Human protein metabolism: its measurement and regulation

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Liu, Zhenqi, and Eugene J. Barrett. Human protein metabolism: its measurement and regulation. Am J Physiol Endocrinol Metab 283: E1105–E1112, 2002; 10.1152/ajpendo.00337.2002.—The body's protein mass not only provides architectural support for cells but also serves vital roles in maintaining their function and survival. The whole body protein pool, as well as that of individual tissues, is determined by the balance between the processes of protein synthesis and degradation. These in turn are regulated by interactions among hormonal, nutritional, neural, inflammatory, and other influences. Prolonged changes in either the synthetic or degradative processes (or both) that cause protein wasting increase morbidity and mortality. The application of tracer kinetic methods, combined with measurements of the activity of components of the cellular signaling pathways involved in protein synthesis and degradation, affords new insights into the regulation of both protein synthesis and breakdown in vivo. These insights, including those from studies of insulin, insulin-like growth factor I, growth hormone, and amino acid-mediated regulation of muscle and whole body protein turnover, provide opportunities to develop and test therapeutic approaches with promise to minimize or prevent these adverse health consequences.

protein synthesis; proteolysis; insulin; insulin-like growth factor I; growth hormone; amino acids

IN HUMANS, THE BODY PROTEIN MASS provides architectural support, enzymes to catalyze metabolic reactions, signaling intermediates within and between cells, and fuel to allow survival. Because there is no protein storage pool, proteins that serve vital roles are catabolized when the body is faced with a need for additional fuel expenditure. Subsequently, the body replaces previously sacrificed proteins during periods of caloric/nitrogen surfeit, thereby keeping a delicate and dynamic protein balance that maintains homeostasis in the face of environmental challenge.

Protein synthesis and degradation are closely regulated in vivo, and each is affected by physiological and pathophysiological conditions, such as fasting, feeding, exercise, disease, and aging. Many diseases can decrease protein synthesis and/or enhance protein degradation, causing negative protein balance. While transiently this may afford benefit by supplying amino acids for gluconeogenesis, wound healing, and synthesis of antibodies and acute-phase proteins, prolonged negative protein balance is associated with significantly increased morbidity and mortality. Our understanding of the cellular and integrated regulation of protein synthesis and degradation has advanced considerably over the past two decades. Here, we briefly review selected aspects of this progress, particularly isotopic methods used to measure whole body and tissue-specific protein turnover as well as newer tracer-independent methods for studying the regulation of body protein metabolism. Finally, we will briefly review how these methods are being applied to studies of insulin, insulin-like growth factor I (IGF-I), growth hormone (GH), and amino acid-mediated regulation of muscle protein turnover.

METHODS FOR MEASUREMENT OF PROTEIN METABOLISM IN VIVO

For any cell or tissue, protein balance reflects the net of protein synthesis and degradation. The rates of protein synthesis and degradation differ drastically.
among organs/tissues, and between the cytosolic, nuclear, and mitochondrial compartments of a cell. Whole body protein balance is the integral of these processes. Many attempts to quantify either whole body or tissue-specific protein metabolism in humans in vivo have been made during the past two to three decades. These efforts have been almost exclusively based on use of tracer measurements. However, these complex dynamic processes require model assumptions that limit the accuracy of all available methods, and each method has inherent shortcomings and limitations.

Whole Body and Organ-Specific Nitrogen Balance

Early studies of whole body nitrogen balance simply employed chemical analyses of nitrogen intake and excretion (8). With the development of automated amino acid analyses, the net balance of all amino acids across a tissue bed (muscle, liver, brain, and so forth) became measurable (44). These estimates at steady state provide a net measurement of the protein accretion/loss, but no insight into the processes of protein synthesis and degradation at either the whole body or tissue level. However, because these measurements do not require the modeling assumptions that accompany the use of tracer methods, they remain a useful, independent method for assessing whole body or organ responses to nutrient, hormonal, and other manipulations.

