Leucine's General Effects on Muscle Growth and Protein Balance

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Abstract

Leucine appears to be a powerful modulator of protein balance, perhaps accounting for 80 % of the differences in protein synthesis after consumption of a mixed meal. The purpose of this paper will be to provide a more in depth look into what leucine's overall role is in muscle growth. Specifically the paper discusses (1) how leucine effects protein synthesis, (3) protein breakdown (3) how a leucine rich diet can swing protein balance into a positive state for the majority of a 24 hour period (5) the amount of leucine that can be consumed in a single setting (6) and finally what the role of other amino acids are in working with leucine to support the process of protein synthesis.

Introduction

> *Go stand in your kitchen for a minute. Look around, you are completely surrounded by the most anabolic substance in the world. You can almost feel its power as if the cabinets were about to explode. For if you consume the correct amount of macronutrients you will undoubtedly experience a surge of muscle growth that you never imagined possible.*
>
> *Quote – Wilson J. 2000*

Over six years ago I started abcbodybuilding.com, and introduced what was then known as "Beyond Failure Magazine." The quote above is from the first issue ever released. It's interesting to note how I stressed consumption of the correct amount of macronutrients, because six years worth of magazines / journal productions latter I find myself submitting a paper which attempts to go beyond correct macronutrient ratios. In fact the purpose of this article is to separate the precise components intrinsic to those macronutrients that science suggests are directly responsible for muscle growth.

It has been known for some time that a mixed meal stimulates protein synthesis. However after substantial research it appears that over 80 % of the stimulatory effect seen from a meal can be attributed to amino acids (Rennie & Wolfe, 2002), particularly the amino acid leucine (Garlick, 2005). In a classic study to investigate the components responsible for protein synthesis, Garlick and Grant (1988) fed rats glucose, glucose with both essential (EAA) and non essential amino acids (NEAAs), and glucose along with just the branched chain amino acids (BCAAs). Briefly, NEAAs are amino acids (aas) that can be synthesized in the body, while EAAs cannot be produced by the body and must be provided by the diet (for an depth reviews of these concepts see Wilson, 2005, Wilson, 2006, and Wilson & Wilson, 2006). BCAAs are three special cases of the Essential Amino acids. Each Branched chain amino acid contains 'side chains' comprised of one carbon atom and three hydrogen atoms (CH_3). These are known as a methyl groups.

In the study it was found that rats consuming glucose alone did not increase protein synthesis. What was interesting was that all three of the conditions containing amino acids increased protein synthesis equally. This suggested that the BCAAs played a critical role in protein synthesis. As a follow up the authors performed an experiment in which they compared a mixture of amino acids and glucose to a mixture of all of the amino acids subtract the BCAAs. Results indicated that protein synthesis was not stimulated in the absence of the BCAAs (see figure 1).

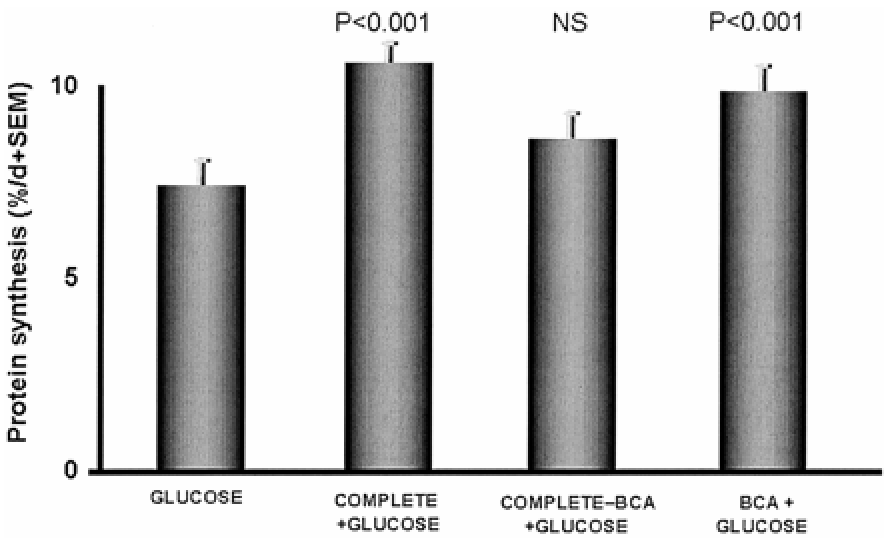

Figure 1. The effects of Branched Chain Amino acids on protein synthesis. Adapted from Garlick (2005)

Perhaps most amazing however were the authors' final experiment. They compared each individual BCAA (leucine, isoleucine, and valine) alone, and to a combined mixture of BCAAs and found that leucine was able to stimulate protein synthesis to the same magnitude as the mixture. No significant increases in protein synthesis were found with isoleucine and valine.

