

Nutritional Regulation of Protein Metabolism

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Abstract

Protein homeostasis depends on the balance between protein synthesis and protein degradation. In muscle of growing animals, and in the whole body of human preterm infants, protein synthesis is especially sensitive to nutrient intake. This effect has been shown to depend on amino acid supply and insulin secretion. In healthy adults there is no growth, but although there is a need to retain protein, muscle protein synthesis appears to be less sensitive to food intake or insulin infusion. In humans, there has been much interest in the responses to pathological conditions, such as trauma and infection, when protein is lost from muscle. Much effort has been spent on strategies for reversing muscle wasting by nutritional and pharmacological means. However, nutritional support supplemented with branched chain amino acids or glutamine has not been shown to restore protein synthesis after surgery. Although treatment of healthy subjects with growth hormone (GH) stimulated muscle protein synthesis, GH may not be effective for reversing protein wasting in AIDS patients, as it inhibited muscle protein synthesis in those patients who were most wasted, perhaps through a TNF related mechanism. Acidosis is also factor which is often associated with wasting, and has been shown in humans and animals to inhibit muscle protein synthesis.

Introduction

During growth, there is an accumulation of tissue protein, whereas in the adult, the tissue protein content is in balance. On the other hand, injury and disease are frequently accompanied by loss of protein from body tissues, and one of the aims of treatment is to minimize this loss and promote its recovery by nutritional and other therapies. A better understanding of the processes that influence tissue protein gain and loss can be achieved by direct measurement of protein synthesis and degradation in tissues. These two complex and opposing processes are controlled by a variety of hormonal, chemical and physical signals, resulting in growth in

the immature, and maintenance of protein balance in the healthy adult individual. An imbalance resulting from modulation of either synthesis or degradation is the cause of protein gain or loss, but in this article we will concentrate mainly on studies of protein synthesis in human muscle, and its modulation by nutritional factors in health and in pathological states.

Measurement of Protein Synthesis.

Rates of protein synthesis have been measured for many years in cells and tissues in vitro and in animal models using radioactively labeled amino acids. These studies have made an invaluable contribution to our understanding of protein metabolism, but their relevance to human disease can only be assessed by direct measurements in humans, using isotopic labeling techniques. Risk of radiation limits the use of radioactive isotopes in humans, however, and they have now been largely replaced by their stable isotope counterparts. The limitations of tissue sampling in humans and the continual improvement of the mass spectrometers used to measure stable isotopes have resulted in the development of a variety of approaches for measuring rates of protein synthesis and degradation in the whole body. These techniques have been used extensively (for reviews see Waterlow et al, 1978, Garlick et al, 1994, Young 1987) and have given rise to much useful information, but suffer from the limitation that changes in whole body rates of protein turnover cannot be ascribed with any certainty to any particular organ, tissue or body compartment. For example, animal studies have shown that inflammation resulting from subcutaneous injection of turpentine is associated with a fall in protein synthesis in skeletal muscle, but a rise in the liver (Ballmer et al, 1991). These changes would not be detected by the whole-body technique. Over the last decade or so, therefore, there has been an increasing interest in measurements of protein synthesis rates in individual tissues of human subjects.

The general procedure for measuring protein synthesis in an individual tissue or organ in vivo is to

1 inject or infuse an amino acid labeled with the chosen isotope into the bloodstream and to make measurements
2 during the period that it is incorporated into tissue protein. To determine rates of protein synthesis directly, a
3 tissue sample is taken at the end of a period of time t (days) and the enrichment of the amino acid in protein (E_p)
4 is determined. The fractional rate of synthesis (k_s , in % per day) is then calculated from the equation: $k_s \times E_f \times t$
5 $= E_p \times 100$. The other necessary information is the average enrichment of the free amino acid that is being
6 incorporated into protein. This latter measurement has given rise to much debate. Theoretically this
7 measurement should be made on the pool of aminoacyl tRNA in the tissue, but this is complicated by the
8 extremely rapid rate of turnover of this pool and its small size, requiring large tissue samples. For most practical
9 purposes the alternative is to use either the plasma or the tissue (intracellular) free amino acid. However, the
10 isotopic enrichment in these two compartments is not necessarily the same, which has led to uncertainty in the
11 calculated values for protein synthesis and has been influential in determining the way in which the labeled amino
12 acid is administered. Both of the procedures commonly used today were originally suggested by the work of
13 R.B. Loftfield in the 1950's. When a labeled amino acid was given to rats by continuous intravenous infusion,
14 the isotopic enrichments (specific radioactivities) in the plasma and tissues rapidly rose to constant (plateau)
15 values, but in the tissues the values remained substantially lower than in the plasma (Loftfield and Harris, 1956).
16 The interpretation made was that the intracellular pool was derived partially by transport of amino acid into the
17 cell from the plasma and partially from the degradation of unlabeled protein. Moreover, it would be difficult to
18 calculate rates of protein synthesis with confidence, because it was not known which, if either, of these two
19 values would be appropriate as the precursor enrichment. Rates of protein synthesis were not therefore
20 calculated, and instead a different method of label administration was devised, with the aim of making the intra-
21 and extracellular enrichments the same, thus minimizing the ambiguity. This was achieved by injecting the
22 labeled amino acid together with a large amount of unlabeled amino acid, sufficiently to dominate (flood) the