Whole Body and Organ-Specific Protein Turnover

To extend investigation beyond the limitations of the whole body nitrogen or tissue amino acid balance techniques, several approaches were developed. Each involves the use of an essential amino acid tracer and is based on the assumption that the kinetics of the essential amino acid tracer used represent the kinetics of the protein pool. Because essential amino acids are not synthesized in vivo, the steady-state dilution of the infused tracer-specific activity/enrichment in blood reflects the inflow into blood (or rate of appearance, Ra) of unlabeled amino acids derived from protein degradation and dietary intake. Conversely, the rate of tracer disappearance (Rd) from blood represents the sum of amino acid oxidation to CO2 and nonoxidative disposal, which, with the appropriately chosen tracer, will principally reflect body protein synthesis.

Whole body protein turnover using isotopic tracers.

Whole body protein turnover is now commonly estimated using L-[^14C]leucine (36) or L-[^15N]phenylalanine labeled with either radioactive or stable isotopic tracers and given as a primed, continuous intravenous infusion. With a continuous intravenous tracer infusion, the arterial plasma tracer enrichment/specific activity reaches a plateau (steady state) in ~2 h. At steady state, whole body protein turnover equals the rate of tracer appearance (Ra) in the plasma free amino acid pool and the rate of tracer disappearance (Rd) from the free amino acid pool, and each can be estimated from the tracer infusion rate divided by the arterial plasma-specific activity/enrichment. When labeled leucine is used as the tracer, venous α-ketoisocaproic acid (α-KIC), the transamination product of leucine, may better reflect the enrichment of leucine in the cellular free amino acid pool (52). In addition, with leucine labeled in carbon 1, provided the loss of label to CO2 is measured, both protein synthesis and degradation can be quantified. This approach provides the most accurate method currently available to simultaneously estimate whole body protein synthesis and degradation. With this method, because of the rapid equilibration of the infused tracer, it is possible to “perturb” the steady state and estimate dynamic changes in response to specific interventions (see Hormonal and Nutritional Regulation of Protein Turnover in Vivo).

However, there are limitations (6). This method certainly underestimates the total turnover of tissue proteins. This is partially compensated in the case of skeletal muscle by measuring the specific activity (or enrichment) of α-KIC (24). It is less clear that this compensation is satisfactory for other tissues with lower transaminase activities. The estimates of synthesis are also affected by assumptions regarding the body bicarbonate pool. These issues aside, the steady-state [L-^14C]leucine infusion method is currently the accepted standard for measurement of whole body protein turnover.

Measurement of protein turnover in specific tissues/organisms.

Measurements of both protein synthesis and degradation within specific tissues/organisms are obtained by using a combination of organ balance and tracer approaches. The methods require arterial sampling together with sampling of venous blood draining the tissue being studied. With this method, one gives either a leucine (12) or phenylalanine (5, 21) tracer by primed, continuous intravenous infusion until a steady state is achieved in plasma. During steady-state infusion of tracer, measurement of the uptake of tracer by the tissue (Ra) and of the dilution of its specific activity as it traverses the tissue (Rd) can be used to estimate protein synthesis and breakdown, respectively. The calculation is based on the measurement of the concentration and specific activity (SA)/enrichment of systemically infused tracer in both arterial blood entering and venous blood draining the tissue under study, together with blood flow to the tissue. The net balance of leucine or phenylalanine simply reflects the difference between its uptake for protein synthesis and its release from degraded protein. The formulas used for these calculations are detailed elsewhere (4). In these estimates it is assumed that either the arterial or the venous blood SA/enrichment at steady state approximates that of the pool being used for protein synthesis. The amino acid selected as tracer is dictated somewhat by the tissue under study. For skeletal muscle (5), heart (48), and adipose tissue (14), phenylalanine appears most appropriate. Phenylalanine is neither synthesized nor concentrated by the muscle and is well represented in muscle protein; its only metabolic fate in muscle is to be incorporated into protein, and the only endogenous source of phenylalanine released into blood traversing muscle is protein. For liver, leucine would be a preferred tracer, because the fraction of its metabolic fate

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directed other than to protein synthesis (42) is less than that of phenylalanine.

The major advantage of this method is that it allows simultaneous estimation of both synthesis and degradation. Because it relies on steady-state conditions being reached in the plasma pool, repeated sampling can be performed to assure this condition, and because this requires only ~2 h, it is amenable for the study of the effects of physiological interventions.

