Fed State Protein Metabolism in Diabetes Mellitus

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ABSTRACT Current knowledge concerning the physiologic regulation of protein anabolism during feeding is both limited and debated; little information is available on the effects of pathological conditions such as diabetes mellitus. This is due largely to methodological problems and the technical difficulties associated with the isotope dilution techniques used to estimate protein kinetics in the fed state. Data that are available suggest that meal intake induces protein anabolism in healthy subjects by reducing endogenous proteolysis and selectively stimulating protein synthesis, with amino acids and insulin playing the major regulatory role. Data obtained in subjects with noninsulin-dependent diabetes mellitus demonstrate that the impaired insulin action on glucose metabolism characteristic of that disease may not be associated with abnormal protein metabolism in the fed state. Severe insulin deficiency, as occurs in insulin-dependent diabetes, adversely affects nitrogen balance; at present, however, the effects of poor postprandial glycemic control on protein kinetics in subjects with insulin-dependent diabetes remain to be established. J. Nutr. 128: 328S–332S, 1998.

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Protein deposition occurring after meal ingestion is the net result of a complex interplay among the effects of substrates and several hormones on the rates of protein synthesis, breakdown and amino acid oxidation. Research in this area became feasible after the introduction of amino acid isotope dilution techniques (Schoenheimer and Rittenberg 1938). Carbon- and/or hydrogen-labeled essential amino acids or nitrogen-labeled nonessential or essential amino acids are administered by oral or intravenous routes; after an equilibration period, their dilution by exogenous and endogenous amino acids is used to estimate the rates of protein breakdown, synthesis and amino acid oxidation (Bier 1989, Fern et al. 1981, Millward et al. 1991, Waterlow 1967 and 1981, Waterlow et al. 1978). The technique employing C- or H-labeled essential amino acids uses a precursor product approach whose accuracy relies on correct estimation of the intracellular tracer/tracer ratio, the compartment in which protein kinetics occur. The technique of N-labeling of amino acids uses an end-product approach to estimate whole-body nitrogen turnover, in which the ratio between labeled and unlabeled nitrogen is measured in end products of protein metabolism such as urea and ammonia (Fern et al. 1981). Both the precursor and the end-product approaches are imperfect research tools because some of their assumptions are not fully validated (Bier 1989, De Feo and Haymond 1994, Millward et al. 1991, Waterlow et al. 1978).

ISOTOPE DILUTION TECHNIQUES IN THE FED STATE

The acute changes of protein kinetics induced by meal intake are more readily explored using essential amino acid kinetics than end-product approaches, which require a longer equilibration time for distribution of 15N tracer in the large whole-body nitrogen pool. Among the essential amino acids, leucine is the one most frequently employed for several reasons (De Feo and Haymond 1994). Intracellular and plasma leucine concentrations are quite similar; moreover, leucine is reversibly transamminated only intracellularly to its ketoacid (ketoisocaproic acid, KIC) which freely exchanges between the intracellular and the extracellular compartments. During a [1-13C]leucine infusion, the enrichment of plasma KIC is used as a surrogate for intracellular leucine enrichment (precursor pool enrichment). Because the first C atom of leucine is irreversibly oxidized and expired as CO2 when KIC is oxidized, the fraction of tracer expired as 13CO2 may be used to estimate leucine oxidation rate. Under steady-state conditions, the classic equation: B + I = S + O is used to determine whole-body protein synthesis and breakdown (Golden and Waterlow 1977), where B is leucine derived from protein breakdown, I is leucine derived from ingestion, S is leucine entering endogenous proteins and O is leucine undergoing irreversible oxidation. The total rate of leucine appearance (Ra = B + I) is calculated by dividing the tracer ([1-13C]leucine) infusion rate by the plasma KIC enrichment. The rate of leucine oxidation is calculated with the precursor-product approach by multiplying the frac-
tion of tracer expired as CO₂ by the total leucine flux. B and S are estimated indirectly by subtracting from total leucine flux (Q) the rates of leucine intake (B) and oxidation (S), respectively. This model is based on the following assumptions: 1) ingested leucine is completely absorbed and contributes entirely to total leucine Ra; 2) the rates of leucine appearance and disappearance are the same (true steady-state condition); 3) 1-C of oxidized leucine is completely expired as CO₂; and 4) plasma KIC enrichment reliably indicates intracellular leucine enrichment. The first assumption may be inspected by giving volunteers dual tracers (isotopomers) of the same amino acid. For example, oral deuterated leucine may be mixed with the meal and [¹³C]leucine infused intravenously to compare leucine Ra calculated with the two tracers. Such dual-tracer experiments show that leucine Ra calculated by the enteral tracer is greater than that calculated by the intravenous tracer, indicating that part of the enterally absorbed leucine tracer undergoes first-pass splanchnic removal (Biolo et al. 1992a, Hoerr et al. 1991, Horber and Haymond 1990, Matthews et al. 1993). These studies suggest that ~10–25% of dietary leucine does not contribute to total leucine Ra because it is sequestered by splanchnic tissue (gut and liver). Consequently, calculations based on intravenous leucine tracer enrichments overestimate meal-induced suppression of endogenous protein synthesis (B) because, in contrast to the postabsorptive state (Ra = B), in the fed state B is indirectly estimated by subtracting dietary leucine (I) from Ra.

