

# Leucine's Effects and Interaction with Insulin and Muscle Growth

Researched and Composed by Jacob M. Wilson BSc. (Hons), MSc., CSCS

Address correspondence to: [jwilson@abcbodybuilding.com](mailto:jwilson@abcbodybuilding.com)

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## Abstract

*Leucine is known for its capacity to stimulate insulin release. In turn a number of investigators have sought to analyze how much of leucine's effects are mediated by insulin. The purpose of the following paper is to analyze the role that insulin has in modulating leucine's effects on protein synthesis and most importantly how these effects can be manipulated for optimizing protein balance.*

## Introduction

In part one of this series, leucine's general role in protein balance was discussed. In particular it was found that this Branched Chain Amino Acid (BCAA) appeared to be the primary regulator of protein synthesis. It has long been known that leucine has insulinogenic properties (stimulates insulin secretion). Currently the three major components of protein balance studied include an analysis of leucine, insulin, and exercise. The following paper will discuss the role that insulin has in modulating leucine's effects on protein synthesis and most importantly how these effects can be manipulated for optimizing protein balance.

## Leucine and Insulin Secretion

Studies from Anthony and colleagues (2000a, 2000b) have found that leucine administered alone to fasting rats can stimulate protein synthesis and the major machinery responsible for protein synthesis (e.g. initiation factors and ribosomal protein 6, see article 1 for an explanation) absent of other macronutrients (e.g. carbohydrates & fats). Further Anthony et al. found that the administration of leucine in both studies did not elicit an increase in insulin secretion. While these findings suggest that leucine enhances protein synthesis independent of insulin, the situation is a bit more complicated than it appears. This is due to the fact that other studies have reported that leucine administration can stimulate insulin secretion (Greiwe et al., 2001; Koopman et al., 2005). Anthony et al (2002) therefore suggested that leucine may enhance insulin secretion early and only transiently after administration, which leads to enhanced protein synthesis. An alternative role is that lower levels of insulin may play a permissive role in leucine's effects.

To further analyze the role of insulin in modulating leucine's effects, Anthony et al. (2002) administered leucine to food deprived rats along with somatostatin, which inhibits the secretion of insulin from the pancreas. Without somatostatin results indicated that leucine transiently increased insulin secretion between 15 and 45 min,

followed by a return to basal levels by 60 minutes. During this process all of the markers of enhanced protein synthesis were increased (e.g initiation factors, and S6). When somatostatin was administered leucine's effects on ribosomal protein S6 (the protein responsible for enhancing the protein synthetic capacity of the cell) was completely inhibited. Further leucine's effects on enhancing initiation factor assembly was partly, but not fully inhibited. A number of studies have further investigated the role of insulin needed to maximally facilitate leucine's effects on protein synthesis. Administration of insulin antibodies, which cause a rapid fall in plasma insulin either partly or fully inhibits leucine's effects on protein synthesis (Preedy, 1986; Svanberg et al., 1996), while Diazoxide administration, which blocks insulin can lower protein synthesis to values below fasting despite being fed a complete meal (Balage, 2001). These results suggest that leucine has both insulin dependent and independent effects on protein synthesis.

### The amount of Insulin Needed to Maximally Stimulate Protein Synthesis

Perhaps the most relevant studies for the amount of insulin needed to maximally stimulate protein synthesis are found in studies analyzing leucine administration in diabetics. Studies indicate that in mildly diabetic conditions, when insulin is only slightly lower than controls that leucine and other anabolic stimuli are still able to maximally stimulate protein synthesis (Kimball et al., 2002). However, in severely diabetic rats, when insulin is extremely low protein synthesis is attenuated, and even lowered (Kimball et al., 2002).

An interesting and classic study was performed by Fedele et al. (2000) to investigate the amount of insulin needed to promote the full benefits of exercise's stimulatory effects on protein synthesis. While this is slightly different than leucine administration, both appear to operate by up regulating the mTOR system. The results are displayed in figure 1.