In studies directed more specifically at examining the process of protein synthesis in tissues in vivo, several methods are currently regularly used. The first is an adaptation of the steady-state leucine or phenylalanine tracer method just described. It involves a continuous tracer infusion that persists until a steady state of enrichment or specific activity is reached in the pool considered to be the precursor for protein synthesis (43). Then a tissue sample is taken, and a second sample is taken hours later, with the tracer remaining at steady-state enrichment between samplings. The proteins in the biopsy samples are analyzed for tracer enrichment. The synthesis rate is calculated by dividing the change in tracer enrichment in the protein-bound amino acid by the steady-state tracer enrichment in the precursor pool over a given period of time. Optimally, labeling of the amino acyl-tRNA pool would provide the precursor pool for protein synthesis. However, its small size relative to the bulk amino acid pool and technical difficulties with its measurement have precluded this approach in most circumstances. Instead, enrichment or specific activity of the amino acid label in the total tissue extract is typically used hopefully to approximate that of the tRNA pool. To circumvent this problem, Garlick and colleagues pioneered in animals (20) and later in humans (38) the so-called "flooding dose" technique, which employs a bolus intravenous injection of large doses of labeled (tracer) and unlabeled (tracee) amino acid to rapidly force equilibration of labeling between the intra- and extracellular amino acid pools. The tracer enrichment in these pools is assumed to be nearly identical to the true precursor, i.e., the aminoacyl-tRNA pool, in all tissues. Protein-bound tracer amino acid is then extracted from tissues (or proteins), and the fractional synthesis rate (FSR) is calculated. A potential drawback of this technique is that rapid injection of a large amount of amino acid tracer may itself stimulate protein synthesis. Most investigators use phenylalanine as the tracer, since large doses of leucine are capable of activating translation initiation and increasing the FSR of mixed muscle protein. Despite use of phenylalanine, the FSR measured with this technique is usually 1.5- to 2-fold higher than that calculated from the continuous infusion method, a finding not yet adequately resolved.

These latter two methods afford the advantage of likely providing a more accurate estimate of protein synthetic rates. However, limitations include the invasive nature of the studies, requiring multiple biopsies that (at least for clinical investigations) limit studies to muscle, adipose tissue, and skin; the lack of information regarding protein degradation; and the concern that it is not trivial to ascertain when the precursor pool labeling has achieved a steady state.

For muscle, an additional method has been used to estimate proteolysis that involves measurement of urinary excretion of 3-methylhistidine (3-MH). Histidyl residues in actin, and to a lesser extent in myosin, are posttranslationally methylated, and methyl-histidines are released when these proteins are degraded. More than 90% of 3-MH residues are in skeletal muscle, and the urinary excretion rate of 3-MH provides noninvasive in vivo estimates of muscle protein degradation in humans that are consistent with rates estimated by other methods. However, the analysis requires quantitative collection of urine, a carefully controlled meat-free diet for several days, and normal renal function and is limited by the quantities of 3-MH in urine contributed by nonskeletal muscle sources, which vary with the 3-MH pool sizes (depending on lean body mass), their relative contents of 3-MH, and the protein turnover rates of different tissues as well (7).

**Measurement of the Synthesis of Specific Proteins or Classes of Proteins**

For some relatively abundant plasma (albumin, fibrinogen, apolipoprotein B-100, and the like) (15) or tissue (myosin and actin in muscle) (11) proteins, as well as for several classes of cellular proteins ( mitochondrial and contractile proteins in muscle) (49), it is possible to incorporate sufficient amino acid label (generally as a stable isotope) to allow measurement of their synthesis rates. These estimates are based upon many of the same assumptions as the whole body and whole tissue measures. Despite the slow turnover of these proteins, their abundance allows reasonable quantitation of their turnover and responses to interventions like insulin and feeding.

