The Role of Leucine in the Regulation of Protein Metabolism$^{1,2}$

Peter J. Garlick$^3$

Department of Animal Sciences, University of Illinois, Urbana, IL 61801

ABSTRACT Studies both in vivo and in vitro have shown that leucine at a very high dose can stimulate muscle protein synthesis, an effect that is enhanced in vivo by insulin secreted in response to the leucine dose. High leucine can also inhibit protein degradation in skeletal muscle, as well as in liver. In contrast, at normal physiological levels, increasing leucine concentration by infusion stimulates muscle protein synthesis by enhancing its sensitivity to insulin. It is concluded that the role of leucine in vivo is to provide a signal that amino acids are available, which in combination with the signal of energy availability from insulin, stimulates muscle protein synthesis.


KEY WORDS: • leucine • isoleucine • valine • branched-chain amino acids • protein synthesis • insulin • muscle

In the 1970s, a number of laboratories were performing in vitro investigations of the factors that control protein turnover in tissues. Among these factors were hormones, e.g., insulin, and the substrates for protein synthesis themselves, amino acids. These studies showed that high concentrations of all the amino acids stimulated protein synthesis and inhibited protein degradation, most notably in skeletal muscle (1–3) but also in cardiac muscle (4). In particular, it was shown in heart that the stimulation by amino acids could be reproduced with only the 3 branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) (5), whereas in isolated diaphragm muscle, leucine alone, as well as the complete amino acid mixture, stimulated protein synthesis (2). This group of studies, from several laboratories, initiated a continuing series of investigations into the role of leucine in the control of tissue protein mass, its mechanism of action, and its possible value for enhancing muscle protein deposition in healthy subjects or for moderating muscle protein loss in catabolic states.

Attempts to demonstrate the effects of leucine in vivo

On the basis that if leucine stimulates muscle protein synthesis and inhibits degradation, then leucine supplements might be effective in limiting protein loss in human patients with pathological conditions, the potential for supplementary leucine to improve protein balance during fasting was examined in several laboratories. Infusion of leucine or keto acid analogues of BCAA into fasting patients was shown by several groups to improve nitrogen balance (6–8), suggesting that leucine might indeed spare body protein. However, this effect did not appear to result from an improvement in protein balance in skeletal muscle of fasting subjects, because the leg outflows of the amino acids phenylalanine and tyrosine were not altered in subjects with femoral arterial and venous catheters (9). These amino acids are not metabolized in skeletal muscle, and so their outflow is a indicator of net negative protein balance in the tissue. Nonetheless, these early results in humans suggesting that BCAA supplementation could moderate the protein loss that occurs in many pathological states has led to a large number of studies of their effectiveness in patients suffering from conditions such as sepsis and trauma, as well as for improving muscle function in athletes.

Studies of the effects of leucine or BCAAs in intact rats were inconclusive. Buse et al. (10) injected leucine plus glucose plus insulin into starving rats and observed a stimulation of muscle polyribosome aggregation, which was indicative of an increase in muscle protein synthesis. However, in a series of studies on growing rats injected with leucine alone (1 mmol/kg), no changes in protein synthesis in gastrocnemius muscle, heart, jejunal serosa, jejunal mucosa, or liver were detected (11). In these studies, protein synthesis was measured by intravenous injection of a flooding dose of $[^3]$Hphenylalanine (12), followed by killing of the rats after 10 min; leucine (or saline) was injected together with the isotope. Separate groups of rats were either fed, food deprived for 2 d, or given a protein-free diet for 9 d, but in no group was a change in muscle protein synthesis detected. In an additional experiment designed to show whether a longer period than 10 min was required to demonstrate a change in protein synthesis, 2-d food-deprived rats were injected with leucine intraperitoneally, and protein synthesis was measured after 30 min. As before, no change was detected. Overall, these studies showed...
that administration of leucine at a dose of 1 mmol/kg (resulting in plasma concentrations of ~1 mmol/L; about 8-fold higher than that in fed rats) in growing rats had no detectable effect on muscle protein synthesis.

