Protein Metabolism in Diabetes Mellitus: Implications for Clinical Management

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Diabetes mellitus is characterized by derangements in the metabolism not only of glucose and fat but also of protein (1). However, protein has always received less attention than fat and glucose, both in terms of alterations in its metabolism and in its nutritional implications. Although hyperglycemia and its consequences have always been the hallmark of the disease and plasma glucose the main index of diabetic control, altered protein metabolism was recognized even in the preinsulin era because of the severe muscle and other protein depletion that occurred, even with an apparently adequate protein intake. In 1906, the German investigator Bernhard Naunyn observed an increase in glycosuria with increased dietary protein and recommended that protein intake as well as carbohydrate intake should be restricted (2). In the preinsulin era (1922), Marsh et al. addressed the issue as to whether “the laws that govern protein metabolism of non-diabetic subjects applied equally to those with diabetes” (3). These investigators concluded, in contrast to Benedict and Joslin [cited in (3)] who preceded them, that protein requirements for achieving nitrogen equilibrium were similar to those of nondiabetic persons. However, this was in a setting in which glycosuria had been eliminated by the high-fat, low-carbohydrate diet that was then the only way of achieving metabolic control.

There intervened a long period in which a disproportionately small amount of attention was paid to the question of protein metabolism. It is informative to trace the trends in nutritional recommendations for persons with diabetes from the preinsulin era to the present. With the advent of insulin and oral agents and with data emerging from clinical and experimental research, nutrition recommendations have evolved from low-carbohydrate, high-fat to high-carbohydrate, high-fibre, low-fat, low-cholesterol diets (4,5). From 1935 until the current decade, guidelines for protein intake had remained at 85–90 g/day or approximately 18% of energy intake. One report of intakes during World War II gave values of 68 g protein and 2150 kcal/d, which were associated with general improvement of “adult diabetes” (diabète gras) (6). The benefit was most likely to have been related to the energy restriction. The
current dietary recommendations in many countries are meant to apply whether diabetes results from an absolute or a relative deficiency of insulin. Protein intakes of the order of 1.5 g/kg ideal body weight appear to be habitually consumed by the diabetic and nondiabetic populations alike in North America. This represents nearly twice the amount recommended for healthy diabetic and nondiabetic individuals, which, at 0.8 g of complete protein/kg/day, provides a margin of safety in the latter.

What remains uncertain at present is the appropriateness of either the usual or the recommended intakes, because the state of protein metabolism of both type 1 and type 2 diabetic persons receiving conventional treatment is not yet clearly defined. Various studies of fasting amino acid and protein kinetics using isotopic amino acid turnover methods have been done, beginning in the 1980s, and these are reviewed below. The integrated fasting-fed cycle has not been the subject of so much investigation, however, since methodological problems have precluded precise study of the fed state. It has been presumed that, since no specific clinical disorder in persons with diabetes can yet be ascribed to altered protein metabolism, the habitual intakes are safe. Clearly, the rates of accumulation of advanced glycosylation end products in structural and other proteins will be affected not only by the prevailing plasma glucose concentrations but also by the rates of turnover of proteins. For this and other reasons, definition of fed-fasted protein metabolism at different levels of diabetes control, as well as different protein intakes, is mandatory.

The goals of nutrition therapy for diabetes are to achieve and maintain a normal metabolic state. By attaining an optimal body weight and normal blood glucose, glycosylated proteins, and desirable plasma lipids, it is hoped that it will be possible to retard or reduce complications such as retinopathy, nephropathy, and neuropathy that are specific to the disease (4). In this regard, the recent Diabetes Control and Complications Trial (DCCT) results strongly support the value of intensive control in persons with type 1 diabetes (see Crofford in this volume). Of the estimated 14 million North Americans with diabetes mellitus, type 2 diabetes accounts for 90–95% of all cases. Since 70–80% of these are obese, treatment should include weight reduction through energy restriction. Energy restriction itself has an impact on the efficiency of utilization of dietary protein even in persons without diabetes, such that more is required as the energy deficit increases. This is another good reason for studying protein-energy relationships at different levels of diabetes control in obese subjects.