**Nontracer Techniques Applied to Studies of Protein Metabolism in Vivo**

Because of difficulties that appear intrinsic to tracer estimates of protein metabolism, efforts to study the regulation of protein synthesis and degradation in vivo have recently been extended to methods that examine the activity of the pathways involved in synthesis (mRNA transcription and activation of translational regulatory elements) and degradation. In both experimental animals and humans, measurement of transcription of specific mRNAs has been accomplished by classical Northern blotting, PCR, and RNase protection assays, and more recently by use of microarray methods. For translation, the phosphorylation states of proteins involved in regulating translation initiation have been coupled with estimates of protein synthesis to provide a more comprehensive picture of the regulation of this portion of the synthetic pathway.

Efforts to study the regulation of protein degradation have also progressed and include estimates of the activities of one or more of the pathways involved in proteolysis or transcription of genes that code for pro-
Progress with these nontracer measures of proteolysis has not kept pace with measures of transcription/translation, in part because of the complexity of the multiple pathways involved in protein degradation, and this will be an area for future investigation, especially as molecular probes for components of the calpain, proteasomal, and lysosomal pathways are developed. This will be particularly important because one action of inflammatory cytokines appears to involve a marked increase in proteolytic activity, and this might contribute to the wasting that occurs in chronic inflammatory disorders (13).

All of these methods involve tissue sampling and provide information on regulatory changes that are relatively stable in nature, i.e., changes that are preserved during tissue sampling and assay. To the extent that allosteric mechanisms are involved in modulating the rates of either synthesis or degradation, their impact remains elusive.

**SELECTED APPLICATIONS OF IN VIVO METHODS TO STUDIES OF HUMAN PROTEIN METABOLISM**

**Whole Body Protein Turnover and Energy Balance**

With their significant limitations, what information can be obtained from whole body protein turnover methods? Here we point out one simple phenomenon that we have come to call the “protein paradox,” which derives quite simply from steady-state measurements of fuel turnover in postabsorptive humans. It begins with a comparison of the turnover rates of the body’s three principal fuels: protein, carbohydrate, and fats. Figure 1 gives the R in plasma of amino acids, free fatty acids, and glucose in the circulation after an overnight fast in healthy humans. In each case, the estimates are derived from steady-state tracer infusion methods. Among these three fuels, protein is unique in that there is no storage form that is not already serving another significant purpose. On this basis, it might be expected that it would be relatively preserved. This would seem more advantageous given the large amount of energy required to synthesize a protein relative to that required to store energy as either carbohydrate or fats (a minimum of 4 ATP equivalents per peptide bond made and broken vs. 2 for a glycosidic linkage in glycogen and 2 per ester linkage in triglyceride). Paradoxically, the turnover rate of the body’s protein pool is substantially higher than that of either of the other two principal fuels (see Fig. 1). However, the fraction of amino acids liberated from body protein via one or another proteolytic pathway that is subsequently oxidized fully is substantially less than that fraction for either fat or carbohydrate. Thus a selective advantage appears to accrue to the organism when the unitary building blocks of proteins (amino acids) are used sparingly as an oxidative fuel, whereas the protein pool per se turns over rapidly at considerable cost of energy to the body. The energy expenditure involved in this turnover of the body protein pool is not insignificant when compared with total body resting energy expenditure. It can be estimated that, at a minimum, the turnover of the body protein pool will account for 5% of our resting energy expenditure (33). The nature of the selective advantage afforded by this plasticity of our body protein pool is unclear. However, estimates of this sort require the use of tracer measurements as described above and cannot be derived by other methods.

**Hormonal and Nutritional Regulation of Protein Turnover in Vivo**

Protein synthesis and degradation are each regulated by multiple hormonal and nutritional factors, and protein balance of individual tissues and the whole body changes constantly. Recent studies at the molecular and cellular levels, coupled with tracer methods, have dramatically advanced our understanding of the regulation of protein metabolism in vivo. Here we will selectively review studies relating to the actions of three peptide hormones, insulin, IGF-I, and GH, and amino acids that have been more extensively studied and that affect body protein metabolism acutely and hence are amenable to study by use of some of the tracer and biopsy methods we have described. Other hormones (e.g., thyroid hormones, glucocorticoids, gonadal steroids), as well as cytokines and interleukins, also impact body protein metabolism, but information on the actions of these effectors is much less complete; review of these is beyond the scope of the current work. Our focus in reviewing these three hormones and amino acids is in part to provide a “status report” of current information but also to highlight the unknowns that will hopefully be areas for further investigations.