1 small endogenous pool of unlabeled free amino acid (Loftfield and Eigner, 1958). This approach has become
2 known as the "flooding method". Both of these approaches have been used extensively in animals with
3 radioactively labeled amino acids (eg. Garlick et al, 1983, McNurlan et al, 1979), and in humans with stable
4 isotopes (Garlick and McNurlan, 1998, Garlick et al, 1994, Rennie et al, 1994).

5 The constant infusion method has been used for many investigations of muscle protein synthesis in
6 volunteers and patients Rennie et al, 1994). Its advantages are that simultaneous measurement can also be
7 made of whole-body protein turnover, and that the labeled amino acid is given as a tracer, unlike the flooding
8 method, and therefore should not disturb metabolism. Its disadvantages are the need for a metabolic steady
9 state during the infusion, which might preclude observations when acute metabolic changes in are occurring (eg.
10 after a meal, in acute illness or during surgery), and the uncertainty regarding the precursor enrichment when
11 direct measurements of aminoacyl tRNA are not made. However, as most of the work described here
12 employed the flooding approach, this method will be described in more detail.

13 Early investigations with the flooding approach in rats showed that 0.1mmol/100g body weight of
14 [¹⁴C]leucine or 0.15 mmol/100g body weight of [³H]phenylalanine was adequate to equalize the plasma and
15 tissue free amino acid specific radioactivities over a 10 min. period following intravenous injection (McNurlan et
16 al, 1979, Garlick et al, 1980). Similarly, preliminary experiments in human volunteers showed that 4g of [1-
17 ¹³C]leucine per 80kg body weight resulted in almost complete equalization of enrichment in the plasma and
18 intracellular pools in muscle of healthy volunteers over a two hour period (Garlick et al, 1989). The rate of
19 muscle protein synthesis was calculated from the enrichment of leucine in protein of a muscle biopsy taken at 90
20 min. and the average value for precursor enrichment, derived from serial measurements on plasma leucine or
21 ketoisocaproate (KIC, the transamination product of muscle leucine, Matthews et al, 1982) at intervals

between 0 and 90 min (Garlick et al, 1989). The value obtained for muscle protein synthesis rate was 1.86 sem 0.12 %/day when the precursor was taken to be the plasma leucine and 1.95 sem 0.12 %/day when the plasma KIC was used as an index of intracellular labeling (Garlick et al, 1989). These values are expressed as fractional rates, in the units of % per day, which represent the amounts of protein synthesized as percentages of the amount in that tissue.

Although the flooding method successfully equalizes the rates calculated from intracellular and plasma enrichments, these values are higher than those measured by constant infusion of [1-¹³C]leucine using KIC as the precursor, eg. 1.10 sem 0.07 %/day (Halliday et al, 1988). This discrepancy has led to much debate about the validity of these two methods (Garlick et al, 1994, Rennie et al, 1994), which is not yet resolved (Garlick and McNurlan, 1998). However, the discrepancy is much lower when leucyl tRNA or intracellular leucine are used for the estimate of precursor enrichment (Watt et al, 1992, Ljungqvist et al, 1997, Adey et al, 1997]. Deuterated phenylalanine ([²H₅]phenylalanine), which has been used in more recent studies employing the flooding method (McNurlan et al, 1994a), yields rates of muscle protein synthesis that are comparable to those obtained by flooding with leucine (Garlick and McNurlan, 1998).