The importance of leucine as the primary amino acid responsible for protein synthesis has been confirmed in numerous studies (Garlick et al., 2005). The purpose of this paper will be to provide a more in depth look into what leucine's overall role is in muscle growth. Specifically the paper discusses (1) how leucine effects protein synthesis, (3) protein breakdown (3) how a leucine rich diet can swing protein balance into a positive state for the majority of a 24 hour period (5) the amount of leucine that can be consumed in a single setting (6) and finally what the role of other amino acids are in working with leucine to support the process of protein synthesis.

How Leucine Effects Protein Synthesis

Protein synthesis can be defined as the constructive process of building new proteins from individual amino acids (Figure 2). This process first requires the information for specific muscular proteins housed in DNA to be copied onto a messenger RNA (mRNA) molecule. Copying of the instructions is known as transcription. Subsequently the mRNA enters into the interior of the cell (sarcoplasm, see Wilson, 2001 for a review of the basic anatomy and physiology of a muscle fiber), and used to construct said protein. This process is known as translation.

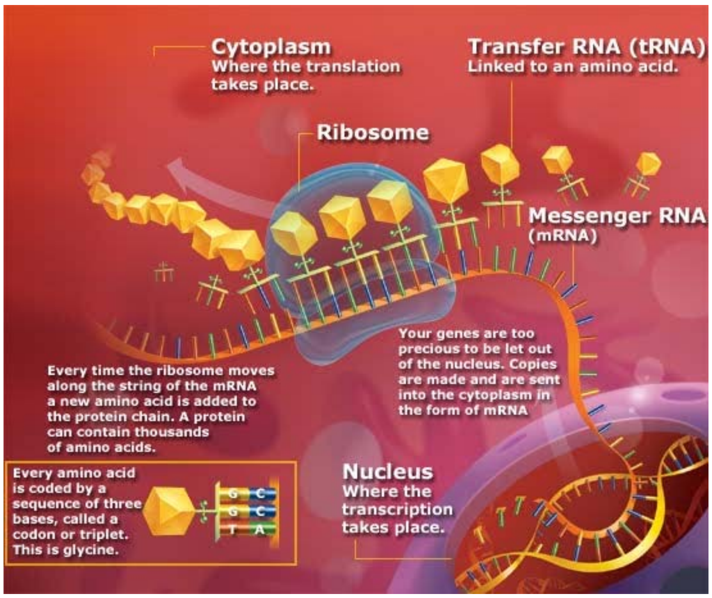

Figure 2. Diagrammatical representation of protein synthesis.

There are three steps to translation, including (1) initiation, (2) elongation, and (3) termination. It is important to note that specific cellular machinery known as ribosomes and their associated proteins (ribosomal proteins) are responsible for production of the protein. In initiation the mRNA containing the instructions for said protein is assembled to a ribosome. During elongation transfer RNA (tRNA) transfers individual amino acids to the Ribosome, and they are linked one after the other until a stop codon is reached and the protein is released (termination).

Immediately following a meal, during, or after exercise individuals have immediate changes in protein synthesis. Evidence suggests that these changes are primarily controlled at the level of translation initiation (Norten & Layne, 2006). The advantage is that initiation is easily manipulated and can be adjusted rapidly in accordance with the basic needs of the cell. For example during fasting, when energy and amino acids are low, the highly energy costly process of protein synthesis is counter to survival; similarly during endurance exercise when energy needs to be partitioned towards contractile activity, the working muscles need to be able to rapidly lower protein synthesis if it threatens the energy supply critical for contractile function (Atherton & Rennie, 2006).