**Insulin.** In humans, the rates of whole body (54), splanchnic (42), skeletal (21), and heart (37) muscle protein degradation are decreased in response to physiological increases in plasma insulin. Conversely, the rates of protein degradation increase transiently in heart and skeletal muscle in diabetic animals (2), and...
the whole body protein turnover is increased in insulin-withdrawn type 1 diabetic patients (10). Decreasing insulin availability secondary to either experimental diabetes or starvation reduces by 40–50% the rate of protein synthesis in rat skeletal muscle. This is rapidly reversed with insulin treatment. Numerous in vitro experiments in cultured cells or isolated perfused tissues have consistently demonstrated that insulin at high concentrations stimulates protein synthesis and abrogates proteolysis (26, 28). Surprisingly, whereas the in vivo antiproteolytic effect of insulin in adult animals or humans has been repeatedly shown, the anabolic effect of insulin on protein synthesis has not been consistently demonstrated. A satisfactory explanation for this apparent discrepancy between in vivo and in vitro studies is wanting. Factors including the insulin concentrations used in vitro (typically between 2 and 10 mU/ml), preincubation of tissues in the complete absence of insulin in vitro before insulin addition, or the overall highly catabolic state of tissues incubated in vitro may all contribute. On the other hand, an effect of insulin in vivo may be underestimated if insulin’s antiproteolytic effect significantly decreases amino acid availability, as amino acids themselves regulate the rate of mRNA translation (55).

Recent studies that build on early work from the laboratory of Jefferson and colleagues (Kimball et al., Ref. 29) have shed light on the effect of insulin to regulate mRNA translation, a critical step in insulin’s action on protein synthesis in isolated muscle, heart, and liver. Availability of phosphospecific antibodies and measurement of electrophoretic mobility shift assays have allowed linkage of insulin action to stimulate protein synthesis with its action on key signaling molecules downstream of phosphatidylinositol 3-kinase (PI-3 kinase) and protein kinase B (PKB or Akt) in the insulin-signaling cascade. At the translational level, insulin has been shown to modulate the phosphorylation states of several key intermediates in the protein synthetic pathway, among them eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase (p70S6K). The phosphorylation of 4E-BP1 and p70S6K, both downstream of PI 3-kinase-Akt-mammalian target of rapamycin (mTOR) in the insulin-signaling pathway, is closely correlated with the rate of protein synthesis (Fig. 2). Inhibition of PI 3-kinase with wortmannin or mTOR with rapamycin blocks insulin-stimulated phosphorylation of 4E-BP1 and p70S6K and partially inhibits protein synthesis. Readers are referred to several excellent recent reviews that have detailed the regulation of protein synthesis by insulin and nutrients through modulating translation initiation (39, 47).

We have examined the effects of insulin, at both pharmacological and physiological concentrations, on the phosphorylation of 4E-BP1 and p70S6K in both humans and laboratory rats. Interestingly, we found that insulin stimulated the phosphorylation of p70S6K, but not 4E-BP1, at physiological concentrations (22), whereas the phosphorylation of both proteins is enhanced at pharmacological concentrations (33). Because ribosomal S6 protein is a principal physiological substrate for p70S6K, and phosphorylation of S6 enhances the translation of a restricted subset of proteins with oligopyrimidine sequences at the transcriptional start site, it may be that p70S6K is required for maintaining the apparatus required for ongoing protein synthesis. This is consistent with previous suggestions that physiological concentrations of insulin play a significant role in maintaining the protein synthetic apparatus (2). We have also found that infusion of IGF-I increases the phosphorylation of both 4E-BP1 and p70S6K (W. Long and E. J. Barrett, unpublished observation), again raising the issue of possible cross-stimulation of IGF-I and/or IGF-I/insulin hybrid receptors by high-dose insulin.