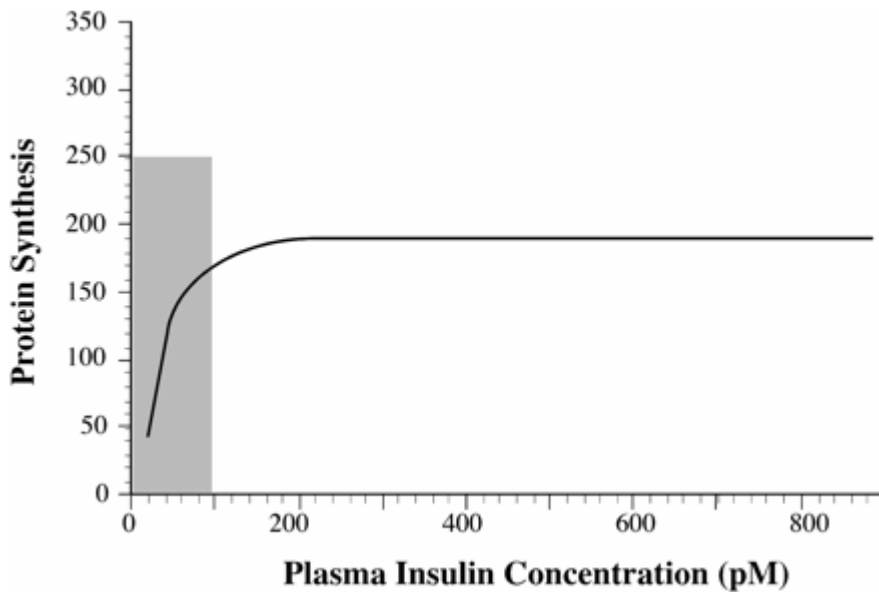


Figure 1. Plasma insulin levels and their effect on protein synthesis.

As can be seen protein synthesis is maximally stimulated at low plasma insulin levels which correspond to fasting conditions.

In summary results from diabetic models suggest that insulin plays either a direct or passive role in maximizing the effects of both leucine and exercise on protein synthesis. However this effect is maximally stimulated at very low physiological concentrations corresponding to fasting levels of insulin.

### **Insulin's capacity to stimulate protein synthesis independently of Leucine and Other Amino Acids**

Insulin's capacity to stimulate protein synthesis has been a confusing problem for some time. Wolfe (2000) summarizes by stating that

"In vitro studies document a stimulatory effect of insulin on muscle protein synthesis, but in vivo results are conflicting. Everything from decreased muscle protein synthesis to increased muscle protein synthesis in response to insulin has been reported. A recent publication suggests that the response of muscle protein synthesis to insulin is dose dependent, and that only supraphysiological dose of insulin stimulate muscle protein synthesis. On the other hand, some studies show a stimulatory effect of insulin in low doses."

However the situation may be able to be resolved when amino acid availability is considered (Wolfe, 2000). Evidence suggests that when amino acid levels are maintained that insulin infusion can increase protein synthesis. An efficient techniques to measure this is by infusing insulin systemically (e.g. to the whole body) or locally in the arm region for example. Systemic increases in insulin without amino acids lower plasma amino acid concentrations, while local infusion does not (amino acid concentrations are maintained).

In an intriguing study Bell and colleagues (2005) infused glucose, lipids, and insulin in a high energy (162 kcals per hour) condition systemically to a group of 6 participants. They then had a low energy condition (35 kcals per hour), in which insulin was infused locally to the thigh region (specifically to the femoral artery). Results found that the high energy condition lowered protein breakdown, but did not affect protein synthesis. In contrast the low energy condition had no change in protein breakdown, but increased protein synthesis. Overall the high energy condition had a net negative protein balance, while the low energy condition had a net positive protein balance (figure 2). This suggests that the maintenance of amino acids is critical for insulin to be able to exert any effects on protein synthesis, but it is not necessary for insulin to decrease protein degradation (discussed further on in the article).