**Leucine and the response to feeding in muscle of growing rats**

In the natural state, leucine is not given alone but is provided as part of a meal and so is accompanied by a balanced mixture of other amino acids and increased glucose and insulin concentrations. Measurements in young rats (~100–150 g body weight) have shown that muscle protein synthesis is stimulated by the intake of nutrients, either intragastrically or intravenously (13). Intravenous infusion of insulin, plus glucose to prevent hypoglycemia, also stimulated muscle protein synthesis in food-deprived rats, but only when the plasma insulin concentration was raised above that normally seen in fed rats (14). Moreover, feeding of rats given anti-insulin serum failed to elevate muscle protein synthesis (13), showing that insulin was essential for protein synthesis to respond. Similarly, infusion of a complete amino acid mixture did not enhance muscle protein synthesis unless, in addition, the insulin concentration was raised by infusion of glucose, leading to the hypothesis that the increase in protein synthesis after feeding resulted from an enhancement of the tissue sensitivity to insulin brought about by amino acids (13). This hypothesis was confirmed by measuring the dose-response of muscle protein synthesis to insulin infusion, in the presence and the absence of infusion of a complete mixture of amino acids [Fig. 1, ref. (15)]. It can be seen from the curves that without amino acids, protein synthesis increased curvilinearly but did not reach a maximum at the highest rate of insulin infusion, which yielded a plasma insulin concentration of ~160 μU/mL. This contrasted sharply with the curve in rats that were given infusions of a mixture of amino acids in addition to insulin. This curve was maximal at a much lower insulin concentration, which was similar to that in fed rats (~15–25 μU/mL).

In the study described above (15), additional experiments were performed to characterize the specificity of the enhancement of insulin sensitivity for individual amino acids or groups of amino acids. A concentration of insulin of about 20 μU/mL, which is insufficient to stimulate protein synthesis by itself, was achieved by infusion of glucose at a low rate in all groups. The dose-response of muscle protein synthesis in growing rats infused with glucose alone (to provide insulin at a level typical of fed rats) or with glucose plus a complete amino acid mixture, only the essential amino acids, only the nonessential amino acids, or only the 3 BCAAs. Incomplete mixtures contained individual amino acids at the same concentrations as in the complete mixture. Error bars represent the SEM of groups of 5 or 6 rats, and $P$-values are in comparison to the “glucose alone” group. Data from (15).

Figure 2 shows that compared with glucose infusion alone, protein synthesis was stimulated equally by the complete mixture, by only the essential amino acids, and by only the BCAAs (15). This demonstrates that it is the BCAAs that enhance the sensitivity of muscle protein synthesis to insulin. This conclusion was confirmed in the experiment shown in Figure 3, which shows that a mixture of amino acids lacking the BCAAs was not effective in stimulating protein synthesis. Moreover, as shown in Figure 4, the effect of the BCAAs can be attributed entirely to leucine, which had the same effect as the 3 BCAAs together, whereas isoleucine and valine had no effect.

The conclusion of the above studies was that during feeding, leucine enhances the insulin sensitivity of protein synthesis, bringing about a stimulation of muscle protein synthesis. A subsequent study has tested the hypothesis that the blunted response of muscle protein synthesis to feeding in old rats can be restored by feeding a leucine-supplemented diet (16). Adult and old rats were deprived of food and then were again fed a normal diet for 1 h or a leucine-supplemented diet. With the
 mechanisms. Also, evidence for the existence of a noninsulin dependent muscle protein synthesis in the perfused rat hind limb (21) is due to stimulation (20). The observation that leucine stimulates effect on protein synthesis, in addition to the insulin dependent synthesis in diabetic rats, suggesting that leucine can have a direct effect of leucine administration was shown to stimulate protein synthesis (19). This implied that the response to leucine is insulin dependent. However, in a different study, leucine is not unique but is the most potent of a group of 8 amino acids that are termed "regulatory" (25). This group also includes tyrosine, phenylalanine, glutamine, proline, histidine, tryptophan, and methionine (25).