The cellular mechanisms underlying insulin’s antiproteolytic actions are poorly understood. Mounting
evidence suggests that the ATP-dependent ubiquitin-proteasome proteolytic pathway plays a central role in insulin-regulated protein degradation, particularly in muscle (46). Blockade of lysosomal and calcium-activated proteases resulted in only minimal decrease in proteolysis, whereas depletion of ATP or inhibition of proteasomal activity with either MG-132 or lactacystin blocked the increase in proteolysis due to insulinopenia. In insulin-deficient rats, increased mRNAs for ubiquitin and proteasome subunits have also been demonstrated (31, 34). However, insulin may also exert a direct inhibitory effect on proteasomal peptidase activity independent of an effect on transcription.

For insulin, there appears to be a dissociation between the doses of insulin that affect protein synthesis vs. those that affect protein degradation. Whether we consider whole body measures of synthesis and degradation as estimated with labeled leucine or measures of these processes in skeletal muscle with phenylalanine tracer and the limb balance method, it appears that proteolysis is more sensitive than synthesis to small changes in plasma insulin within its physiological range. Unknown is where in the insulin-signaling system the pathway for regulation of synthesis and degradation diverges. Dissecting this will require a more detailed understanding of the early signaling events that mediate insulin’s antiproteolytic action.

IGF-I. Like insulin, IGF-I has been repeatedly shown to have anabolic properties on protein metabolism (30). Systemically administered IGF-I inhibits whole body protein breakdown and causes hypoglycemia and hypoglycemia. When given acutely in vivo at doses that have no stimulatory effect on glucose uptake, IGF-I stimulates muscle protein synthesis (17) and is very effective in activating 4E-BP1 and p70S6K phosphorylation in both skeletal and cardiac muscle (W. Long and E. J. Barrett, unpublished observation). In contrast, there is a lack of stimulation of whole body protein synthesis with systemic infusion of IGF-I (16), which likely results from the fall in circulating insulin and/or amino acid concentrations that accompanies systemic IGF-I infusion in vivo. When amino acids (50) or insulin (25) is replaced simultaneously, IGF-I clearly stimulates whole body protein synthesis. Muscle protein breakdown seems to be inhibited by the higher doses of IGF-I that also promote glucose uptake.

Inasmuch as insulin and IGF-I exert anabolic actions on both protein synthesis and proteolysis, the question naturally arises as to whether there is coupling between the processes of synthesis and degradation. In this regard, it is particularly intriguing to examine the dose-response characteristics of each. Thus modest increases in plasma insulin diminish whole body and skeletal muscle proteolysis (35) and increase glucose disposal in muscle, but higher doses appear required to stimulate protein synthesis. In contrast, increases in plasma IGF-I that are without effect on glucose uptake stimulate protein synthesis in muscle and the whole body (17) and, at least in muscle, higher doses are required to retard proteolysis. This suggests that the cellular signals that mediate insulin and IGF-I action in muscle, although similar, diverge significantly, yet both can influence both the synthesis and degradation pathways.

GH. Chronic GH treatment increases lean body mass and decreases fat mass, especially when used to treat antecedent GH deficiency (27, 51) and/or prevent glucocorticoid-induced protein wasting (23). In healthy humans, acute infusions of GH lasting from 6 h to 3–7 days modestly stimulate muscle (18) and whole body protein synthesis without significantly affecting plasma amino acid concentrations in healthy humans. The mechanisms of GH-stimulated protein synthesis remain unclear. Systemic infusion not only increases systemic IGF-I concentrations but also stimulates local generation of IGF-I. GH may also exert its protein anabolic actions directly, independently of IGF-I generation. GH has been shown to phosphorylate insulin receptor substrate 1 (IRS-1), the principal substrate of the insulin receptor, activate PI 3-kinase, and increase the phosphorylation of p70S6K, actions similar to those of insulin and IGF-I. When GH is infused locally into the brachial artery, forearm muscle protein synthesis increases, whereas systemic IGF-I concentrations and muscle protein degradation are unaffected. This suggests that GH-stimulated protein synthesis is not dependent on increases in systemic IGF-I concentrations, but the local generation of IGF-I in the skeletal muscle remains a possible mechanism (30). Although GH infusion provokes an increase in serum insulin concentration, this does not retard protein degradation in human muscle. Indeed, when GH and insulin are co-infused, GH can block the effect of insulin to retard proteolysis (19). Thus, for protein metabolism, both an “insulin-like” and an insulin-antagonistic action of GH can be seen when GH is given acutely to humans.

