The Metabolic Response of Subjects with Type 2 Diabetes to a High-Protein, Weight-Maintenance Diet

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In a randomized, crossover 5-wk study design, we recently reported that a weight-maintaining diet in which the percentage of total food energy as protein was increased from 15–30% resulted in a decrease in postprandial glucose and glycohemoglobin in people with untreated type 2 diabetes without a significant change in insulin. Protein was substituted for carbohydrate in the diet. The fat content remained unchanged. In this publication, we present data on substitution of protein for carbohydrate in the diet.

The mean fasting plasma GH and total IGF-I concentrations were elevated on the 30% protein diet. The urinary free cortisol also was increased. However, the urinary aldosterone was unchanged. Although urinary pH was decreased, calcium excretion was not significantly increased. The plasma postprandial α-amino nitrogen concentrations were increased, but the 24-h integrated concentration was unchanged, indicating an accelerated amino acid removal rate. The plasma urea nitrogen was increased as expected. The urea production rate also was increased such that a new steady-state fasting value was present. The calculated urea production rate accounted for 97% of the protein ingested on the 15% protein diet, but only 80% on the 30% protein diet, suggesting net nitrogen retention on the high-protein diet. In conclusion, an increase in dietary protein results in a number of metabolic adaptations in addition to reducing the circulating glucose concentration. Serum TSH, total T₃, free T₄, B₁₂, folate, homocysteine, uric acid, and creatinine concentrations were unchanged. (J Clin Endocrinol Metab 88: 3577–3583, 2003)

Subjects and Methods

Twelve subjects (10 males, two females) with mild, untreated type 2 diabetes were studied in a Special Diagnostic and Treatment Unit (SDTU, similar to a Clinical Research Center). All subjects met the National Diabetes Data Group criteria for the diagnosis of type 2 diabetes mellitus (5). The patient characteristics and study design have been reported previously (1). Written informed consent was obtained from all subjects, and the study was approved by the Department of Veterans Affairs Medical Center, and the University of Minnesota Committee on Human Subjects. None of the subjects was being treated with oral hypoglycemic agents or insulin.

The control diet consisted of 55% carbohydrate, with an emphasis on starch-containing foods, 15% protein, 30% fat (10% monounsaturated, 10% polyunsaturated, 10% saturated fat). A second diet was designed to consist of 40% carbohydrate, 30% protein, and 30% fat (10:10:10). It is referred to in the text as the high-protein or 30% protein diet. Thus, the protein content of the diet was increased at the expense of carbohydrate. The fat content of the diets was similar. The diets were based on a 6-d rotating menu. All of the food was supplied to the subjects. Examples of each diet have been published previously (1). Subjects were randomized to the 15% protein or 30% protein diet by the flip of a coin. There was a 2- to 5-wk washout period between diets, at which time the subjects ingested an ad libitum diet. They were requested to maintain their calculated caloric intake and activity level so that they remained weight stable.

Subjects returned to the SDTU every 2–3 d to pick up food. At that time, they provided a morning fasting urine specimen
for analysis of creatinine and urea, to determine dietary compliance. They also were weighed. As reported previously (1), dietary compliance was excellent, and the weight was stable throughout the study. The mean body weight was 97 kg (212 lb), range 75–121 kg (164–266 lb).

At the beginning and the end of each 5-wk diet period, the subjects were admitted to the SDTU and blood was drawn at various times throughout a 24-h period. A 24-h urine specimen also was obtained. The control or high-protein meals (breakfast, lunch, dinner, and two snacks) were given, as appropriate. The subjects continued on the rotating menu during the SDTU admission. Therefore, the foods were not identical from patient to patient on either the control or the high-protein diet each time. The distribution of calories was 21% breakfast, 27% lunch, 34% supper, 1600 h snack 13%, and 2100 h snack 5%. The amount of carbohydrate in the meals and snacks for the 15% protein diet was approximately 82 g for breakfast, 69 g for lunch, 36 g for the 1600 h snack, 79 g for dinner, and 35 g for the 2100 h snack; for the 30% protein diet, it was approximately 65 g for breakfast, 49 g for lunch, 22 g for the 1600 h snack, 67 g for dinner, and 20 g for the 2100 h snack.