Furthermore, 25% of new-onset, chronic renal failure is related to diabetes, and protein restriction is now being recommended for both diabetic and nondiabetic persons with incipient renal failure (7). Some investigators have been concerned with the generous protein recommendations for diabetes because studies in experimental animals show that dietary protein contributes to the development of azotemia and renal failure (8), and there is a growing body of literature that suggests that dietary protein restriction is of value as a preventive or therapeutic measure for renal insufficiency (7). Protein intakes of 0.62 g/kg body weight, with associated reductions in phosphate, fat, and sodium, have been shown to retard the rate of decline of glomerular filtration rate in diabetic nephropathy (9). However, one study of protein intakes
in a cohort of type 1 diabetic subjects compared by degree of nephropathy failed to show significant differences (10). It has been reported that protein consumption influences glomerular filtration rate but not microalbuminuria (11). One contributor to the present uncertainty is that diabetes control is likely to be a major regulator of the disposition of ingested protein, a variable that has not been controlled for systematically. This makes it even more important to determine the extent to which the relative or absolute reduction in insulin action in diabetes alters protein metabolism, and if optimal control of diabetes (as indicated by normoglycemia, glycated hemoglobins, or fructosamine) will normalize protein metabolism in the setting of the current standards of diabetes practice.

Other settings in which the appropriate levels of protein intake in relation to level of diabetic control need to be defined include infancy, childhood, and adolescence, when there is a need for rapid rates of body protein accretion. Likewise, during pregnancy and lactation, the changed physiological demands have well-defined effects on nutrient requirements, but, with either pregestational or gestational diabetes, these may well be altered, in particular the requirements for protein.

There is clear experimental evidence dating from the 1950s (12) that the diabetic state affects protein metabolism and that insulin is an important regulator of the response of protein metabolism to nutrient intake in achieving net protein retention. Thus a deficiency of insulin or insulin action may be associated with an abnormal handling of dietary protein such that minimum requirements may differ from those in normal persons if they are to be considered safe. Concern has been expressed (7,13,14) as to what the safe levels of protein intake are in diabetes and to what extent restrictions can be recommended in the establishment of a prudent nutrition plan. Such concerns are supported by the work of the group of Hoffer, who showed an 18% increase in obligate minimum nitrogen excretion in very intensively treated type 1 diabetic subjects compared with normal controls, using the protein-free diet technique (15). A significant correlation between average plasma glucose and minimum nitrogen excretion was found even in a range of plasma glucoses that spanned the normal and minimally elevated range. These authors suggest that even with rigorous glycemic control, optimal protein metabolism might require higher protein intakes, and these could be even higher in patients treated with conventional insulin regimes. Proof that the concern is justified was provided by the recent study by Brodsky et al. that clearly showed that protein restriction to 0.6 g/kg/d in type 1 diabetic subjects with early nephropathy compromised their protein metabolism (16).

Data obtained from nitrogen (N) balance studies have shown that insulin withdrawal is associated with an increase in urinary nitrogen loss, which is reversed by restoration of insulin therapy. However, nitrogen balance studies do not distinguish between changes in the rates of protein synthesis and breakdown, and total insulin withdrawal is a very coarse stimulus that does not necessarily give clinically relevant information applicable to conventionally controlled diabetic persons (13). Because of the limitations of the nitrogen-balance method, studies carried out during the past decade particularly have estimated whole body protein turnover using the $^{13}$C-labeled essential amino acid leucine (or occasionally other amino acids and other labels) as
a tracer. Recent studies of protein metabolism inferred by leucine kinetics (flux, synthesis, and breakdown) have shown that it is the cooperative action of the increases in plasma concentrations of insulin and amino acids (branched chain amino acids, possibly leucine) that mediates the response of protein metabolism to food intake. The combination of increased insulin along with exogenous amino acids enhances the rate of protein synthesis and decreases the rate of whole body protein breakdown, resulting in a net retention of amino acids in body protein (17). Such observations have been made in humans (17,18) and in dogs (19) with infused insulin and/or amino acid mixtures that would result in an increase in endogenous insulin (17,18).

Controversy exists as to whether insulin alone can increase protein synthesis (20). Studies by Garlick and Grant [reported in (20)] in postabsorptive rats indicate that infused insulin producing concentrations well above those found in the normally fed animal stimulated protein synthesis but had a maximal effect on muscle protein synthesis at concentrations within the physiological range when given in combination with amino acids. The authors concluded that the response of muscle protein synthesis to feeding arises from an increase in the sensitivity to insulin brought about by amino acids. In vitro studies in skeletal muscle of the effects of amino acid and insulin treatment on protein synthesis and breakdown suggest that myofibrillar protein degradation may be inhibited by dietary protein but not by circulating insulin (21) [the inhibition possibly being brought about by the α-keto acid of leucine (22)], and that insulin stimulates protein synthesis. In contrast, in vivo postabsorptive studies of insulin infusion (in the absence of an exogenous source of protein) have shown that the primary effect of insulin is to restrain whole-body proteolysis, as assessed by a decrease in leucine release from protein. Such studies have not shown an effect of insulin on synthesis or leucine incorporation into protein (23–25). In the absence of exogenous amino acids, insulin, by inhibiting proteolysis, decreases the release of many amino acids from insulin-sensitive tissues, thereby decreasing the substrate available for protein synthesis. This would appear to be responsible for the apparently paradoxical whole-body decrease in protein synthesis found in such experiments. Insulin deficiency has also been associated with a decrease in the albumin fractional synthetic rate (25).

