Latency, Duration and Dose Response Relationships of Amino Acid Effects on Human Muscle Protein Synthesis

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ABSTRACT The components of the stimulatory effect of food on net deposition of protein are beginning to be identified and separated. One of the most important of these appears to be the effect of amino acids per se in stimulating muscle anabolism. Amino acids appear to have a linear stimulatory effect within the range of normal diurnal plasma concentrations from postabsorptive to postprandial. Within this range, muscle protein synthesis (measured by incorporation of stable isotope tracers of amino acids into biopsied muscle protein) appears to be stimulated approximately twofold; however, little further increase occurs when very high concentrations of amino acids (>2.5 times the normal postabsorptive plasma concentration) are made available. Amino acids provided in surfeit of the ability of the system to synthesize protein are disposed of by oxidation, ureagenesis and gluconeogenesis. The stimulatory effect of amino acids appears to be time dependent; a square wave increase in the availability of amino acids causes muscle protein synthesis to be stimulated and to fall back to basal values, despite continued amino acid availability. The relationship between muscle protein synthesis and insulin availability suggests that most of the stimulatory effects occur at low insulin concentrations, with large increases having no effect. These findings may have implications for our understanding of the body’s requirements for protein. The maximal capacity for storage of amino acids as muscle protein probably sets an upper value on the extent to which amino acids can be stored after a single meal. J. Nutr. 132: 3225S–3227S, 2002.

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It has been known for many years that protein balance in limb muscle can be switched from negative to positive by the ingestion of a mixed meal, but the exact contributions by the components within a meal and by the metabolic and hormonal consequences of an ingestion of carbohydrates, fats and proteins are still not understood in detail. Some years ago we showed, to the surprise of many observers at that time, that amino acids per se stimulate human muscle protein synthesis (1). Subsequently we showed that substantial doses of essential amino acids appeared to stimulate protein synthesis (2), and Tipton et al. (3) demonstrated that provision of essential amino acids without nonessential amino acids could stimulate protein synthesis immediately after resistance exercise. A substantial amount of work from our laboratory demonstrated that ~80% of the effect of feeding appears to reside in the stimulation caused by amino acids (4–6). Therefore, in the past few years we have attempted to treat amino acids as if they were a single pharmacological entity and we have attempted to obtain information about the latency and duration of their stimulatory effect, and then, having obtained this information, to measure the dose response of muscle protein synthesis to amino acids supplied during the time of their maximal efficacy.

Latency and duration of the effect of amino acids

To gain some insight into the underlying control processes involved in regulation of the effects of amino acids on muscle protein turnover, we needed to know how long it took before the amino acid effect turned on and how long it lasted. We conducted studies in which the resting postabsorptive plasma amino acid concentration was increased by ~70% through the infusion of a commercial parenteral amino acid solution (Aminofusin; Abbott Laboratories, Abbott Park, IL). There appeared to be a lag period of ~30 min followed by a very steep rise in muscle protein synthesis to a value four times that
of the resting value within 2 h. Thereafter, muscle protein synthesis fell back to a value near basal, despite amino acids being infused at a high rate to maintain the high plasma amino acid concentration (7).

The fate of the excess amino acids that were not incorporated into protein appeared to be ureagenesis, according to a large linear increase in blood urea concentration. Plasma glucose concentration also increased, suggesting that a major fate of some of the amino acids was gluconeogenesis.

These results have important implications. First, the rise in muscle protein synthesis is greater than we have observed when making measurements over 4–8 h, and presumably this is because the previous measurements effectively integrated the rise and fall to produce an apparently smaller change. This is important because, when looking for differences as a result of some physiological event or intervention or a pathophysiological condition, the longer the period of infusion of tracer the less likely it will be that any change or difference will be discerned, given the size of the group variances observed in some studies. A large number of the results of studies currently in the literature, including our own earlier studies, suffer this insensitivity and are open to this criticism.

We measured the effects of amino acids on different classes of muscle proteins (those present in myofibrils, sarcoplasm and mitochondria) and showed that all three classes of proteins showed a similar pattern of change (i.e., up then down) in response to the provision of exogenous amino acids. We were disappointed not to see a differential pattern between the rates of stimulation (8), presumably because the method we used, which depends upon incorporation of deuterated leucine into muscle protein, is inherently more variable than that using \(^{13}\)C-leucine with either preparative gas chromatography and off-line isotope ratio mass spectrometry or chromatography combustion mass spectrometry, the tools with which we have seen a differential effect of amino acids and exercise (9).

