:00 h: meal 2 with 0.4 g/kg (0.18 g/lb) protein and 20% of energy intake
15:30-17:00 h: training session
17:30 h: meal 3 with 0.5 g/kg (0.23 g/lb) protein and 30% of energy intake

22:30 h: meal 4 with 0.6 g/kg (0.27 g/lb) protein and 30% of energy intake

If somebody trains early in the morning, they can generally spread their macros out equally across the day, starting with a pre-workout meal.

If you prefer a standardized nutrient timing formula, you can use the tables below to determine what percentage of your daily protein and energy intake you allocate to each meal based on when a strength trainee trains in the day. However, we recommend you create your own method based on your own understanding of the science. In the case study module, you'll see far more elaborate examples of protein timing to give you ideas on how to implement the science into practical programs. Depending on your personal preferences, you may prefer either a more sophisticated and customized implementation or something more simple and convenient. The research that synchronizing your protein intake to your anabolic windows improves your gains is not strong, so you can also opt to simply spread out your protein intake evenly across the day. The results probably won't differ majorly compared to the below set-ups if total daily protein intake is sufficient.

You can also compensate for lack of nutrient timing with excess protein intake. If you consume at least 0.6 g/kg protein in at least 4 meals a day, protein timing is most likely completely irrelevant, because you should maximize MPS throughout the entire day regardless. In this sense, protein timing's main benefit is allowing you to set lower minimum necessary protein intakes per meal.

		Protein and energy intake allocation per meal			
The workout takes place after meal…		**Meal 1**	**Meal 2**	**Meal 3**	**Meal 4**
	1	25%	25%	25%	25%
	2	20%	20%	30%	30%
	3	20%	20%	20%	40%

A standardized method of allocating calories and protein across the meals of the day for a trained individual with 4 meals a day, depending on which of those meals fall in the post-workout anabolic window (shaded in the table).

		Protein and energy intake allocation per meal		
The workout takes place after meal…		**Meal 1**	**Meal 2**	**Meal 3**
	1	25%	37.5%	37.5%
	2	25%	25%	50%

A standardized method of allocating calories and protein across the meals of the day for a trained individual with 3 meals a day, depending on which of those meals fall in the post-workout anabolic window (shaded in the table).

Practical applications

Protein is arguably the most important macronutrient (remember the word literally means this?), so you learned a lot of theory in this module. However, the practical applications are not difficult (although you can make protein timing complicated if you want to).

- Assuming you get at least 50% of your protein from high-quality sources, most people don't need more than 1.8 g/kg (0.82g/lb) of protein per day for body recomposition or strength development, but the following populations may require more.
    - Anabolic-androgenic steroid users: 2.1-3.7 g/kg (0.95-1.68 g/lb) per day.
    - Vegans: 2.3 g/kg (1.05 g/lb) per day with heavy reliance on an 80:20 pea-rice protein supplement.
    - Trainees with muscle memory and concurrent athletes: 2.2 g/kg (1 g/lb) per day.
- There are generally no adverse health effects of consuming such protein intakes, even though some blood and urine biomarkers may be elevated in strength trainees on high-protein diets compared to sedentary individuals.
- This protein intake should be spread across at least 3 meals a day for maximum muscular development.
- Every meal ideally contains at least 0.3 g/kg (0.14 g/lb) protein to cover the leucine threshold and maximize MPS over the whole day.
- Protein intake can be spread equally across the day in novice trainees, but more advanced trainees may benefit from synchronizing their protein intake over time roughly with their anabolic windows. As a rule of thumb for trained individuals, allocate 50% more protein to any meals in the post-workout

period, or 100% if there's only a single meal in between the workout and bedtime.

- Fasted training is inadvisable: you should sandwich your workouts in between a 5-hour inter-meal interval for maximum performance and gains. For example, if you train at noon, 12:00 h, and your pre-workout meal was at 10:00 h, you should have your post-workout meal no later than 15:00 h.