Taken together, the in vivo and in vitro experiments are conclusive that amino acids, as well as insulin, elicit responses in protein synthesis and breakdown similar to those resulting from feeding, these two factors acting cooperatively (20). What modifications must be made in one factor when the other is deficient (such as insulin in diabetes), to ensure the maintenance of normal body composition and function? What modifications are also indicated when energy restriction is superimposed on the relative insulin deficiency, such as during weight reduction in the obese type 2 diabetic population? Conventional, energy-restricted diets for weight reduction, providing 1500 kcal with 18% of energy derived from protein, would supply only 67 g of protein. Recent recommendations for low-energy balanced diets, e.g., less than 1200 kcal/day, have included as much as 28% of energy from protein to provide 0.8–1.2 g protein/kg body weight. We have shown a return to nitrogen equilibrium
Altered postabsorptive-state amino acid metabolism has been well documented in type 1 diabetic human subjects using isotopic tracer techniques: rates of leucine turnover and oxidation are reported to be increased during insulin withdrawal (27-31) and are not completely corrected by conventional insulin therapy (28,29,31,32). The lowering of plasma glucose to normal levels by intensive insulin therapy reduced leucine turnover and oxidation rates (28-31). These observations indicate that, in type 1 diabetes, poor glycemic control is associated with altered leucine kinetics, suggesting increases in protein breakdown greater than those in protein synthesis; the consequent net protein loss can be returned toward normal with optimized insulin therapy, a result of a reduction in protein breakdown. The apparently paradoxical increase in protein synthesis is thought to be caused by greater availability of amino acids (from the increased catabolism). The tissues in which such increases in synthesis occur appear to be mainly splanchnic, from recent regional catheterization studies by Nair et al. (33). Likewise, these authors showed the in vivo effect of insulin to decrease protein turnover to be due to splanchnic tissues.

Glycine fluxes were significantly higher in "adequately treated" normoglycemic pregestational pregnant diabetic women, suggesting that plasma glucose levels do not necessarily reflect the static or dynamic parameters of other fuels affected by insulin, such as amino acids. Greater glycine fluxes were associated with delivery of fetuses with higher birthweights than those of control nondiabetic and gestational diabetic women (34).

In contrast to the considerable number of studies in type 1 diabetes, few previous studies have reported the kinetics of protein metabolism in type 2 diabetes. Those available led Bier in 1991 to conclude that "body protein metabolism is, by and large, essentially normal in" this type of diabetes, when studied in the fasted state (35). For example, Staten et al. (36), in studies of leucine turnover and oxidation, have found no difference between conventionally controlled, insulin-treated type 2 diabetic subjects, matched obese nondiabetic subjects, and normal weight subjects. Intensive insulin therapy sufficient to normalize blood glucose did not alter leucine flux and oxidation. This contrasts with the increased leucine flux and oxidation reported in hyperglycemic type 1 diabetic subjects, which could be normalized with insulin therapy. The authors concluded that despite similar levels of hyperglycemia, it is possible that in type 2 diabetes during conventional insulin therapy sufficient insulin action is present to control amino acid metabolism (36). In contrast to the other studies cited by Bier (35), Umpleby et al. showed increased leucine oxidation (although no other aspect of leucine kinetics was increased) when studied diurnally (37). Intensive insulin therapy was shown to decrease plasma glucose and glycerol levels, which, when taken together with the leucine data, was interpreted to suggest that the sensitivity of leucine metabolism to insufficient insulin action may differ from that of glucose.
and fat metabolism (36,38). Differential effects of insulin are known to occur at different plasma concentrations. Acute physiologic increases of insulin had no effect on nitrogen accretion in human skeletal muscle in healthy men during a protein meal. The major determinant of amino acid uptake across human forearm tissue appears to be the amino acid concentrations. However, more prolonged hyperinsulinemia (36–48 h) resulted in a significant increase in nitrogen accretion, indicating that a time-dependent induction of enzymes may be required for protein synthesis (39). Furthermore, Staten et al. (36) investigated a very specific obese diabetic population requiring insulin studied in the fasting state and during insulin therapy that maintained blood glucose at levels averaging 9.7 mmol/liter.