**Dose response effects of amino acids**

Having established the time window within which amino acid stimulation of protein synthesis is maximal, we moved on to investigate the dose response relationship between infused amino acids and muscle protein turnover. The results suggested that muscle protein synthesis was stimulated almost linearly within the normal physiological range of plasma amino acids and that very high plasma concentrations of amino acids (2.5-fold normal) were required to saturate the system. We discovered that the relationship could be fitted to a rectangular hyperbole in which the half maximal stimulatory concentration of amino acids was ~60% above the normal postabsorptive concentration. In fact, the best relationship could be observed between the extracellular essential amino acids and a stimulation of protein synthesis. There were a number of surprising findings. First, the relationship held only for extracellular amino acids and, in fact, examination of the time course of intracellular amino acids suggested that the provision of exogenous amino acids caused a disappearance of amino acids from the sarcoplasmic space. This has been observed previously by Bergström et al. (10) and is, in fact, what would be expected if amino acids present extracellularly stimulated the flux through the intracellular pool into protein and resulted in a greater use of the intracellular amino acids. This is an example, in fact, of the crossover theorem identified by workers in the 1960s during studies of glycolysis and mitochondrial respiration. However, it also suggests that the intracellular pool can not be the monitored or metered pool that has a controlling influence on muscle protein turnover, because the rise in muscle protein synthesis occurs when the intracellular essential amino acid concentration falls. Indeed, this suggests that there must be some extracellular or membrane-associated sensor that influences mechanisms of muscle protein synthesis, possibly via intracellular signaling processes (11,12).

**The puzzling role of insulin**

There has been substantial controversy over the role of insulin in the control of muscle protein synthesis. Some early studies (13) suggested that insulin did not stimulate muscle protein synthesis. However, the weight of evidence suggests that, when arteriovenous tracer flux studies are carried out correctly with the appropriate handling of the data or when measurements of direct incorporation of tracer amino acids are carried out, then insulin can be shown to stimulate muscle protein synthesis so long as there is a sufficiency of amino acids as substrates (5,14–16). Nevertheless, our results suggest that only a small amount of insulin (10–20 \(\mu\)U per millilitre of plasma) is required to cause the stimulation and that the role of insulin appears to be permissive rather than modulatory; in other words, large increases in insulin have no further effect on muscle protein synthesis. Our understanding of the relationship between insulin and muscle protein turnover is complicated by the fact that protein breakdown appears to be exquisitely sensitive to insulin (17), and it may be that the linearity of the suppressive effect extends to a higher insulin concentration than occurs for protein synthesis. This would mean that the presence of insulin could, under normal circumstances, deprive protein synthesis of substrate by the suppression of protein breakdown, thus limiting the stimulatory effect of food.

**Relationships with protein breakdown**

Amino acids inhibit muscle protein breakdown (17), although not as powerfully as they promote protein synthesis. They also inhibit protein breakdown in other tissues, especially the liver, perhaps more powerfully than they do in muscle (18). It has been suggested that the effect in liver is also due to an extracellular signaling effect, as we hypothesize for muscle. Thus, any effect of exogenous amino acids in inhibiting protein breakdown would decrease the size of the free pool of amino acids available for protein synthesis and also inhibit protein breakdown, providing a link between the two arms of the processes of protein turnover via the extracellular pool, which would effectively integrate the action on a whole body basis.

**Implications for requirements**

Our results suggest that the mechanisms of control of muscle protein synthesis contain physiological devices that limit the amount of protein that can be stored in muscle. Thus, it makes no sense to supply more than the amount required to cause a “muscle full” situation. By our calculations from the infusion rates and the time over which the infusion was applied, amounts of amino acid as small as 3.5 g given on a single occasion would result in this “muscle full” situation. This does not take into account the amounts of amino acid sequestered by splanchnic protein during feeding.

However, there may be circumstances in which the sensitivity or capacity of the process is altered in some way. For example, it has been shown in studies of aged rats that the slope of the relationship between muscle protein synthesis and
the availability of leucine is shifted to the right, as a decrease in sensitivity, or capacity, or both (19). If such a circumstance exists in human muscle, it would help to explain the sarcopenia that occurs in the elderly.

LITERATURE CITED