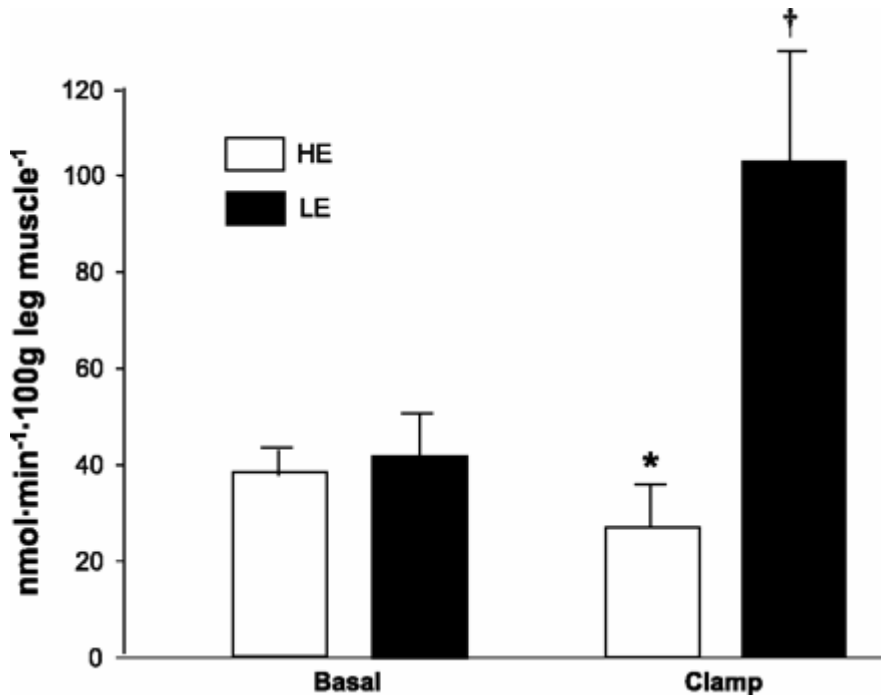


Figure 2. Protein synthesis in low and high energy conditions.

### Insulin's Mechanisms of Action and Synergistic Effects with Leucine

Like leucine, insulin's actions appear to be mTOR dependent (see article 1). Specifically it is thought that insulin binds to its receptor, which leads to downstream events that activate Protein Kinase B. Protein Kinase B then activates mTOR, leading to up regulation of initiation factors and ribosomal protein S6 (see article 1).

In an interesting study Greiwe et al. (2001) infused insulin alone, leucine alone, or insulin + leucine to human participants. The investigators then measured 70-kDa ribosomal protein S6 kinase (p70<sup>S6k</sup>). p70<sup>S6k</sup> is the protein kinase that mTOR stimulates, which activates ribosomal protein S6. So as not to complicate the situation however, I should again stress that measures of either p70<sup>S6k</sup> or ribosomal protein S6 are really measures of the cells capacity to enhance protein synthesis. Results can be found in figure 3.