As noted above, one important limitation of the use of the $^{13}$C-leucine kinetic methodology at present is that it can give useful data only in steady-state conditions. Thus, most studies in both type 1 and type 2 diabetes have reflected the fasted state and cannot be readily extrapolated to the fed-fasted, nonsteady-state that prevails in daily life. For this reason, we have chosen to use the longer and more tedious $^{15}$N glycine method, because it gives data that are integrated over the fed and fasted states. For this (and other) reasons, we realize that the approach yields data that give rather imprecise inferences about the rates of protein synthesis and breakdown. It relies on end product $^{15}$N enrichment in urine urea nitrogen, and on a number of assumptions relating to compartments of amino acids and to urea and its excretion. The calculations are based on the model of Picou and Taylor-Roberts [cited in (26)]. Of particular interest is the fact that this approach allows for repeated measurements in the same individual in different nutritional states and at different levels of diabetes control. It involves 3-hourly oral $^{15}$N-glycine dosing, with corresponding timing of urine collections and meal consumption, over a total of 60 hours. We have chosen to include 3 days and 2 nights in the 60 hours, with nutrients in formula form divided into six equal meals during the day. The only other way of using the stable isotopic methodology with $^{13}$C-leucine in the fed state would be to assure a continuously fed state during the tracer infusion, and this does not approximate to the physiological nonsteady-state related to meal consumption. Thus we have sacrificed the somewhat greater precision in estimating turnover parameters for the capacity to make “integrated feeding-fasting” estimations. We are not aware that anyone else has used this approach in persons with diabetes mellitus.

We first quantified protein turnover in this manner in obese nondiabetic persons in the isoenergetic weight-maintained state and then during the state of maximum endogenous fat mobilization with a protein-sparing modified fast (a very low energy, all protein diet, or VLED) (26). This approach was used because others have previously shown that such diets are capable of markedly reducing (and even normalizing) plasma glucose levels in people with type 2 diabetes (e.g., 40,41). Our strategy was thus to study such obese type 2 diabetic subjects first while markedly hyperglycemic and off all therapy, and then after 4 and 6 weeks of a VLED, to compare their nitrogen balance and $^{15}$N-glycine results with those of the control obese subjects. The VLED contains a generous amount of protein. Having shown considerable differences, we
then undertook to determine responses over one isoenergetic diet period in comparable subjects in whom intensive insulin therapy was employed, after which we allowed them to become hyperglycemic off insulin (again at isoenergetic intakes), and then again after 4 weeks of a VLED. This allowed us to compare the effects of rigorous short-term glycemic control with more moderate hyperglycemia, and then with near-euglycemia during the VLED.

In the first study, the isocaloric diet in the obese and type 2 diabetic subjects contained a fixed 80 g/day of good quality protein, and the VLED consisted entirely of 93 g/day of a collagen hydrolysate-based diet that provided 1.7 MJ/day (400 kcal/day). Collagen hydrolysate was used because of its palatability; it was methionine and tryptophan supplemented and provided sufficient essential amino acids to meet the current WHO recommendations. (We have since shown that such diets result in rather similar nitrogen balance and $^{15}$N-glycine kinetic responses to those obtained during diets with the same protein content but with a higher proportion of essential amino acids, unpublished.) Since the subjects were comparable with respect to anthropometric variables and since the protocol was otherwise identical, the results of the seven nondiabetic subjects (26) can be directly compared with those of the seven type 2 diabetic subjects (42).

Figure 1 shows that off-treatment mean fasting and mean daily (premeal and bed-
time) glycemia in the diabetic subjects was markedly increased, at $15 \pm 2 \text{ mmol/liter}$, compared with $5.9 \pm 0.6 \text{ mmol/liter}$ in the control subjects. Such hyperglycemia is not uncommonly encountered in certain treated patients. It was associated with polyuria and polydipsia (mean 24-h urine glucose $580 \text{ mmol/d}$) but with no undue discomfort in our subjects for the short duration of the study. Additional carbohydrate was given to approximate the measured urine glucose losses. Whereas fasting plasma free fatty acids were mildly raised ($860 \mu\text{mol/liter}$), blood, breath and urine ketone bodies were not increased in the diabetic subjects during the isoenergetic $^{15}$N-glycine study.