The requirement for steady state imposes one of the greatest methodological problems for the researcher investigating protein metabolism in the fed state. In fact, this requirement is in open conflict with the acute perturbation of substrates and hormones that occurs when food is ingested. If the investigator chooses to respect the steady-state assumption, the meal may be administered over several hours, giving nitrogen preferentially as a solution of free amino acids by means of an enteral infusion. Using this approach, a near-steady state can be achieved after 3–4 h of infusion (De Feo et al. 1995, Horber and Haymond 1990, Volpi et al. 1996). However, initial changes induced by meal absorption are ignored, and the real effect of acute substrate and hormonal effects on protein kinetics is not precisely estimated. On the other hand, if the investigator prefers to closely reproduce the effects of the intake of a regular meal at the expense of the steady state, strange results, such as negative rates of endogenous leucine appearance, can be obtained (Boirie et al. 1996).

Another methodological factor that differentiates the absorptive from the postabsorptive state is the estimate of leucine oxidation. A portion of the leucine tracer C is not expired in the time frame of the experiment because it is retained within the body CO₂ pool. Because gluconeogenesis represents an important pathway for CO₂ recycling, the relative amount of labeled C retained will be greater during the fed state (~20%) than during the absorptive period (~10%) because of insulin-induced suppression of gluconeogenesis (Hoerr et al. 1989). Thus, a valid comparison of leucine oxidation in the fasted and fed states requires different correction factors for CO₂ fixation. This requires an additional study to measure the effects of meal intake on body CO₂ recycling by infusing [¹³C]-labeled bicarbonate (Hoerr et al. 1989).

The assumption that plasma KIC enrichment reliably indicates intracellular leucine enrichment at the site of leucine metabolism is crucial for the calculation of kinetic parameters, because all metabolic events occur in the intracellular compartment. The use of plasma KIC enrichment has been validated by Watt et al. (1991) for the measurement of muscle protein synthesis in postabsorptive humans by measuring muscle leucyl-tRNA enrichment. Enteral absorption of amino acids complicates the question because this perturbs the system by further diluting intracellular tracer leucine enrichment. It is possible that differences in the rates of entry of dietary amino acids into the cells of different organs (i.e., liver or muscle) or of different areas of the same organ (i.e., hepatic zonation, peri-portal cells preferentially using enteral amino acids) result in different precursor pool enrichments. The larger the difference, the greater the error involved in using plasma KIC enrichment, because this is an average value that is thought to be more influenced by muscle leucine metabolism than by splanchnic leucine uptake and metabolism (Bier 1989). In theory, the use of plasma KIC as a precursor pool enrichment for liver protein synthesis underestimates the rates of protein synthesis in the fed state because of the dilution of intrahepatic leucyl-tRNA enrichment caused by portal amino acids. Some experiments in humans support this hypothesis. Hepatic leucyl-tRNA enrichment has been indirectly measured during a continuous tracer leucine infusion using the enrichment of leucine derived from the hydrolysis of VLDL-apolipoprotein (apo)B100, a liver protein with a fast turnover rate (Cayol et al. 1996, Parhofer et al. 1991, Reeds et al. 1992, Volpi et al. 1996). It is reasonable to assume that leucine-apoB enrichment equals its leucyl-tRNA when apo enrichment reaches a plateau, i.e., when the plasma apoB pool has been replaced by newly synthesized protein. In the absence of dietary amino acids, leucine-apoB and plasma KIC enrichments are not statistically different (Cayol et al. 1996, Reeds et al. 1992, Volpi et al. 1996); however, during protein feeding, plasma KIC has been reported to be ~30% greater than leucine-apoB enrichment (Cayol et al. 1996, Reeds et al. 1992).