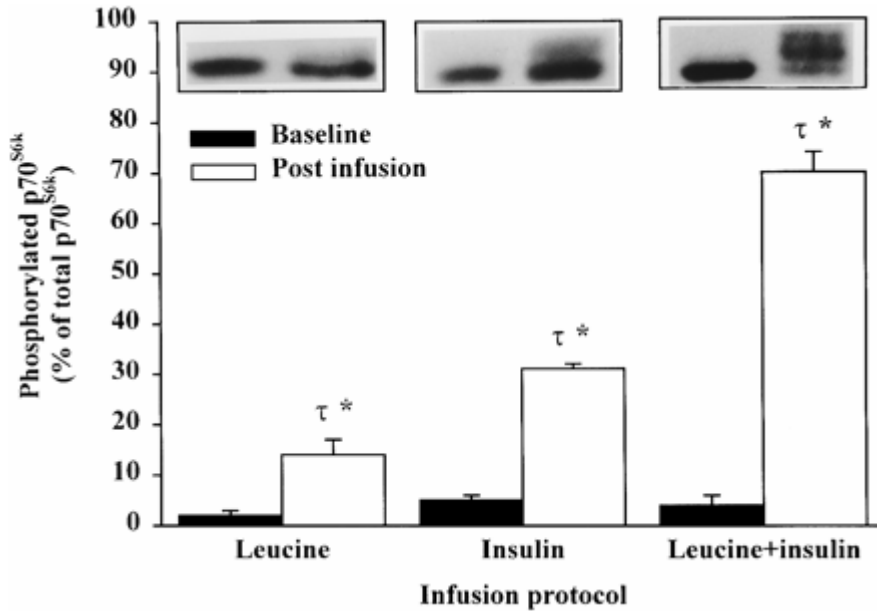


Figure 3. Level of phosphprylated p70<sup>S6k</sup>

As can be seen, leucine alone increased the phosphorylation (a measure of activation) of the protein kinase 4 fold, while insulin increased it 8 fold. If their effects were additive then we would expect a 12 fold increase in the combined condition. However, instead an 18 fold increase was found. This means that their combined effects were greater than their effects alone.

There are a number of explanations which can be derived from these results. First the investigators analyzed to see if insulin and leucine operated through the activation of protein kinase B. Results found that only insulin stimulated protein kinase B, suggesting that they operate through independent mechanisms. Secondly plasma insulin concentrations were measured in each condition. Here results indicated that leucine alone did not stimulate insulin, while leucine combined with insulin raised insulin in the plasma by 20 %. However it was found that the increase in plasma insulin did not further increase activation of protein kinase B, meaning that the rise in insulin may not explain the synergistic effects seen.

However the synergistic effects seen may be related to insulin's capacity to increase blood flow and amino acid availability to the musculature. This was supported by a 2006 study by Volpi and colleagues. These investigators used the local infusion technique of insulin. They postulated that insulin could not stimulate protein synthesis unless it increased amino acid delivery and thus availability to muscle tissue. They had low, intermediate, and high insulin conditions. They found no increases in amino acid delivery in muscle tissue in the low condition. However blood flow and amino acid delivery increased in the moderate condition as well as protein synthesis. In the high condition amino acid concentration began to lower and protein synthesis was not as pronounced. Finally correlations between changes in blood flow ( $r = 0.79$ ) and amino acid delivery ( $r = 0.80$ ) and changes in protein synthesis were both high, supporting the authors hypothesis.

Returning to the Greiwe et al. (2001) study, it is important to emphasize that leucine administration alone did not increase plasma insulin levels, yet when combined with insulin leucine did increase plasma insulin concentrations. One possible explanation is that leucine may have inhibited the degradation of insulin. Because degradation (specifically receptor mediated degradation) is the primary mechanism to clear insulin, leucine's anticatabolic effects may have enhanced insulin availability. It would be interesting to research the possibility of HMBs capacity to enhance insulin levels.

### Insulin decreases protein degradation

While leucine appears to be the major dietary regulator of protein synthesis, insulin appears to be the major regulator of protein degradation. Studies indicate that insulin's effect are dependent on blood flow to muscle tissue. Briefly, muscle tissue receives blood slowly during rest relative to the liver and kidneys, but during exercise this can increase up to 30 %. This increase in blood flow has been demonstrated to be significantly correlated to enhancing positive protein balance (Wilson & Wilson, 2006).

However recently the effects of insulin and other hormones have been analyzed in their capacity to enhance blood flow to skeletal muscle tissue. Liu et al (2006) found that insulin, IGF-1, and GH infused locally all significantly increased blood flow to skeletal muscle tissue (figure 4). Further, along with an increase in blood flow insulin and IGF-1 increased protein balance. What was interesting is that they appeared to do so through different mechanisms. Insulin did not increase protein synthesis, but greatly lowered protein breakdown, while IGF-1 had opposite effects.

The mechanism is that insulin is able to hinder proteolytic pathways through protein kinase B (specifically the Ubiquitin pathway discussed in article 1). The investigators also found that administering insulin at increasing concentrations (low, moderate, and high) increased protein balance from low to moderate, but not from moderate to high.