The advantage of the flooding method is that there is less uncertainty regarding the precursor enrichment, there is no strict requirement for a metabolic steady state to be maintained during the measurement, and the procedure is rapid, typically about 90 min, compared with 4-6h for the infusion. The latter 2 advantages are important when studies are made on patients who might be unstable, such as during the perioperative period (eg. Essén et al, 1992a, Barle et al, 1999). Both the infusion and flooding methods have been used to investigate the control of protein synthesis in a variety of human tissues. Fig. 1 illustrates the wide range of fractional rates of synthesis (FSR) observed in various healthy tissues taken from volunteers and from

patients during surgery (Essén et al, 1992a and b, 1995, 1998, McNurlan et al, 1997, Tjäder et al, 1993).

Nutritional control of muscle protein synthesis in health.

Much information about the control of muscle protein synthesis by food intake has been gained from studies in small rodents. In growing rats, the effect of short periods of starvation on protein synthesis is much greater for skeletal and cardiac muscle than for liver, intestine, kidney and brain (Garlick et al, 1975; McNurlan et al 1979), demonstrating the high sensitivity of skeletal muscle to nutritional changes compared with other tissues. The mediators of the effects of nutrients on muscle protein synthesis have been examined by studying the responses of growing rats to the consumption of meals. When postabsorptive (food deprived for 12h) rats were refed, protein synthesis was rapidly elevated, and this effect could be reproduced by infusion of insulin (Garlick et al, 1983). Moreover, administration of insulin antiserum before refeeding abolished the effect on protein synthesis (Preedy and Garlick, 1986). This suggested that insulin is an important mediator of the response to feeding, but it could not be the only factor, as supraphysiological concentrations were needed to stimulate muscle protein synthesis when insulin alone was given. Similarly, infusion of a mixture of amino acids did not affect protein synthesis, although amino acids plus insulin at a physiological concentration resulted in a stimulation similar to that induced by feeding (Garlick and Grant, 1988). The conclusion from these results was that amino acids enhanced the sensitivity of muscle protein synthesis to insulin. Later studies suggested that the main effect of amino acids was the result of the branched chain amino acids, and possibly only leucine (Garlick et al, 1992).

Studies of feeding and insulin infusion have also been performed in adult rats (1 year old), showing that starvation periods of 12h or 36h had little effect on muscle protein synthesis, in stark contrast to the results from growing animals (Baillie and Garlick 1992). Moreover, there was little effect of refeeding or insulin

infusion after fasting. It has therefore been suggested that the sensitivity of muscle protein synthesis to nutrients in the young animal might be related to the growth process (Baillie and Garlick, 1992). In the adult, by contrast, the much smaller retention of protein after feeding might be brought about not by an increase in synthesis, but instead by a depression of protein degradation. Whether this distinction between responses in the young and adult is also true in humans is not yet clear. When measurements of whole-body protein synthesis were made daily in preterm neonates over the first few days of life, during which the dietary intake was increased daily, there were corresponding increases in protein retention, as a result of an increase in protein synthesis and a smaller increase in degradation (Mitton et al, 1991). This suggests the same sensitivity of protein synthesis to feeding as in the growing rat. Moreover, when measurements of the response of muscle protein synthesis to feeding or short periods of starvation have been made in adult humans, only small changes have been demonstrated in some studies (McNurlan et al, 1993; Essen et al 1992b, Fig 2.), similar to the adult rats. However, other studies have suggested much larger effects of nutrient intake (eg. Biolo et al, 1997, Rennie et al, 1982), and these differences in response need to be resolved.

Muscle Protein Synthesis in Pathological States

Much of the work on human protein metabolism has been directed towards an understanding of the mechanisms that underlie the loss of body protein, particularly from skeletal muscle, in pathological conditions such as injury and infection. In an attempt to elucidate the muscle wasting that takes place after surgical injury, measurements of muscle protein synthesis were made in patients before and after gall bladder surgery (cholecystectomy). A short period of anesthesia without surgery did not affect muscle protein synthesis, but immediately after the completion of surgery (open cholecystectomy, Fig. 2), there was already a 30% fall (Essén et al, 1992a). By day 3 after surgery, the decrease in protein synthesis had enlarged to 50% (Essén et al,