With this protein intake, which was of the order of $0.85 \text{ g/kg/d}$, nitrogen balance was in equilibrium in both diabetic and nondiabetic subjects during the isoenergetic diet (Fig. 2). Had this been the only information available, the temptation would have been [as in the type 2 diabetic subjects previously studied, including the leucine kinetic data (35)] to infer a relative "resistance" of the untoward effects of type 2 diabetes on protein metabolism. However, as shown by the $^{15}$N-glycine-derived data (Fig. 3), whole body N flux was increased by 13%, protein synthesis was increased by 16%, and protein breakdown was increased by 21%. This resulted in net synthesis (S-B) that was negative in the diabetic subjects and significantly different from that of the control subjects. Thus the maintenance of N balance in the diabetic subjects under the conditions of this study was at the cost of increased rates of protein turnover. We cannot extrapolate these results to what happens in a more chronic state.
FIG. 3. Whole body protein kinetics measured by the $^{15}$N-glycine method during isoenergetic feeding and at weeks 4 and 6 of a 1.7 MJ/day collagen hydrolysate-based protein diet (VLED) in obese subjects without (solid bars) and with (shaded bars) type 2 diabetes. (A) N flux (Q); (B) rate of protein synthesis (S); (C) rate of protein breakdown (B); (D) net protein synthesis (S-B). Data are means ± SE.
of hyperglycemia, nor can we speculate upon whether the prior state of glycemic control was such that the subjects had already accommodated to a chronic challenge to maintain their protein metabolic status. Notably, within the group of seven diabetic subjects, there was no significant correlation between mean glycemia and the protein turnover variables. This is probably because their levels of hyperglycemia were rather comparable. However, significant correlations emerged when the data for the control and diabetic subjects were combined. Thus the possibility of there being such an association is raised, but clearly requires the study of a dose-response between graded levels of diabetic control and their effects on protein metabolism. This is presently being done in our laboratory. Having shown that comparing best (i.e., normal) with worst case glycemic conditions, we now address the responses of both groups to one approach to normalizing the hyperglycemia, the VLED.

There was a rapid decline in plasma glucose during the first week of the VLED to values similar to, but remaining slightly above, those of the control subjects (Fig. 1). Several of the subjects' values were superimposable upon those of the control subjects. The initial pattern of N balance response was similar to that of the control subjects, namely a shift toward greater negative balance, but it was sustained at a greater level in the diabetic subjects. Whereas a progressive improvement occurred in the control subjects, with N equilibrium being achieved at the third to fifth weeks, the mean values always remained negative in the diabetic subjects (Fig. 2). In both groups, the S and B declined, and total flux decreased in the diabetic subjects, such that at 4 and 6 weeks they no longer differed significantly (Fig. 3). However, S-B was more negative at week 4, and remained less than in controls at both 4 and 6 weeks.

The greater negative N balance for the first 2-3 weeks might have been due to the finite time required for the diet, and the weight loss it caused, to restore plasma glucose to near normal. The continued negative N balance, and the less favorable S-B at 4 weeks, was not due to the diabetic subjects with higher plasma glucose values at this phase. Nor was it directly attributable to plasma total immunoreactive insulin levels, since those were comparably high during the isoenergetic diet, falling rapidly during the first week of VLED and slightly thereafter in both groups (not shown). Clearly, the insulin resistance/insensitivity was markedly greater in the diabetic subjects while they were hyperglycemic, but we have no independent direct measure of this later in the VLED. It remains a tenable (and testable) hypothesis, however, that there is resistance to the effects of insulin on protein turnover (those components that are affected by insulin), and that this persists to a lesser degree during the VLED. Our insulin assay does cross-react with proinsulin, so the totals measured could also obscure the possibility of a greater proportion of proinsulin in plasma of the diabetic subjects. Whether or not this is a factor, hypoinsulinemia relative to that required for the maintenance of normal glucoregulation was also present. Insulin secretion was not assessed directly by other methods in this study. We cannot exclude the possibility that the diabetic subjects had been chronically adapted to the abnormal protein metabolism, and that their ability to respond to this protein conservation challenge had thereby been impaired. Although the protein
intakes given were previously presumed to be in the range appropriate for both control and diabetic subjects, the present data further support the notion that a clinically inapparent abnormality is present, obscured by the fact that the amounts habitually ingested represent a surfeit (13). Finally, for analogous reasons, though 93 g/day (1.0 g/kg/day or 1.3 g/kg/day adjusted to a body mass index of 25 kg/m^2 per day) of the protein used was sufficient for control subjects to achieve a successful adaptation (at least until 5 weeks of VLED), it is conceivable that a higher quality protein might be required in the diabetic subjects. Nonetheless, these results add considerably to what has been reported previously with VLED (40,41).