Translation initiation (combining the mRNA to a ribosome) is a process which is guided by a family of proteins known as "Initiation Factors" (eIFs). As would be expected eIFs are sensitive to changes in the surrounding environment, including intracellular amino acid content, availability of energy, and growth factors (eg insulin like growth factor) (Norton et al., 2006) (figure 3).

Essentially leucine stimulates an increase in the concentration of these initiation factors. Increased leucine concentration also stimulates a specific ribosomal protein known as ribosomal protein S6 (S6) (Sans et al., 2006). S6 preferentially increases translation of proteins in the cell responsible for protein synthesis including initiation factors, and other ribosomal proteins. In sum Leucine increases translation and the capacity of the cell to produce new proteins (via S6).

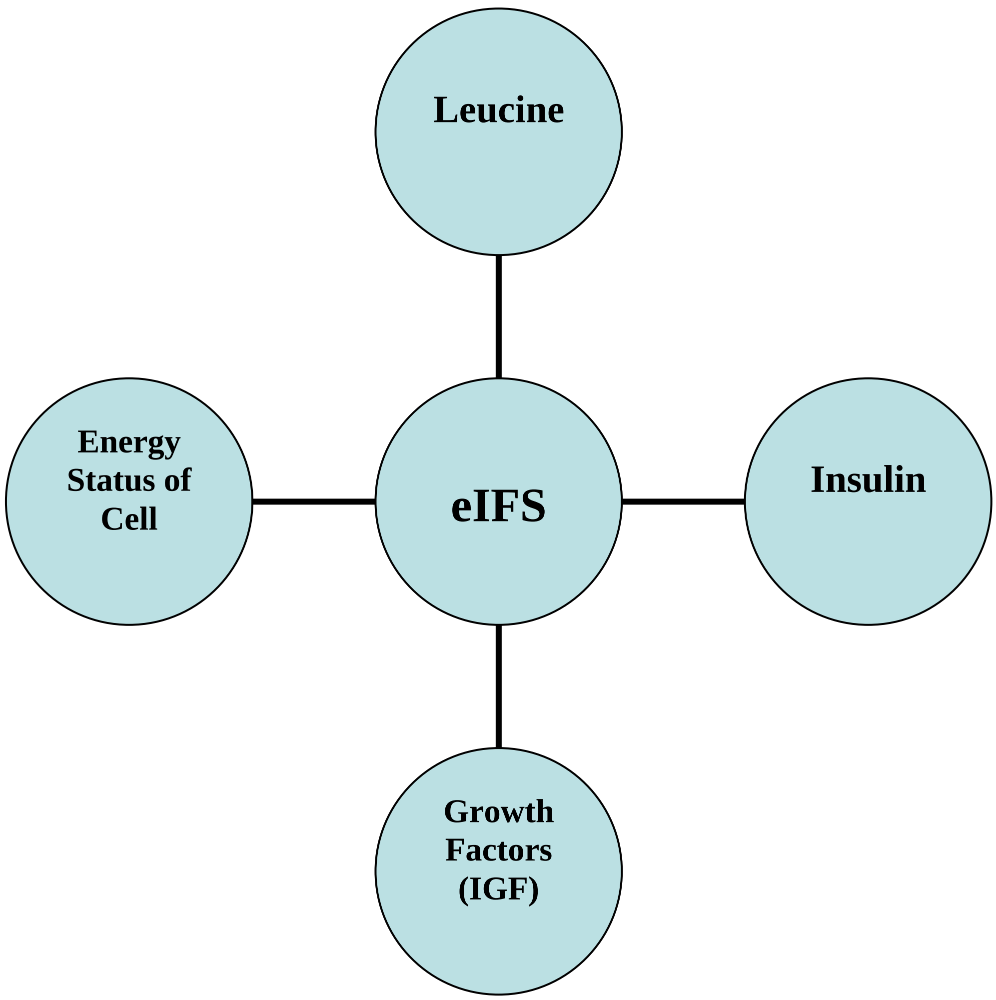

Figure 3. Factors which effect the concentration of eIFS in the cell.

Mammalian target of rapamycin (mTOR)

It is now clear that leucine as well as the factors listed in figure 3 are integrated together either directly or indirectly through Mammalian target of rapamycin (mTOR) (Norton et al., 2006). Briefly mTOR is a protein kinase which is responsible for controlling levels of protein synthesis in the cell. A protein kinase is a molecule which activates or deactivates cellular machinery by adding phosphate groups to the molecule. These phosphate groups therefore act as *molecular switches*.