Using a different experimental approach (a liquid amino acid meal given by naso-gastric infusion), we were unable to disclose significant differences between plasma KIC and leucine-apoB specific activities (Volpi et al. 1996). Taken together, these data suggest that when liver protein synthesis is measured, it is important to determine the plateau leucine-apoB enrichment. Unfortunately, apoB reaches the plateau only after 5–8 h, requiring prolonged nonphysiologic periods of meal administration. The effect of these different methodological problems is substantial enough to prevent exact quantitative measurements of whole-body or single-protein kinetics in the fed state. However, when the study protocol is well designed, currently available techniques can at least indicate the direction of metabolic changes due to substrate intake and hormonal responses to feeding.

**FED STATE PROTEIN METABOLISM IN HEALTHY SUBJECTS**

The effects of meal intake on protein kinetics in healthy humans are not clearly established. There is agreement that protein feeding increases amino acid oxidation, but controversy exists concerning the mechanism(s) responsible for protein anabolism. In several studies, it was concluded that postprandial protein anabolism is due entirely to a reduced rate of endogenous proteolysis (Beaupre et al. 1989, Melville et al. 1989, Motil et al. 1981a and 1981b, Young et al. 1987), whereas others suggested that increased protein synthesis occurs as well (Clague et al. 1983, Collin-Vidal et al. 1994, De Feo et al. 1995, El-Khoury et al., 1995, Hoffer et al. 1985, Horber and Haymond 1990, Pacy et al. 1988 and 1994, Rennie et al. 1982, Volpi et al. 1996). These discrepancies might be more apparent than real for a number of methodological reasons reviewed in detail by McNurlan and Garlick (1989). In addition, the relative role played by decreased endogenous
proteolysis and increased protein synthesis is influenced by the amount and the form of nitrogen intake. Pacy et al. (1994) found protein synthesis to be increased during high (1.59 g/(kg·d)) but not lower (0.77 g/(kg·d)) protein meals. Collin-Vidal et al. (1994) demonstrated that oligopeptides induced greater estimates of protein synthesis than the equivalent amount of dietary nitrogen given as casein, but a lesser positive leucine balance because of higher leucine oxidation. Thus, when examining the mechanisms responsible for meal-induced protein anabolism, differences in meal composition and delivery might well account for differing results. For instance, leucine kinetics measured after the administration of a 4-h mixed liquid meal containing a mixture of free amino acids indicate poorly controlled diabetic patients and whether or not intensive insulin treatment is necessary to normalize protein metabolism. Answers to this question should provide sound information about protein dietary requirements for well- or severely prandial insulin deficiency on meal-induced protein anabolism. The latter was partially corrected by intensified insulin therapy. Thus, their results suggest that impaired prandial protein anabolism in IDDM is due at least in part to increased proteolysis, decreased protein synthesis or a combination of these.

Amino acids and insulin play the major roles in promoting postprandial protein anabolism, and it is therefore highly relevant to establish their relative contribution in this process when considering insulin and dietary treatment of diabetic patients. We have examined this issue by comparing the effects on leucine kinetics of an intragastric infusion of water (control), a liquid glucose-lipid-amino acid meal (Meal + AA) or an isocaloric glucose-lipid meal (Meal – AA) that induced the same insulin response as the Meal + AA (Volpi et al. 1996). The results of the study suggest that dietary amino acid account for almost 90% of fed state protein anabolism by decreasing proteolysis and increasing protein synthesis, whereas endogenous hyperinsulinemia accounts for the remaining 10%, primarily by decreasing amino acid oxidation. In comparison to the direct intravenous infusion of insulin and/or amino acids (Castellino et al. 1987, De Feo et al. 1993, Inciostro et al. 1992, Luzi et al. 1990, Tessari et al. 1987), our data show that prandial endogenous hyperinsulinemia is less effective in reducing whole-body proteolysis and suggest, in agreement with those of Biolo et al. (1995b) and Collin-Vidal et al. (1994), that meal-induced reduction of protein breakdown is due primarily to hypermimacidemia.