1993). These data suggest that muscle wasting after surgery results at least in part from an immediate and sustained decrease in muscle protein synthesis. Further experiments were performed to see whether this change was dependent on the degree of trauma. Minor surgery for breast lump removal did not affect muscle protein synthesis (Fig. 2)(Tjäder et al, 1993), but the effect of major surgery was no greater than that of cholecystectomy. Moreover, the rates in critically ill patients in the intensive care unit were on average less depressed than those after surgery, although the range of values, shown in Fig. 2 by the upper part of the bar, was in this case considerable (Essén et al, 1998). Surprisingly, laparoscopic cholecystectomy did not produce a smaller depression of protein synthesis than conventional, open surgery (Fig.2), even though its beneficial effects on recovery are well accepted (Essén et al, 1993). Muscle wasting also occurs in chronic diseases, such as HIV/AIDS. In this case we have shown that protein synthesis is not depressed; even patients who show overt muscle wasting do not have lower rates of muscle protein synthesis (Fig 2, McNurlan et al, 1997)).

The factors that cause the decrease in protein synthesis and protein wasting are therefore not clear at present, as the rate of protein synthesis does not appear to be related to the degree of stress or trauma. This is particularly apparent in critically ill patients, who might display either reduced or elevated rates of protein synthesis (Fig.2), which do not relate well to the clinical condition of the patient (Essén et al, 1998, Gamrin et al, 2000). As modulation of protein degradation is also thought to be important in pathological states, combined measurements of synthesis and degradation may be needed in future studies.

Because protein wasting contributes substantially to morbidity and mortality, there has been much interest in the potential benefits of nutritional support. However, studies in patients following cholecystectomy showed that postoperative intravenous nutrition did not diminish the inhibition of protein synthesis (Essén et al, 1993). The inability of conventional nutritional support to stem protein wasting has led to the search for new

1 strategies, such as nutritional support supplemented with specific amino acids, eg. BCA or glutamine, or
2 treatment with growth hormone, which might be needed to minimize protein loss after surgery and optimize
3 recovery. In general, these strategies have not proved very successful. Although BCA have been shown in
4 growing animals to enhance the stimulation of muscle protein synthesis by insulin (see above), and in vitro to
5 stimulate muscle protein synthesis directly (Li and Jefferson, 1978), in patients they have not consistently been
6 shown to improve nitrogen balance (Brennan et al, 1986). Moreover, in colorectal cancer patients, BCA
7 supplemented nutrition did not enhance muscle protein synthesis (McNurlan et al, 1994b). Similarly, it has been
8 suggested from work in animals that glutamine can stimulate protein synthesis (Jepson et al, 1988), but direct
9 measurement of muscle protein synthesis in patients supplemented with glutamine after surgery (Januszkiewicz et
10 al, 1996) did not reveal any beneficial response. However, because of glutamine's potential role in preserving
11 gut integrity and immune function (Jonas et al, 1999, Wilmore et al, 1999), this amino acid is still under active
12 investigation.

13 Protein wasting is also a characteristic of chronic infectious diseases, in particular in patients with
14 AIDS. However, as indicated above, protein synthesis is not depressed. As shown in Fig 3, there are no
15 significant differences in postabsorptive rates of muscle protein synthesis between healthy controls and patients
16 who are HIV positive but asymptomatic, or who have AIDS with or without wasting. There have been a
17 number of studies of the effectiveness of GH treatment to restore muscle mass in AIDS patients, with varying
18 results (Krentz et al, 1993, Mulligan et al, 1993, Schambelan et al, 1996)). In the study illustrated in Fig 3,
19 muscle protein synthesis was measured in patients at various stages of HIV infection compared with healthy
20 controls. When these patients were given a 2-week course of treatment with growth hormone, the reason for
21 the wasting became clear, as there was a significant stimulation in muscle protein synthesis in the healthy controls
22 and in asymptomatic HIV patients, but an inhibition of protein synthesis in AIDS patients, particularly those who

were wasted (Fig. 3). This suggests that the trophic mechanisms involved in maintaining muscle mass, such as growth hormone and IGF-I, have become progressively deficient as the HIV disease advances (McNurlan et al, 1997). In an attempt to discover the mechanisms involved, the plasma from these patients was analyzed for IGF-I and a number of cytokines. The GH treatment caused a similar elevation of IGF-I levels in all groups, suggesting that declining response to GH reflected a decreased responsiveness of the muscle to IGF-I. The plasma levels of TNF α did not differ between groups, but the level of the soluble TNF receptor Type 2 (sTNFR-2) correlated negatively ($p=0.01$) with the stimulation of protein synthesis by GH (Gelato et al, 2002). The plasma concentration of sTNFR-2 may be a measure of the ongoing level of immune activation, as its plasma half-life is much longer than that of TNF α . Hence, this correlation suggests that resistance to GH treatment, and hence the decrease in muscle responsiveness to IGF-I, might be the result of an inflammatory process involving TNF.