Evidence for leucine's effects occurring through stimulating the activation of mTOR comes from studies which administer mTOR inhibitors such as rapamycin. These studies show that a great majority of leucine's effects are inhibited when rapamycin is administered (Anthony et al., 2000). However, it appears that leucine is able to still partially increase the formation of key initiation complexes independent of mTOR, suggesting that leucine operates through mTOR dependent and independent mechanisms (Anthony et al., 2000). However, if the mTOR mechanism is hindered than the overall effect on protein synthesis is attenuated.

Summary: Leucine → mTOR → Protein Synthesis → Skeletal Muscular Growth

Leucine's Role in regulating protein degradation

While leucine's role in protein synthesis has received the majority of attention it also has been demonstrated to inhibit whole body proteolysis (Frexes-Steed *et al.* 1992). For example Frexes-Steed et al. (1992) administered insulin (a potent antiproteolytic hormone) and insulin and leucine to fasted animals. They found that insulin alone decreased proteolysis by 40 %, while the addition of leucine decreased it by 80 %. Leucine administration has also been conducted on athletes at extremely high dosages and been found to inhibit proteolysis. For example MacLean et al. (1994) found that 77 mg / kg of bodyweight of BCAAs to participants training at 70 % of their 1-RM in leg extensions decreased protein degradation. In another study participants were administered 12 grams a day of BCAAs for 2 weeks prior to an exhaustive cycling bout. Then markers of muscle damage measured included lactate dehydrogenase, and creatine kinase before, and after the bout. Results found that the BCAA supplementation significantly reduced LDH from 2hrs to 5 d posttest, and CK from 4 hrs to 5 d post-test, indicating that leucine lowered protein degradation. Finally, as will be discussed in the anabolic resistance paper, leucine supplementation is able to reduce protein degradation in the elderly in response to a meal to levels comparable to young participants!

There are a number of rationales for leucines anticatabolic effects. In a recently submitted paper on HMB supplementation, we discussed the possibility that leucine may exert its anticatabolic effects through its metabolite HMB (Wilson & Wilson, in Press). One of the key findings we reported was that leucine's effects on proteolysis have been inhibited in studies where it cannot be transaminated into alpha ketoisocaproate (KIC). KIC is the precursor to HMB. One of the problems with this is that only 2-10 percent of leucine is turned into HMB. However at high dosages, such as 12 grams of BCAAs, it is possible to obtain enough HMB to elicit its anticatabolic effects.

A second possibility is that leucine is actually able to directly inhibit specific proteolytic pathways in human muscle tissue. Briefly, there are a number of pathways responsible for proteolysis or the breakdown of tissue. The pathway which appears to have the greatest effect on muscle tissue breakdown is known as the Ubiquitin-proteasome-proteolytic pathway. Ubiquitin is a protein molecule which is attached to proteins in the cell to be degraded. Once a Ub molecule is attached to a protein, the protein is recognized by specific machinery in a cell responsible for protein breakdown known as the proteasome. This pathway is increased during catabolic states such as fasting, or even many exercising conditions (Wilson & Wilson, in Press). However, studies suggest that leucine administration is able to lower components of the Ub-pathway (discussed in the anabolic resistance paper).

In the paper mentioned above we also discussed extensive evidence, indicating that HMB inhibits the Ub-pathway.

How Diets Rich in Leucine can Stimulate Net Positive Protein Balance

Ultimately muscle tissue growth or loss is a factor of net muscle protein balance. Protein balance can be defined as the difference between protein synthesis and protein degradation (breakdown).

Protein balance = Protein synthesis – Protein degradation

If protein synthesis exceeds degradation you experience growth, which in reality is what the focal point of this journal is. Clearly leucine is able to manipulate both processes, but we will review its overall role in protein balance here, with particular emphasis on practical applications.