The total α-amino nitrogen concentration was determined by the method of Goodwin (7), which is a measure of the total amino acid concentration. The plasma TSH (Abbott Architect, Abbott Park, IL), GH (Quest, New Brighton, MN), B12 and folate (Diagnostic Products Corp., Los Angeles, CA) were determined by chemiluminescence. Total T₃ and free T₄ were determined by RIA (Quest). IGF-I was determined using a Beckman-Coulter (Fullerton, CA) Array 360 analyzer. Urinary free cortisol was determined in the laboratory of Dr. B. Pearson Murphy using an HPLC purification analyzer. Microalbumin was determined using a Beckman-Coulter (Fullerton, CA) Array 360 analyzer. Urinary calcium and magnesium were measured by atomic absorption spectrophotometry (Perkin-Elmer, Boston, MA). Urinary phosphorus was measured colorimetrically on a J & J Vitros instrument (J & J Engineering, Poulsbo, WA).

The total amount of protein oxidized was determined by quantifying the urine urea nitrogen excreted over the 24 h of the study in association with the change in the amount of urea nitrogen retained endogenously. The latter was calculated by determining the change in plasma urea nitrogen concentration between the fasting baseline and at the end of the 24-h study period, and correcting for plasma water by dividing by 0.94. In this calculation, it is assumed that there is a relatively rapid and complete equilibration of urea in total body water (9). Total body water as a percentage of body weight was calculated using the equation of Watson et al. (10). The overall assumption is that a change in plasma urea concentration is indicative of a corresponding change in total body water urea concentration. However, in this 24-h study, the beginning and ending urea nitrogen concentrations were essentially identical (see Fig. 2). The sum of total urea nitrogen in urine and body water was divided by 0.86 to account for 14% lost to metabolism in the gut (11).

The net 24-h area responses were calculated using a computer program based on the trapezoidal rule (12). Statistics were determined using Student’s t test for paired variates, or Wilcoxon’s rank sum, with the StatView 512+ program (Brain Power, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA). A P value of <0.05 was the criterion for significance. Data are presented as the mean ± SEM.

Results

α-Amino nitrogen

The 30% protein diet resulted in a lower mean overnight fasting α-amino nitrogen concentration. However, during the subsequent 24 h when the subjects were consuming the 30% protein diet the postmeal α-amino nitrogen concentrations were higher, as expected (Fig. 1, top). The concentration integrated over 24 h using the fasting value as a base line was approximately 2-fold greater than when the 15% protein diet was ingested (Fig. 1, bottom left). Nevertheless, when the absolute areas were calculated the integrated, 24-h responses were quantitatively similar (Fig. 1, bottom right). This was...
due to a more rapid decrease on the 30% protein diet during
the night when the subjects were not eating.

**Plasma urea nitrogen**

The 30% protein diet resulted in a 38% increase in the
morning fasting value. After an 8-h delay, there was a grad-
ual further small increase until the 17-h time point. There-
after, the urea nitrogen decreased back to the original fasting
value. When the subjects ingested the 15% protein diet, there
was little change in urea concentration throughout the day
(Fig. 2, top).

**Calculated amount of protein metabolized.** The calculated total
amount of protein ingested during the 24-h study period was
compared with the total protein metabolized. Following in-
gestion of the 15% protein meals, 90 g of protein were cal-
culated to have been ingested and 87 g were calculated to
have been metabolized, i.e. 97% of that ingested. Following
ingestion of the 30% protein meals, 181 g of protein were
calculated to have been ingested, and 144 g were estimated
to have been metabolized or only 80% of that ingested
(Fig. 3).

**GH and IGF-I**

The mean fasting GH concentration in the subjects when
ingesting the 15% protein diet was $0.15 \pm 0.03$ ng/ml ($\mu g$/
liter). On the 30% protein diet, the mean concentration was
$0.32 \pm 0.1$ ng/ml ($\mu g$/liter), i.e. the mean was approximately
2-fold greater. However, this was not statistically significant
($P = 0.10$, Wilcoxon’s sign-rank test; $P = 0.19$, Student’s $t$
test). The mean IGF-I concentration also was increased when
the subjects ingested the 30% protein diet ($149 \pm 16$ vs. $205 \pm$
36 ng/ml) ($19.4 \pm 2.1$ vs. $26.7 \pm 4.7$ nmol/liter). This differ-
ence was significant ($P < 0.05$ Student’s $t$ test) (Fig. 4).

**Urinary free cortisol and urinary aldosterone**

The 30% protein diet resulted in a 39% increase in mean
24-h free cortisol. This approached significance with Stu-
dent’s $t$ test ($P = 0.06$) ($P < 0.02$ Wilcoxon’s sign-rank test)
(Fig. 5, top). There was little change in the 24-h urinary aldosterone excretion (Fig. 5, bottom).

Other results

A number of other fasting serum, or plasma laboratory tests were done. These are included in Table 1. The change in diet did not affect any of these results.