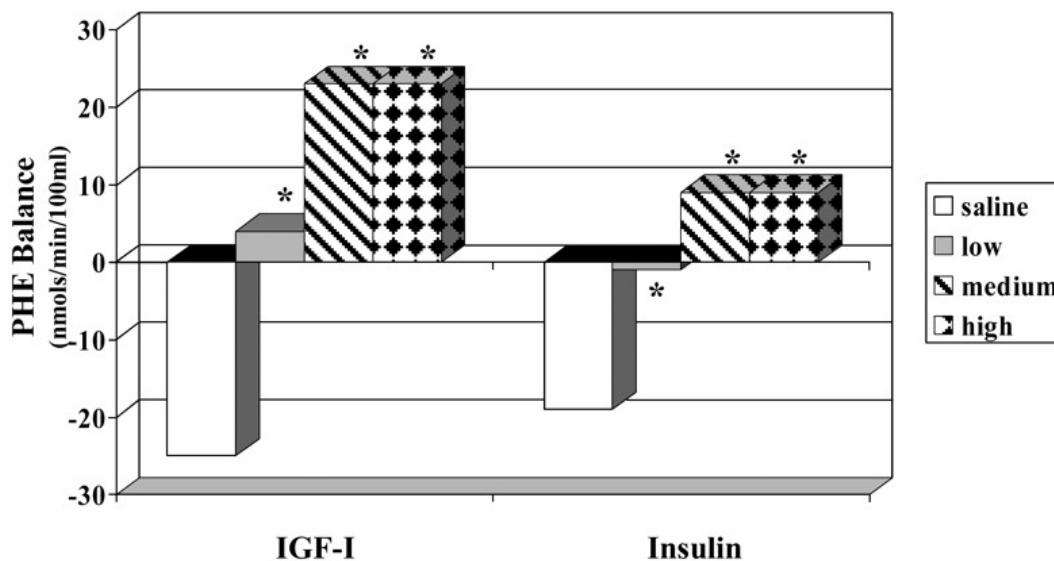


Figure 4. Protein balance at low, moderate, and high levels of insulin and IGF-1.

The low, moderate and hi conditions corresponded to increased insulin levels at ~20, 60, and 110  $\mu\text{U}/\text{mL}$  above fasting levels.

It is interesting as a recent study conducted may further shed light on the insulin protein balance relationship. The study was conducted by Koopman et al. (2005), and they had participants consume three different beverages after exercise and analyzed their effects on protein synthesis, degradation, and net protein balance. The first beverage was comprised of a 0.3 grams of dextrose and maltodextrin combination per kg of bodyweight (CHO condition). The second beverage was the CHO combo plus 0.2 grams of whey protein per kg of bodyweight. Finally in condition three participants consumed the CHO-PRO drink with 0.1 grams of leucine added per kg of bodyweight. For a 91 kg bodybuilder (200 pounds), this would correspond to a beverage of 27 grams of carbohydrate, 18.2 grams of whey, and 9.1 grams of leucine per hour for approximately 330 minutes following exercise. Results found that protein balance was negative in the CHO condition, became positive in the CHO-PRO condition, and was greatest in the CHO-PRO-LEU condition.

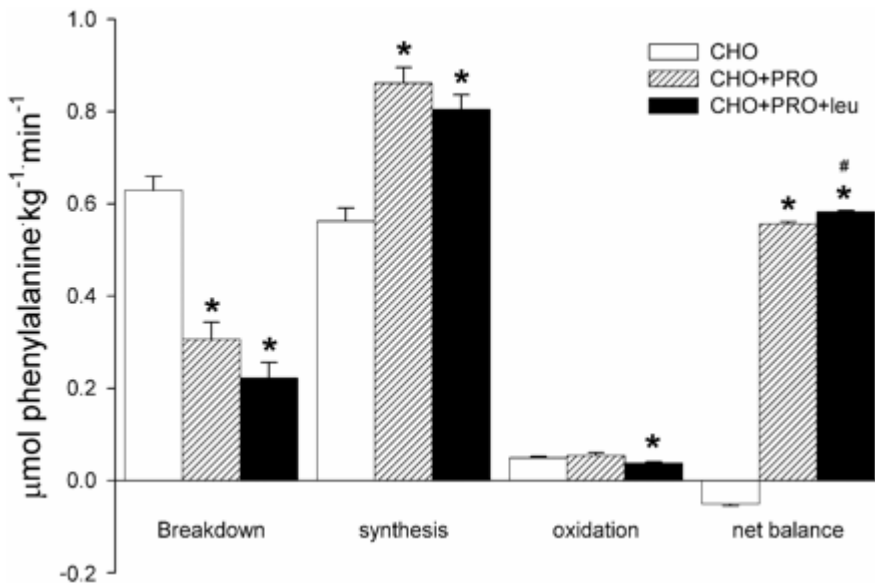


Figure 5.0 Net Protein Balance, Adapted from Koopman et al. (2005)