During the course of a 24 hour period human beings have a flux of postprandial (following a meal) and post absorptive (fasting) conditions. It is during postprandial states when humans enter into a state of net muscle growth, while in fasting conditions they enter into a state of net muscle catabolism. The results of this are summed in an excellent quote by Combaret and colleagues (2005):

> "*Skeletal muscle protein synthesis decreases in the postabsorptive (PA) state and increases in the postprandial (PP) state, while protein breakdown follows the inverse pattern. In adults,* ***net positive protein balance in the PP state and net negative protein balance in the PA state cancel each other.***

They conclude on exactly how this cancellation process occurs:

> *"In humans protein mass increases in daytime and decreases overnight so that muscle protein mass does not change throughout the day and night cycle*. "

The above statements are true, especially in bodybuilders who during the day consume meals every 2-3 hours throughout the day, with protein sources extremely high in leucine content (see leucine requirements section below). However, what should be understood is that muscle tissue growth is a battle of minute gains over 24 hour periods, which chronically add up to notable increases in muscle mass. If we were to analyze a single day of even optimal protein accretion we would think it appeared negligible and not even worth the effort. However if you add those minute changes over a months time, gains become very notable. This is why it is critical that athletes never view small changes as negligible, because in reality bodybuilding is a game of inches, which overtime add up to significant gains. Because gains on a daily basis are so small, even a 3-4 hour period of time in a 24 hour period can mean the difference between net gain, maintenance, or even a loss of muscle tissue during a day! For example, Esmarck et al. (2001) administered protein either immediately after training or 2 hours after training in a resistance training study. After 12 weeks time only the immediate group had gained muscle tissue. Those two hours meant a great deal.

I am suggesting that for the bodybuilder the nighttime sleeping period is one of the most critical to manipulate for optimal gains. The night sleeping period is a time in which movement is slowest and the potential for growth is optimized. The first step to taking advantage of the night period is the pre bed meal, which should consist of a slow digesting source of protein such as casein or meat. The second phase occurs in the middle of the sleep period when the individual enters into the postprandial state. During this time, protein synthesis lowers. Research indicates that protein synthesis can lower with a range of 15-55 % after an overnight fast (Norten et al., 2003; Svanberg et al., 1996)! Further studies suggest that overnight infusion of BCAAs can attenuate the negative effects seen in protein balance (Louard et al., 1995). Without an intervention however the result is net catabolism. If we assume that the average individual who consumes a proper pre bed meal enters into a state of net catabolism for 4 hours per night, the loss does not seem like much, particularly if the individual eats properly for the remainder of the day. However, when added over a year's time this accumulates to approximately 1500 hours or a 60 full days of time! The question each bodybuilder should ask is what is optimal for their program. You be the judge.

60 days of muscle tissue loss or 60 days of muscle tissue growth?

Fortunately evidence clearly demonstrates that even a small amount of leucine corresponding to 3 grams found in 30 grams of whey protein, or a 45 calorie serving of amino acid shooter can completely reverse these negative effects (Layman et al., 2003). This was confirmed in an extensive review by Norton et al. (2006) who found that conditions of lowered protein synthesis (e.g overnight fasting and long duration endurance exercise) are associated with depletion of leucine levels (discussed in detail in the effects of leucine on exercise). Further, when leucine alone or along with a rich protein source is administered these negative effects are completely reversed and the participant enters into a state of positive protein balance (Norton et al., 2006). My recommendations for this meal are given at the end of the paper after leucine is discussed in the context of other amino acids.

How much leucine should an individual consume in a given day

Both protein requirements and individual amino acid requirements are typically determined by the recommended daily allowance (RDA). For both amino acids and protein the RDA is determined by nitrogen balance studies. We discuss the nitrogen balance technique in detail in our publication: Wilson, J. and G.J. Wilson. Contemporary issues in protein requirements and consumption for resistance trained athletes. Journal of the International Society of Sports Nutrition. 3(1):7-27, 2006.

The reader is referred to that paper for an in depth review of the subject. Briefly however protein contains nitrogen. Therefore if the amount of nitrogen leaving the body is equal to the amount entering the body it is assumed that the individual is in a state of zero protein balance (they are neither losing nor gaining proteins). The RDA for protein is approximately 0.8 grams per kg of bodyweight. However we argued (Wilson & Wilson, 2006) that the evidence clearly supports much higher protein intakes for athletes. For example extensive analysis of reviews on the subject demonstrate a range from 1.2 to 2.2 grams of protein per kg of bodyweight daily (2.2 per kg = 1 gram per pound). We also provided evidence that performance may be optimized at even higher levels than this. Ultimately however the papers theme was that a stagnant protein requirement may not be able to be calculated, simply because there are far too many variables involved in a daily period to account for. For this reason we suggested that athletes should attempt to optimize a number of other variables such as the timing of protein intake, and the pattern of protein ingestion (see Wilson, 2006 for practical applications).