In the next experiment, various changes were made to respond to these issues, and to test whether intensive insulin treatment aimed at rapid correction of the hyperglycemia would correct protein turnover in the isoenergetic state. In otherwise comparable type 2 diabetic subjects, high quality protein (45% versus 18% essential amino acids) was used at 93 g/day throughout. The energy intake was kept isocaloric for the first 14 days. Aggressive insulin therapy was used, with multiple daily injections of biosynthetic human regular and NPH, and a rapid increase in dose over the first week (mean 150 ± 13 U/day) aiming for normoglycemia during the last 3 days, during the \(^{15}\text{N}-\text{glycine}\) study. This was followed by a period of equal duration off all therapy and remaining at isoenergetic intake, and finally a 4-week VLED, again off all other therapy. The glycemic responses were such that mean premeal capillary glucose values were about 6.0 mmol/liter with insulin, 13 mmol/liter off insulin, and again near normal with the VLED.

The \( \text{N} \) balance was positive with insulin therapy, suggesting that this group may have been in a state of previous protein depletion since such a response would not otherwise have been predicted at this level of protein intake (Fig. 4). N balance reverted to equilibrium off insulin, then showed negative values with failure to return to equilibrium during the VLED as was the case in the previous subjects. The \(^{15}\text{N}-\text{glycine}\) results are shown in Fig. 5. A markedly greater total flux (Q) and B were observed off insulin, associated with a significant decline in net synthesis (S-B) despite an increase in mean S. All three parameters of turnover decreased with the VLED, but once again S-B remained negative as it had in the first study (Fig. 3).

The conclusions from these latter unpublished data are as follows: the more modest level of hyperglycemia that followed the insulin therapy is nonetheless still associated with an undesirable trend in N balance, and with the same abnormalities shown in the preceding study. Viewed in the reverse, correction of blood glucose with insulin, even in such insulin-resistant subjects, has marked beneficial effects on protein turnover. These effects occurred rapidly (within days), and at protein intakes well within the range likely to be consumed by a large proportion of type 2 diabetic persons. The VLED that followed the return to hyperglycemia was once again unable to normalize the \(^{15}\text{N}-\text{glycine}\) responses completely, despite its effectiveness in normalizing glycemia and despite the administration of a high-quality protein. The latter again implies that the phenomenon under study is not one that can be demonstrated only under extreme conditions of hyperglycemia or during therapy with very large insulin doses.
FIG. 4. Daily N balance during isoenergetic (ISO) feeding with and without insulin therapy and during the 1.7 MJ/day casein-soy protein diet (VLED) in obese subjects with type 2 diabetes. -INS signifies no insulin treatment; +INS signifies that insulin was given. Data are plotted as means ± SE.

FIG. 5. Whole-body protein turnover measured by the $^{15}$N-glycine method during isoenergetic (ISO) feeding with or without insulin therapy and at week 4 of the 1.7 MJ/day casein-soy protein diet (VLED) in obese subjects with type 2 diabetes. -INS signifies no insulin treatment; +INS signifies that insulin was given. Data are presented in g N/day as means ± SE.
CONCLUSION

In summary, the main thrust of this review has been to present current knowledge on whole-body protein metabolism in human diabetes mellitus. We propose that because protein metabolism can be shown to be abnormal in both type 1 and type 2 diabetes when moderate to marked hyperglycemia is present, this has potential clinical importance. The demonstration that improvement or correction of the protein metabolic abnormalities can be achieved by tight control of plasma glucose has implications for treatment. There are as yet insufficient data to allow us to correlate the magnitude of the abnormalities in protein metabolism with those of glucose (or fat) metabolism. However, data in type 1 diabetes (13,15,16), as well as that presented in this chapter, suggest that the previously held view that protein metabolism is less sensitive to the deficiency in insulin and/or to the actions of insulin than is the metabolism of glucose or fat must now be reassessed.

Hoffer has stated the case in relation to the possible impact on dietary protein requirements (13). The amounts required to optimize protein metabolism as well as the responses to experimental diets may be different in diabetic people and in normal individuals, even if the diabetes is rigorously controlled. The end points currently used in this type of clinical investigation have imprecisions, and none of the whole-body techniques is able to determine which of the multitude of body proteins are most susceptible to the abnormalities shown. What seems apparent to us, however, is that the previous failures to find abnormalities in type 2 diabetes were related to the methodologies used, which restricted the studies to the fasted state. The implication is that greater abnormalities occur in the markedly non-steady-state conditions that are related to ingestion and absorption of the exogenous protein. These are the times of the day when it is most difficult to achieve normalization of glucose metabolism as well, even during glycemic control using the “artificial pancreas” (43). Even with very precise glycemic control, the excursions of certain blood amino acids (including leucine) were actually overcorrected (i.e., they did not rise as much as normal) (43). It is possible that the peripheral blood hyperinsulinemia that is required to normalize glucose excursions may have been responsible. More precise methods are needed to test fed-state amino acid metabolism in this setting. One of these might be the approach recently published by Hoffer’s group (44), and others might be to study regional protein metabolism by the approach developed by Nair (33,45), or the synthesis of specific proteins as reported by De Feo et al. (25).