At the current time, it is difficult to construct an integrated, mechanistic picture of GH’s action on human protein turnover. However, as additional tools have become available for measuring cellular mediators of GH action, including the activity of the families of the janus and signal transducer and activator of transcription kinases, it will become increasingly possible to track responses in accessible tissues in humans and animals that will allow a more detailed understanding of how GH affects protein synthesis and degradation.

Amino acids. Ingestion of proteins or infusion of mixed amino acids (AA) has significant protein anabolic effects in healthy humans. At a systemic level, AA administration stimulates insulin, GH, and glucagon secretion. Independently of these hormonal effects, in vitro AA directly stimulate protein synthesis (9, 53), inhibit proteolysis (41), and enhance the sensitivity of protein synthesis to insulin. Among all AA, branched-chain amino acids (BCAA), especially leucine, have a particularly important role in mediating these protein anabolic effects (1). Recent data in cultured cell and animal studies have demonstrated that both mixed AA and BCAA can act as direct initiators of signal transduction through mTOR-dependent phosphorylation of 4E-BP1 and p70S6K (Fig. 2), thereby enhancing protein
synthesis at a translational level. BCAA or leucine alone can activate the mTOR-4E-BP1/p70S6K pathway. AA deprivation results in reversible inactivation of p70S6K, increased binding of 4E-BP1 to eIF4E and dissociation of the eIF4F complex, decreased phosphorylation of eIF4E, and increased phosphorylation of eukaryotic elongation factor 2 (eEF2). In aggregate, these actions retard protein synthesis. AA also regulate global protein synthesis through modulation of eIF2B activity. Despite these seemingly concordant effects between AA mixture and BCAA on the activation of the mTOR-4E-BP1/p70S6K pathway, in vivo infusions of AA mixtures and BCAA evoke quite different patterns of anabolic response. Combining the forearm arteriovenous tracer balance technique and muscle biopsy, our laboratory has found that, in humans, systemic infusion of a physiological amount of AA increases protein synthesis with concurrent enhancement of 4E-BP1 and p70S6K phosphorylation, without affecting proteolysis (31a), whereas systemic infusion of BCAA decreases proteolysis without affecting protein synthesis, despite significant increase in 4E-BP1 and p70S6K phosphorylation (32). The mechanisms underlying this difference are not yet clear. The lack of anabolic action on protein synthesis during BCAA infusion is possibly due to the decline in plasma (perhaps intracellular) AA concentrations secondary to suppression of protein degradation, because the availability of AA per se regulates protein synthesis. The mechanism(s) by which BCAA retards proteolysis is poorly understood. Moreover, why a complete mixture of AA should be less effective in suppressing proteolysis than BCAA alone is not known. AA mixtures have been shown to modify, or even antagonize, insulin’s action at sites early in the insulin-signaling cascade, including the insulin receptor per se (45). Whether this might account for the lack of antiproteolytic action of infused AA requires further investigation. At this point, it cannot be discounted that the tracer methods being used to study the turnover of protein may be compromised in some manner not currently apparent.

CONCLUSIONS AND FUTURE DIRECTIONS

We are early along in our understanding of how the body regulates its protein mass. Major unanswered questions abound. Some are basic to our understanding of this homeostatic system. For example, why are the turnover rates of proteins so high relative to those of either carbohydrates or fats? Likewise, there is a major question as to whether there is coupling between the processes of protein synthesis and protein degradation that needs to be more directly addressed. This arises from observations that some hormones (e.g., insulin, IGF-I) have anabolic actions on both processes, whereas others (glucocorticoids) have dual anti-anabolic actions. Is there overlap in the signaling pathways mediating this? What are the early steps in the pathway of insulin’s and IGF-I’s anti-proteolytic effects? Questions focusing more particularly on pathological processes involved in body protein wasting also warrant further investigation. Addressing some of these questions will require new tools. These may include newer generations of mass spectrometers that will allow more facile measurement of the synthesis of specific proteins. In addition, better probes for addressing the functional status of the ubiquitin-proteasomal proteolytic pathways will need to be developed to examine the role of this potentially very important pathway in the in vivo regulation of protein metabolism.

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