Other quantitative urinary data are presented in Table 2. The urinary uric acid was increased with the increase in meat protein, as expected (9). This was statistically significant \( (P < 0.002) \). The 30% protein diet also resulted in an increase in phosphorus, but not calcium or magnesium. The urine pH also was only modestly lower when the subjects were ingesting the 30% protein diet. However, this was statistically significant \( (P < 0.05) \).

In this publication, we present the results of a number of other determinations done during that study. We were particularly interested in assessing whether an increase in protein ingestion would stimulate an increase in cortisol production, aldosterone production, and \( / or \) would stimulate an increase in circulating GH and of IGF-I. Production of the latter is stimulated by GH.

Because the carbohydrate content of the diet also may regulate thyroid hormone metabolism (14), we were interested in determining if the decrease in carbohydrate content in the 30% protein diet would influence thyroid function test results.

There also has been concern that a high-protein diet would result in an increased loss of calcium in the urine (9), which in the long term could reduce bone density (mass). A loss of calcium from bone was presumed to be the result of a dietary protein-induced mild metabolic acidosis (15). In a carefully controlled trial, a high-protein diet has been reported to result in a negative calcium balance. Concerns that such a diet could result in a deficiency of some other micronutrients also has been expressed (16, 17). Nevertheless, whether an increased protein content of the diet leads to osteoporosis remains controversial (16). Actually, epidemiological studies suggest that a higher protein intake is associated with a higher bone mineral density, at least in postmenopausal women (19).

The data suggest that a loss of bone mineral is not likely to have occurred. In the present study, the urinary calcium

**FIG. 5.** Top, Urine cortisol and (bottom) aldosterone excretion.

**TABLE 1.** Hormone and metabolite data

<table>
<thead>
<tr>
<th>Test</th>
<th>Common SI</th>
<th>15% Protein SI</th>
<th>30% Protein SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>1.45 ± 0.02 µIU/ml</td>
<td>1.45 ± 0.02 mU/liter</td>
<td>1.49 ± 0.03 µIU/ml</td>
</tr>
<tr>
<td>Total T₃</td>
<td>76 ± 0.4 ng/dl</td>
<td>1.2 ± 0.01 nmol/liter</td>
<td>78 ± 0.4 ng/dl</td>
</tr>
<tr>
<td>Free T₄</td>
<td>0.91 ± 0.001 ng/dl</td>
<td>11.7 ± 0.01 pmol/liter</td>
<td>0.92 ± 0.001 ng/dl</td>
</tr>
<tr>
<td>B 12</td>
<td>449 ± 46 pg/ml</td>
<td>331 ± 34 pmol/liter</td>
<td>463 ± 55 pg/ml</td>
</tr>
<tr>
<td>Folate</td>
<td>21.7 ± 2.1 ng/ml</td>
<td>49 ± 4.8 nmol/liter</td>
<td>18.7 ± 1.9 ng/ml</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>120 ± 6.8 µg/dl</td>
<td>8.9 ± 0.5 µmol/liter</td>
<td>124 ± 9.5 µg/dl</td>
</tr>
<tr>
<td>Uric acid</td>
<td>6.3 ± 0.6 mg/dl</td>
<td>375 ± 36 µmol/liter</td>
<td>6.2 ± 0.6 mg/dl</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.0 ± 0.05 mg/dl</td>
<td>88.4 ± 4.4 µmol/liter</td>
<td>1.0 ± 0.05 mg/dl</td>
</tr>
</tbody>
</table>

**SI, Systeme Internationale.**
excretion was modestly but not significantly increased, although the increased protein content did result in a small decrease in urine pH (Table 2). Unfortunately, blood pH was not determined and dietary calcium was not rigorously controlled in the study. However, the calculated calcium intake was greater when the subjects ingested the 30% protein diet. Thus the significance of the small difference in calcium excretion is difficult to interpret.

Several years ago we reported that a 40% protein diet ingested as three identical meals, over a 12-h period of time, resulted in postmeal rises in serum cortisol and ACTH in normal young subjects (20). However, an index of the effect of increased protein content on the 24-h production rate of cortisol has been observed when subjects were ingesting a high-protein diet (21). Both methods are indirect measures of cortisol production. The mean increase was 39% (Fig. 5). It indeed increases the 24-h urinary free cortisol, an index of cortisol production. The mean increase was 39% (Fig. 5). It is important because the majority of methods currently in use are not specific for cortisol (8). Of interest, a small but significant increase in urinary 17-OH corticosteroids and 17-keto steroids has been observed when subjects were ingesting a high-protein diet (21). Both methods are indirect measures of cortisol production. Definitive data will require direct measurement using an isotope technique.