Studies exploring the effects of feeding on tissue or individual protein synthesis demonstrate that meal intake has differential effects on protein synthesis in humans. Among the plasma proteins, albumin synthesis increases by ~30–50% (Cayol et al. 1996, De Feo et al. 1992, Hunter et al. 1995, Volpi et al. 1996) and that of immunoglobulin (IgG) by ~50% (De Feo et al. 1995, Volpi et al. 1996), whereas fibrogen (Cayol et al. 1996, Volpi et al. 1996) and antithrombin III (Volpi et al. 1996) synthesis do not change. Albumin synthesis is increased by ~20% by isolated postprandial hyperinsulinemia and by ~50% by combined hyperinsulinemia and dietary amino acids, whereas dietary amino acids alone are responsible for the increase in IgG synthesis (Volpi et al. 1996). During meal absorption, mixed muscle protein increases (Rennie et al. 1982), but the contribution of insulin to this increase is debated. Several studies suggest that in the postabsorptive state insulin does not increase muscle synthesis in normal or diabetic subjects (Bennet et al. 1990, Gelfand and Barrett 1986, Pacy et al. 1989). However, the methodology used to estimate muscle protein synthesis has been criticized by Biolo et al. (1995a). By using leg arteriovenous catheterization combined with muscle biopsy, they concluded that insulin promotes protein anabolism primarily by increasing protein synthesis. Further studies are required, perhaps including ones examining the effects of feeding and insulin on individual muscle protein synthetic rates.

**FED STATE PROTEIN METABOLISM IN DIABETES**

Investigations of protein kinetics in diabetic patients in the fed state are limited and inconclusive. A clinically relevant question that still remains to be answered is the effect of partial or severe prandial insulin deficiency on meal-induced protein anabolism. Answers to this question should provide sound information about protein dietary requirements for well- or poorly controlled diabetic patients and whether or not intensive insulin treatment is necessary to normalize protein metabolism. At present, the consequences of a partial impairment of insulin action on protein kinetics can be extrapolated from the studies performed in noninsulin-dependent diabetes (NIDDM). There is agreement that in postabsorptive NIDDM, leucine estimates of whole-body protein kinetics are not different than normal (Luzi et al. 1993, Nair et al. 1983, Staten et al. 1986) in spite of clear alterations in glucose kinetics (Luzi et al. 1993). Biolo et al. (1992b) examined phenylalanine metabolism in non-obese mild NIDDM in both the postabsorptive and absorptive state. In the postabsorptive state, phenylalanine Ra was comparable to that of control subjects. Similarly, in the fed state, total and endogenous phenylalanine Ra, splanchnic uptake, oxidation and nonoxidative disposal of ingested phenylalanine were not different from those of normal controls. Thus, these data, which agree with earlier observations in subjects with NIDDM and a normal lean body mass (Bogardus et al. 1986), suggest that protein metabolism is normal in NIDDM despite the impaired insulin action on glucose metabolism. However, Gougeon et al. (1994), using the [13C]glycine end-product method, reported higher than normal rates of protein breakdown in poorly controlled NIDDM and a more negative nitrogen balance after a hypocaloric diet.

The effects of severe insulin deficiency on interorgan exchange of amino acids during feeding were described 20 years ago by Wahren et al. (1976), who investigated insulin-deprived subjects with insulin-dependent diabetes (IDDM). After the ingestion of a protein meal, the splanchnic release of amino acids was comparable to that observed in controls. However, arterial concentrations of branched-chain amino acids (BCAA) were 30–50% greater in persons with IDDM, and leg amino acid balance demonstrated that, in contrast to controls, persons with IDDM had only a transiently positive BCAA balance. Thus, these data suggest that postprandial muscle protein anabolism is impaired in insulin-deprived IDDM (Wahren et al. 1976). Whether the impairment is due to increased proteolysis, decreased protein synthesis or a combination of these is still unclear. Tessari et al. (1988) compared the rates of whole-body leucine appearance, after the rapid consumption of a chemically defined elemental test meal, in a group of insulin-insufficient IDDM subjects, another of well-controlled IDDM subjects and one of normal controls. These authors confirmed the presence of hyperleucinemia in poorly controlled IDDM and observed normal rates of dietary leucine absorption in the presence of increased leucine Ra from endogenous protein. The latter was partially corrected by intensified insulin therapy. Thus, their results suggest that impaired prandial protein anabolism in IDDM is due at least in part to less effective suppression of whole-body proteolysis (Tessari et al. 1988). However, their conclusion must be regarded as uncertain because the study was performed under nonsteady-state conditions. More recently, Biolo et al. (1995b) re-investigated...
the study, under near steady-state conditions, the effect of mixed-meal ingestion on leucine and phenylalanine rates of appearance in insulin-deprived IDDM subjects and in normal controls. In both the postabsorptive and fed states, IDDM subjects had increased leucine and phenylalanine Ra, but the absolute reduction in leucine and phenylalanine Ra in the postabsorptive state was comparable in the two groups, suggesting that whole-body proteolysis is suppressed in IDDM in spite of insulin withdrawal, likely as a result of hyperaminoacidemia. Because C-labeled leucine was not infused in this study, it could not give information about protein synthesis and leucine balance.

**LITERATURE CITED**