Recent studies of leucine as a regulator of muscle-protein synthesis

In recent years, the subject of leucine and its ability to modify protein synthesis has been reexamined and has led to advances in the understanding of the mechanisms of nutritional regulation of protein synthesis at the molecular level. In a study of the depression of muscle protein synthesis after exercise in rats, it was observed that leucine administration restored protein synthesis to the same value as that in unexercised rats (18). Moreover, this effect was the same when glucose was administered together with the leucine, suggesting that the effect was independent of insulin. The notable difference between this and previous studies was the leucine dose, which was given by intragastric gavage instead of intravenously, and at a 10-fold higher level (10 mmol/kg) than in earlier studies (11). Subsequent work revealed an interaction between leucine and insulin secretion at these higher doses. After oral gavage of leucine (10 mmol/kg) in food-deprived rats, the rate of protein synthesis was stimulated by >50%, not returning to basal levels until after 2 h (19). It was also noted that the insulin level rose transiently, peaking at about 3 times the basal level after 30 min (19). This led to an experiment in which insulin secretion was suppressed by somatostatin infusion, resulting in an abolition of the effect of leucine on protein synthesis (19). This implied that the response to leucine is insulin dependent. However, in a different study, leucine administration was shown to stimulate protein synthesis in diabetic rats, suggesting that leucine can have a direct effect on protein synthesis, in addition to the insulin dependent stimulation (20). The observation that leucine stimulates muscle protein synthesis in the perfused rat hind limb (21) is also evidence for the existence of a noninsulin dependent mechanism.

These studies also provide an explanation why some of the earlier investigations of the effects of leucine on muscle protein synthesis failed to detect a stimulation [e.g., ref. (11)]. The doses of leucine, although large enough to cause an increase in plasma leucine concentration similar to that of feeding, were both too small to enhance protein synthesis by themselves and also too small to induce insulin secretion. By contrast, in the study of Buse et al. (10), leucine was given, together with glucose and insulin, which, as described above, will enhance the effect of leucine.

The work described above has yielded substantial advances in the understanding of the signal transduction pathways involved in the control of muscle protein synthesis by amino acids and insulin. The details of these advances are outlined in a recent review (22) and will not be described here.

Other effects of leucine

The majority of the investigations of leucine's effects on protein metabolism have concentrated on protein synthesis in skeletal muscle. However, there is also evidence that leucine inhibits protein degradation in muscle (2), which in this tissue occurs mainly via the ubiquitin–proteasome pathway (23). By contrast with muscle, in liver there is no effect of leucine on overall protein synthesis, although there is a stimulation of ribosomal protein synthesis (24). The main effect of leucine on liver seems to be on proteolysis, which in this tissue is predominantly lysosomal (23,25). However, unlike in muscle, leucine is not unique but is the most potent of a group of amino acids that are termed "regulatory" (25). This group also includes tyrosine, phenylalanine, glutamine, proline, histidine, tryptophan, and methionine (25).

Conclusions: the role of leucine

Very high concentrations of leucine have the capacity to stimulate protein synthesis and inhibit protein degradation in skeletal muscle of intact rats. This effect on protein synthesis may be enhanced by the transient but small increase in serum insulin that is induced by the leucine dose. However, within the normal physiological concentration range of leucine and insulin in food-deprived and fed rats, the sensitivity of muscle protein synthesis to insulin is enhanced by infusion of leucine, so that protein synthesis is stimulated by the moderately elevated concentrations of insulin and leucine that are typical of the fed rat. The physiological role of leucine is therefore to work with insulin to activate the switch that stimulates muscle protein synthesis when amino acids and energy from food become available. The advantage of this mode of regulation is that the switch requires both amino acids (leucine) and energy (insulin) to be present simultaneously, so is only activated when conditions are ideal.

A role for leucine as an enhancer of insulin sensitivity also implies the possibility that prolonged very high intakes of leucine might lead to insulin resistance, in an analogous way to insulin resistance resulting from prolonged hyperglycemia. This might ultimately lead to a blunting of the stimulation of muscle protein synthesis by food intake. Moreover, because parts of the signaling pathways from insulin to protein synthesis are shared with those involved in the regulation of glucose metabolism, as discussed previously (26), there is the possibility that overstimulation by leucine could lead to abnormalities of glucose metabolism. The search for the “upper level” of dietary leucine might therefore include an investigation of the effects of prolonged high intake of leucine on glucose homeostasis and metabolism.
LITERATURE CITED