Even with a liberal protein intake, as used in our studies, the fact that hyperglycemia is associated with protein-kinetic and even N balance abnormalities provides yet another argument to support the conclusions of the DCCT in relation to the consequences of hyperglycemia and extrapolation of the results to type 2 diabetes. Future studies may define the amounts of dietary protein that seem to be required at different levels of glycemic control. We would argue that for both theoretical and practical reasons the management goals should include normalization of protein metabolism at the current usual intakes, by considering protein in addition to the glycemic, glycosylated protein, and lipid end points that are currently established.
This will require definition of the dose-response of level of glycemic control versus protein metabolism in vivo with the best tools available. As noted above, it could well be that an accelerated rate of protein turnover due to diabetes, even without ongoing net loss of body proteins, could place those proteins at greater risk of abnormal function through increased glycosylation resulting from the concurrent hyperglycemia. For the present, the chief therapeutic end point should still be to normalize glycemia, since this appears to have the greatest chance of normalizing protein metabolism at the usually taken and usually recommended intakes. The conundrum of incipient nephropathy remains; Brodsky et al. (16) have shown the risks of protein restriction, yet its benefits in slowing progression of nephropathy seem at this time to be reasonably well documented. Optimal diabetic control, with no more than 0.8 g/kg of reference body weight/day, would be the best compromise for the present, but this area, as well as the area in general, needs more research. This has been emphasized by the authors of the most recent review of the nutrition recommendations of the American Diabetes Association (46).

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REFERENCES


**DISCUSSION**

**Dr. Jiang:** What kind of natural food that is rich in fiber do you recommend?

**Dr. Marliss:** The source of fiber should be foods containing complex carbohydrates such as whole grain cereals and the like that are fairly typical dietary components in many parts of the world and have always been so. I do not at this stage recommend a supplement of fiber. I think Dr. J. Anderson who is a bit of an evangelist in relation to the use of fiber, probably recommends fiber more than other people do. One problem with very high intakes of the best kind of fiber in terms of plasma glucose control is that there is a substantial increase in flatulence.

**Dr. Drash:** Years ago, I had an interesting conversation with Ted Danowsky with regard to diet in diabetes. He said that diabetics ought to eat whatever a Chinese coolie eats. I think the point is a very good one. What he was saying was that diabetic people should eat a diet that makes them lean, mean, and fit. How does a long-term program of physical fitness change the diet?

**Dr. Marliss:** There are no really substantial differences in the diet required by an individual who engages in a fitness program as distinct from an intensive training program for competitive athletics, which is not I think what you were talking about. The kinds of recommendations that are made (such as in the US Recommended Dietary Allowances, or by various national diabetes organizations) are not meant to be what the mean of the population should be taking, but what the mean plus two standard deviations of the population might take in order to provide a margin of safety for the vast majority of individuals. The question becomes much more complicated when we are talking about diabetic individuals and trying to decide to what extent one should tailor intakes according to the level of diabetic control. The goal for most individuals is find a level that is very close to euglycemia, in which case the standard recommendations should apply. The activity issue is largely a calorie issue as much as anything, and how much more energy needs to be taken in to maintain homeostasis depends on the level of activity. That is pretty straightforward.

**Dr. Karamanos:** In the individuals in whom there is a defect in the balance of protein metabolism, can you change and improve this if you add a certain amount of exercise? And is there any difference between proteins of animal and vegetable origin?

**Dr. Marliss:** These are two very complicated questions. It would appear that acute bouts of exercise stimulate proteolysis in so far as this has been looked at. What then appears to
happen is that between bouts of exercise, and most probably postprandially, repletion takes place, and the muscle will selectively take up the extra amino acids required to build up the necessary amount of protein. However, when you have a non-steady-state condition induced by exercise, it is a very complex problem to examine acute effects on protein balance. One of the points that I did not make about the leucine method is that it is really applicable only in the steady state, which would be either in the overnight fasted state or in a continuously fed state, which implies intravenous provision of amino acids up to the point at which blood levels are constant.