The metabolic consequences of a dietary protein-stimulated increase in cortisol production, if any, remain to be determined, as does the mechanism. Because the ACTH concentration was increased in our previous study (20), presumably the effect occurs at the pituitary, hypothalamus level or higher in the brain. A direct correlation between the protein content of the diet and plasma renin activity has been reported, as well as an increase in aldosterone in a short-term study (22). Aldosterone production is stimulated by angiotensin II, which in turn is regulated by renin. It has been suggested that dietary protein may have a role in regulation of the entire renin-angiotensin system (22). In the present study, the renin activity was not determined. However, the 24-h urinary aldosterone was quantified and was little changed by the difference in dietary protein content (Fig. 5). Thus, the present data indicate that increasing the protein content over an extended period of time does not affect aldosterone production, at least in people with type 2 diabetes. In any regard, more detailed studies using widely varying dietary proteins and variations in the duration of exposure to such diets would be useful in addressing this issue.

In the present study, doubling the protein content of the diet resulted in a doubling of the mean overnight fasting GH concentration. However, this was not statistically significant due to the variance in the data. The IGF-I concentration also was increased and this was statistically significant (Fig. 4).

The majority of the IGF-I (>90%) circulates as a ternary complex composed of IGF-I, IGFBP-3, and another acid-labile subunit. GH stimulates the synthesis of IGF-I as well as the other components of this ternary complex (23, 24). The bulk of GH is secreted during the night, during sleep (25). During the day the concentration is very low. The IGF-I concentration also increased and this was statistically significant (Fig. 4). This needs to be confirmed in more detailed studies. A number of dietary factors, including the carbohydrate content of the diet, alcohol, and a reduction in dietary food energy also are considered to be potential regulators of the IGF-I concentration and these may be independent of a change in GH (26).

The mechanism by which dietary protein may stimulate an increase in GH is uncertain. Administration of a number of indispensable amino acids intravenously, in large, pharmacological amounts, has been reported to stimulate GH secretion (27). Whether specific amino acids independently stimulate GH secretion when ingested in physiological amounts remains to be determined.

A combination of lysine and arginine, ingested in physiological amounts strongly stimulated a rise in GH concentrations, but neither amino acid was effective when ingested individually, even when ingested in a larger amount (28). Ingestion of a protein meal also was reported many years...
ago to rapidly stimulate a rise in serum GH concentration (29–31). However, we were not able to confirm this in a single meal study (our unpublished data). Others also reported that ingestion of 80 g of beef or soy protein in a single meal did not raise the GH concentration (32). We are not aware of data correlating the protein content of the diet with the circulating GH or with the IGF-I concentration in a controlled study in which food energy and the protein content was adequate. An association between the protein intake, as determined by a food questionnaire, and the plasma IGF-I and IGF binding protein 3 concentration was present in the Nurses Health Study. This was largely attributable to milk intake. An association between red meat or poultry consumption was not present (26). Both IGF-I and IGF binding protein-3 are GH dependent as indicated previously (23). An increase in GH and decrease in IGF-I has been reported in people on a low food energy and very low protein diet. These were only corrected when the subjects received a protein adequate diet (23). Data suggesting the insulin and thyroid hormone may play a regulating role in IGF-I production also have been published (23). These are not likely to have played a role in the present study. Increasing the protein content from 15% to 30% with a corresponding decrease in carbohydrate did not affect the serum TSH, free T₄, or total T₃ concentrations (Table 1). The insulin area response also did not change significantly (1).

An increase in GH and IGF-I concentration could have an anabolic effect on bone and muscle when ingested over an extended period of time (33, 34). They, as well as a raised insulin and an amino acid concentration (35), could potentially offset a protein catabolic effect of the increased cortisol (36) resulting from protein ingestion. The increase in IGF-I also may explain (21, 33, 34), and in part, why a high-protein extended period of time (33, 34). They, as well as a raised

Corrected when the subjects received a protein adequate diet (Table 1). The insulin area response also did not change significantly (1).

In summary increasing the protein content of the diet from 30% of food energy in people with untreated, type 2 diabetes. We reported previously that the increase in dietary protein also resulted in a decrease in total glycohemoglobin, and in the 24-h integrated glucose concentration, and an increase in glucagon, but there was little change in insulin concentration (1). The net effect of these changes on metabolic processes in general, and on protein metabolism in particular, over an extended period of time remains to be determined.

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