A number of pathological states involving wasting are associated with acidosis, which has received relatively little attention as a possible mediator of protein wasting. In the study of protein synthesis in muscle of critically ill patients (Fig.2, Essen et al, 1998), the wide variation in rates were partially explained by variations in the arterial pH. Also, there have been studies suggesting that acidosis causes negative nitrogen and leucine balance (Ballmer et al, 1995, Reaich et al, 1992) and that proteolysis in muscle is enhanced (Mitch et al, 1993). Our own work has led to the conclusion that muscle protein synthesis is inhibited by acidosis and may also be stimulated by alkalosis. Seven days of metabolic acidosis, induced in human volunteers by ammonium chloride administration, resulted in negative nitrogen balance and a significant depression of rates of synthesis of serum albumin (Ballmer et al, 1995). A more recent work has shown that human muscle protein synthesis is depressed by 2 days of metabolic acidosis (Kleger et al, 2001). This phenomenon has also been demonstrated in a rat model, involving intragastric gavage of ammonium chloride over 24h (Fig 4, Caso et al, 1999, 2000a), showing

1 that plantaris and gastrocnemius muscles, containing mixed fiber types, are inhibited more by acidosis than
2 soleus, containing mainly oxidative fibers, and that cardiac muscle is unaffected. In addition, no effects were
3 found in liver, gut, spleen and kidney. Moreover, muscle protein synthesis in rats is also depressed by
4 respiratory acidosis (Caso et al, 2000b). Figure 5 shows evidence that pH might be of importance in critically
5 ill patients, and moreover, that alkalosis might stimulate protein synthesis. Measurements of muscle protein
6 synthesis were made in head trauma patients, who were hyperventilated as part of their treatment, resulting in
7 respiratory alkalosis. The measurement was then repeated shortly after hyperventilation was discontinued. The
8 resulting fall in arterial pH was associated with a significant fall in the rate of muscle protein synthesis (Fig.6,
9 Vosswinkel et al, 2000), suggesting that pH might have an important role in modulating protein synthesis in
10 pathological states.

11 Conclusions

12 During growth, muscle protein synthesis is especially sensitive to stimulation by nutrient intake,
13 through the combined actions of insulin and amino acids, particularly the branched chain amino acids. However,
14 these anabolic responses appear to be blunted in adulthood in rats, and possibly in humans also. In the adult
15 human, pathological states, such as injury and infection, result in muscle protein wasting, which in the case of
16 surgery is associated with a fall in muscle protein synthesis. However, this defect in protein synthesis is not
17 effectively reversed by provision of nutritional support, even when supplemented with branched chain amino
18 acids or glutamine. Moreover, attempts to enhance protein synthesis after surgery and in AIDS patients by
19 treatment with growth hormone have also been unsuccessful, possibly because the inflammatory process
20 reduces the responsiveness of muscle to anabolic factors such as GH and IGF-I. In addition to nutrients and
21 hormones, physiological factors such as pH might also have a role to play in the regulation of protein balance,

- 1 especially in pathological states, as changes in arterial pH have been shown to alter protein synthesis in healthy
- 2 rats and humans, and also in critically ill patients.

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Figure legends

Figure 1. Rates of protein synthesis in a range of human tissues, measured by flooding with [1-¹³C]leucine or [²H₅]phenylalanine (Heys et al, 1991, Park et al, 1993,1994, Garlick et al, 1989)

Figure 2.

Effects of various pathological states on the fractional rate of protein synthesis in muscle. Values are expressed as percent of the value measured in the same subject prior to starvation or surgery, or as percent of values obtained in normal healthy subjects for the AIDS and intensive care patients (for references, see text).

Figure 3.

Rates of muscle protein synthesis before and 2 weeks after growth hormone treatment of healthy subjects and patients with asymptomatic HIV infection, AIDS without wasting and AIDS with wasting. (McNurlan et al, 1997)

Figure 4.

The effect of acidosis induced by gavaging rats with ammonium chloride on muscle protein synthesis (Caso et al, 1999, 2000b)

Figure 5.

- 1 The changes in muscle protein synthesis and arterial pH in head trauma patients during hyperventilation and
- 2 shortly after normal respiratory parameters had been restored. (Vosswinkel et al, 2000)

3

1 Figure 1

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