The situation becomes even more complex however when attempting to analyze each individual amino acid. This occurs because the RDA analyzes the use of amino acids as substrates for proteins. Recently Layman (2003), a foremost authority on leucine argued that Nitrogen balance was only useful for amino acids such as lysine, with limited use outside of its role as a building block for protein structures. Leucine however not only serves as a substrate for protein synthesis, but also has numerous metabolic roles including direct actions in stimulating protein synthesis (Anthony et al., 2000), decreasing protein breakdown (Frexes-Steed et al., 1992), regulating glucose homeostasis (Layman et al., 2004), effecting insulin signaling (see article 2), as well as a host of other factors. These metabolic roles appear to be proportional to the amount of leucine consumed (Layman, 2003).

The discrepancy can be seen when comparing the RDA of leucine in healthy individuals to studies which measure the amount of leucine used for metabolism in a single day. While the RDA is approximately 1-4 grams per day, its actual metabolic use is estimated conservatively in the upwards of 12 grams per day (Layman, 2006)! These findings led Layman, (2003) to conclude that “a single definition of minimum requirements may not be adequate to encompass the full range of human needs for each of the nine indispensable amino acids.” Instead he suggested that the more recent Dietary Reference Intakes (DRI) scale would be more suitable. Note however that we (my colleague Gabriel and I) are thinking of proposing a new scale, the *Dietary Growth Index*.

According to the DRI concept there are a range of results which can occur from consuming a given nutrient. This range includes the minimum amount needed for survival (RDA), to levels which are toxic to the body. It is thought that with nutrients without a great number of roles or metabolic functions, such as lysine that the nutrient intake or zone in which optimal functioning of the individual is reached is not much beyond the RDA. However in an amino acid such as leucine, whose metabolic roles are proportional to the amount in the diet provided, optimal function may be reached at levels much higher than the RDA.

What that optimal function is, may not be able to be defined. If we analyze leucine based on its role in the stimulation of protein synthesis, we can infer its optimal range from the scientific communities reccomednations on daily protein intake, which ranges from 1.2 to 2.2.grams per kg daily. For a 200 pound man this amounts to 200 grams of protein daily. Leucine on average in high quality animal derived products is about 10 %. Therefore leucine intake on this scale would most likely be approximately 20 grams a day, and as has been stated, authors in peer reviewed literature have argued that nitrogen balance studies may even suggest that leucine should be higher than this.

However, as will be discussed in subsequent articles there are a number of other metabolic roles for leucine which cloud what exactly can serve to optimize performance in an athlete.

The role of other Essential Amino Acids in Stimulating Protein Synthesis

Leucine alone can stimulate protein synthesis without other essential amino acids (Garlick et al., 1988), however these effects appear to be more transient in nature when analyzed over a longer time scale. For example Kobayashi et al. infused leucine, or all of the essential amino acids into skeletal muscle of rats for 4 hours. Leucine was only able to stimulate protein synthsis for 30 minutes, while the full mixture maintained elevation for the entire time period. This suggests that maintaining levels of the other essential amino acids is critical in the capacity for leucine to maintain its effects on protein synthesis.

Further it is important to understand that leucine administration alone actually lowers the concentration of other essential amino acids, particularly the BCAAs isoleucine and valine. The rate limiting step in the breakdown of all three BCAAs involves an enzyme known as Branched Chain Ketoacid dehydrogenase (BCKD). This enzyme is activated by increased concentrations of any of the BCAAs (see article 5 on leucine and fat metabolism). Therefore a disproportional increase in leucine would have the effect of enhancing the degradation of the remaining BCAAs, and therefore lower overall EAA concentrations.

How much leucine is optimal in a single setting?