What was interesting however is that these results paralleled increases in both leucine and insulin concentrations. It was found that leucine was significantly correlated to protein synthesis, while insulin was not. Further insulin was inversely correlated to protein breakdown (more insulin less breakdown), while leucine was not. Finally, when analyzing the insulin response in figure 6, it can be seen that the greatest protein balance was found when insulin levels peaked at around 60-80  $\mu\text{U}/\text{mL}$ .

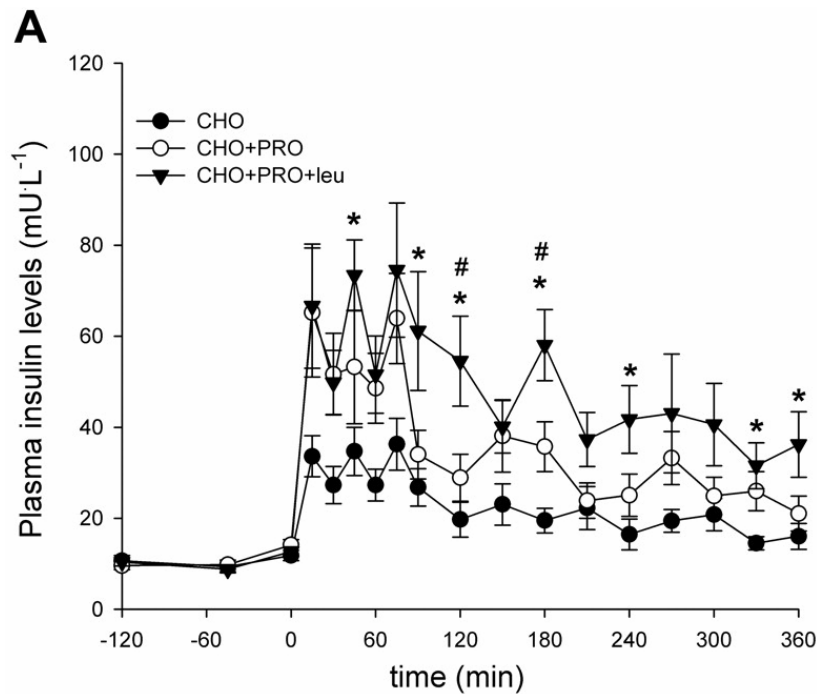


Figure 6. Protein balance as a function of insulin concentration. Adapted from Koopman et al. (2005)

Further discussion as to the optimal dosage of insulin to enhance protein balance is beyond the scope of this paper, but will be addressed in a future series on insulin, and body composition (currently a work in progress for the journal).

What it does demonstrate however is the relationship between positive protein balance and increasing insulin levels, along with the role that leucine plays in regulating this response.

## Summary and Conclusions

Leucine appears to affect the capacity of a cell to increase protein synthesis in both insulin independent and dependent mechanisms. However, in order for these effects to be manifested via significant increases in protein synthesis, both dependent and independent mechanisms must accompany each other.

Leucine and insulin's effects appear to be independent of each other, and when both insulin and leucine levels increase they have a synergistic effect on pKS6, a marker of the cells capacity for protein synthesis. The mechanism appears to be related to insulin's capacity to increase blood flow and amino acid deliver to skeletal muscle tissue.

While insulin's effects on protein synthesis can occur at low levels, its effects on protein degradation appear to increase in hyperinsulemic conditions as indicated by post workout nutrition studies, which show a continual decrease in protein degradation to increasing levels of insulin. Studies also indicate that adding leucine or having a large dose of leucine in a protein containing meal enhances the insulinogenic response to carbohydrates (for an in depth review see Knowlden,

2003). This suggests that higher glycemic index carbohydrates combined with leucine rich protein sources, or protein combined with EAAs for caloric efficiency may be optimal for protein balance post exercise.

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