The problem with vegetarian diets is that the proportion of essential amino acids may be quite low and can in certain instances be limiting, particularly with respect to lysine and methionine. Therefore one would predict that individuals who have compromised protein metabolism to begin with, i.e., persons with diabetes, and particularly those who are not in very good control, would be placing themselves at even greater risk if they chose a vegetarian diet. To my knowledge there are no data on this, but it would be prudent to look at the protein composition of those individuals' diets very carefully and make certain that, if they happen to be strict vegetarians, they are being supplemented with proteins that would complement any lack of particular amino acids in their diet.

Dr. Hoet: I should like to know whether you have any data on the specific patterns of plasma amino acids in vegetarians. The reason for asking this is that vegetarians have the lowest levels of taurine that one can see and in view of the new data accumulating that taurine may have a specific effect on glomerulonephropathy, at least in the rat, one has to be concerned.

Dr. Marliss: One has a tendency to defend fasting amino acid concentrations reasonably well in the face of fairly substantial changes in protein intake, and therefore it would not surprise me if there were no major changes in people on vegetarian diets. But I don’t have data on taurine in particular.

Dr. Phenekos: We all know that apart from insulin there is another hormone with an anabolic action and that is growth hormone/IGF-1. I wonder if some of the abnormalities you have described may be mediated through the growth hormone IGF-1 axis.

Dr. Marliss: The answer is probably yes. It is clear that people with type 1 diabetes, particularly if it is in poor control, have significant abnormalities of growth hormone secretion. Corresponding abnormalities of IGF-1 and its binding proteins and the like have been identified, and it is entirely possible that these may be playing a role as well. To my knowledge, this has not been studied directly in such patients yet.

Dr. Christakopoulos: Would you please comment on protein intake and the progress of nephropathy?

Dr. Marliss: There is unfortunately no definitive evidence from a well-conducted multicenter controlled trial on protein restriction in diabetic nephropathy. In North America, nephrologists have moved en masse in the direction of protein restriction in individuals with early nephropathy. What we are talking about is the notion of protein restriction to prevent progression of nephropathy. Brodsky and Robbins (1) wrote an editorial raising this concern, shared by Hoffer (2), that we simply don’t know enough about protein metabolism in people with diabetes to begin with, and we may be placing them at risk of protein malnutrition while trying to protect their kidneys. A study published in the Journal of Clinical Endocrinology and Metabolism by Brodsky et al. in 1992 (3) has been criticized because it was uncontrolled and because it was very difficult to obtain compliance with an extremely low protein diet of 0.6 g/kg per day. Although the study appeared to show benefit for incipient nephropathy, one cannot accept the evidence as definitive. It did show evidence of protein malnutrition.
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It was the first study of its kind and one needs to be very careful about its interpretation. I think that if our incipient nephropathic patients are asked to restrict protein, they should probably not restrict it to as little as 0.6 g/kg, even if that 0.6 g is high quality protein with 45% essential amino acids.

Dr. Nattrass: One of the problems about metabolism is that it is very difficult to change one variable without altering a host of others, and very low energy intakes are quite ketogenic. I would like to ask you whether you have data on other gluconeogenic precursors in your studies and whether you think that what is responsible for the effect upon protein balance may be mediated through some of those other aspects of metabolism.

Dr. Marliss: Yes, of course we have considered the issue of the alternate sources of energy substrates in a hypoenergetic situation in some detail but we have no answers. As soon as one reduces the energy intake, the protein requirement for maintenance increases, so that in an obese individual on a hypocaloric diet the presumption is that the lower the energy intake the higher the protein requirement because of the well-known protein-energy interrelationship. The issue this raises is that if the protein-energy relationship in obese individuals who are not diabetic is such that there is a progressive increase in protein requirement as the energy intake falls, one would predict that, in a diabetic individual who is obese and receiving a low-energy intake, the slope of the curve of protein requirement with decreased energy intake would be steeper, so this puts another variable into the equation. The question of alternative energy substrates in relation to protein equilibrium is an extremely important one and what one would like to see happening is maximum fat mobilization to make up the energy deficit and minimum endogenous protein mobilization to maintain body protein homeostasis. What I have devoted 20 or more years of my career to trying to understand and still don’t understand is what signals the body interprets to show that it is getting fewer calories than it needs. The other issue that arises is that we all adapt or accommodate to our particular level of protein intake and probably diabetics do this to a certain extent as well. If they then face a challenge such as a very low energy diet, this could be a factor in the sustained inability to maintain or reach a normal level of protein turnover and nitrogen balance, even later on. So what that suggests (which does not answer your question directly although it bears on it) is that individuals with conventionally controlled Type 2 diabetes may be in a state where, when challenged either by a low energy diet or by some other stress to their protein metabolism such as an activity program, they may be unable to mount a normal homeostatic response. This may relate to alternative substrates, or more likely to protein metabolism itself.

REFERENCES