Evidence seems to suggest that leucine has to be consumed with a mixture of essential amino acids. For example young individuals administered 1.7 grams of leucine in a 7 gram Essential Amino Acid mixture experience increased protein synthesis (Katsanos et al. 2006). When lowering other EAAs and increasing leucine content to 2.8 grams no further increases in protein synthesis are seen. However, when increasing EAAs up to 15 grams, with a leucine content of 2.8 grams, protein synthesis is doubled relative to the 7 gram EAA mixture with 1.7 grams of leucine in

it. Therefore approximately three grams of leucine from a 7 to 15 gram mixture of EAAs is beneficial for protein balance.
However, this did not definitively show what the ceiling actually was. Through there is recent evidence that 10 grams of EAAs is able to maximally stimulate protein synthesis
(Cuthbertson et al., 2005). What is also known is that 40 grams of EAAs do not appear to stimulate protein synthesis more than 20 grams of EAAs, suggesting that the limit lies between 10 and 20 grams, at approximately 3 grams of leucine per bolus.

Finally we can also infer optimal leucine intake based on whole protein intake. Dangdin and colleages (2002) had participants consume 22 grams of whey protein compared to 33 grams of whey protein and found that protein synthesis increased. The whey contained approximately 10.1 % leucine content, meaning the participants consumed 2.2 grams to 3.3 grams of leucine and experienced increases in protein synthesis. However, in reality the investigators actually had administered 0.31 g of leucine in the low condition and 0.48 grams of whey per kg of bodyweight, in the high protein condition (the average protein consumed was 33 based on participants weight in the study). In a 200 pound bodybuilder this would amount to 43 grams of protein or roughly 4.3 grams of leucine. Based on this we have an upper limit of 0.048 grams of leucine per kg of bodyweight per serving for a bodybuilder.

From current evidence we can make the following range of conclusions

1. Most likely an individual will be able to maximally stimulate protein synthesis at approximately 3 grams of leucine in a single serving. Evidence suggests that individuals moving from 1.7 to 2.8 grams of leucine increased protein synthesis when other amino acids were adjusted accordingly.

2. The leucine should be consumed in a supporting EAA mixture. The lower limit of which appears to be 10 grams of EAAs (Cuthbertson et al., 2006). However, taking into account variability and a study conducted by Volpi and colleagues (2001) the range of optimal EAA dosage, given consumption of 3 grams of leucine may increase to 10-15 grams. Because Volpi et al. (2001) did not examine further increases in EAAs we assume that the upper-upper range is somewhere between 15 and 20 grams of EAAs, based on the finding that no further increases in protein synthesis are found between 20 to 40 grams of EAAs.

3. The only study that administered a dosage of protein based on bodyweight that has relevance was conduced by Dangdin and colleagues (2003). They found that a whey protein mixture 0.48 grams of protein per kg of bodyweight stimulated protein synthesis greater than a lower dosage. Based on the above evidence this therefore most likely places an upper limit at around 0.048 grams of leucine per kg of bodyweight per serving, which is roughly 4 grams for a 200 pound bodybuilder. A more conservative number would fall at around 0.04 grams.

Assuming that most high quality proteins such as whey and meats are approximately 10 % leucine then roughly 30 to 40 grams of protein will meet the 3-4 gram leucine standard in a single setting, which is roughly 150 to 200 calories. If consuming an essential amino acid supplement such as the Essential Amino Acid shooter, one serving administers 3 grams of leucine at 45 calories per serving.

Mid Sleep Meal

Based on the above suggestions, meals should consist of protein sources rich in leucine. Individuals may benefit from consuming 3-4 grams of leucine per meal (but note that according to Layman et al., 2003 a minimum of 2.5 grams), which can be found in 30-40 grams of high quality proteins, or 45 calories of the amino acid shooter product by Champion Nutrition (note that the formula was based on the above evidence).
It would also be advantageous to add in anticatabolic agents. Suggestions include 5 grams of glutamine, HMB, and fish oil pills. The Glutamine will assist the liver in producing glucose during fasting, and help spare BCAAs in muscle tissue (Wilson G., 2003). The HMB may directly hinder various proteolytic pathways. Finally the fish oil pills are rich in EPA, which may act to hinder the Ub-pathway (Wilson & Wilson, in Press), as well as provide fuel, thus sparing amino acids for protein synthesis.

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