

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

FRANK Q. NUTTALL, MARY C. GANNON, ASAD SAEED, KELLY JORDAN, AND HEIDI HOOVER

*Metabolic Research Laboratory and the Section of Endocrinology, Metabolism, and Nutrition (F.Q.N., M.C.G., A.S., K.J., H.H.), Department of Veterans Affairs Medical Center and the Departments of Medicine (F.Q.N., M.C.G.) and Food Science and Nutrition (M.C.G.), University of Minnesota, Minneapolis, Minnesota 55417*

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 other hormones and metabolites that were considered to potentially be affected by 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  $\alpha$ -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 T<sub>3</sub>, free T<sub>4</sub>, B<sub>12</sub>, folate, homocysteine, uric acid, and creatinine concentrations were unchanged. (*J Clin Endocrinol Metab* 88: 3577–3583, 2003)

WE PREVIOUSLY HAVE reported the effect on the percentage of glycohemoglobin and the circulating glucose, insulin, and glucagon concentrations (1) of a diet in which the protein content was increased at the expense of carbohydrates. In this diet, the food energy ratio was 30:40:30 for protein, carbohydrate, and fat, respectively. The results were compared with those when the same subjects ingested a diet in which the food energy ratio was 15:55:30 for protein, carbohydrate, and fat, *i.e.* a diet recommended for the general public (2–5). The effect of a high-protein diet on blood glucose control was studied because data from several laboratories, including our own indicated that: 1) ingested protein alone resulted in either no increase, or a small decrease in blood glucose concentration, and 2) the protein content of mixed meals was calculated to lower the blood glucose concentration (reviewed in Ref. 1).

Twelve subjects with untreated type 2 diabetes were studied over a 5-wk period on each diet in a randomized, crossover design, with a washout period between. Care was taken to maintain weight stability, and all of the food was supplied to the subjects (1).

In the present publication, the serum urea nitrogen,  $\alpha$ -amino nitrogen, GH, IGF-I, TSH, free T<sub>4</sub>, and total T<sub>3</sub> response is presented as well as the urinary urea nitrogen, creatinine, quantitative urea nitrogen production, uric acid, aldosterone, and free cortisol over 24 h, in the same subjects at the end of each study period.

Part of the data were presented previously in abstract form (6).

## 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

Abbreviation: SDTU, Special Diagnostic and Treatment Unit.

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 33 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  $\alpha$ -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  $T_3$  and free  $T_4$  were determined by chemiflex (Abbott Architect). IGF-I was determined by RIA (Quest). Homocysteine was measured by HPLC (Hewlett Packard, Palo Alto, CA). The plasma and urine creatinine, urea nitrogen and uric acid were measured by an automated method on an OrthoClinical Diagnostic (Raritan, NJ) Vitros 950 analyzer. Microalbumin 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 step followed by a cortisol binding assay (8). Urinary aldosterone was determined by RIA (Diagnostic Products Corp). 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 nitro-

gen 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 trapezoid 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  $\pm$  SEM.

## Results

### $\alpha$ -Amino nitrogen

The 30% protein diet resulted in a lower mean overnight fasting  $\alpha$ -amino nitrogen concentration. However, during the subsequent 24 h when the subjects were consuming the 30% protein diet the postmeal  $\alpha$ -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

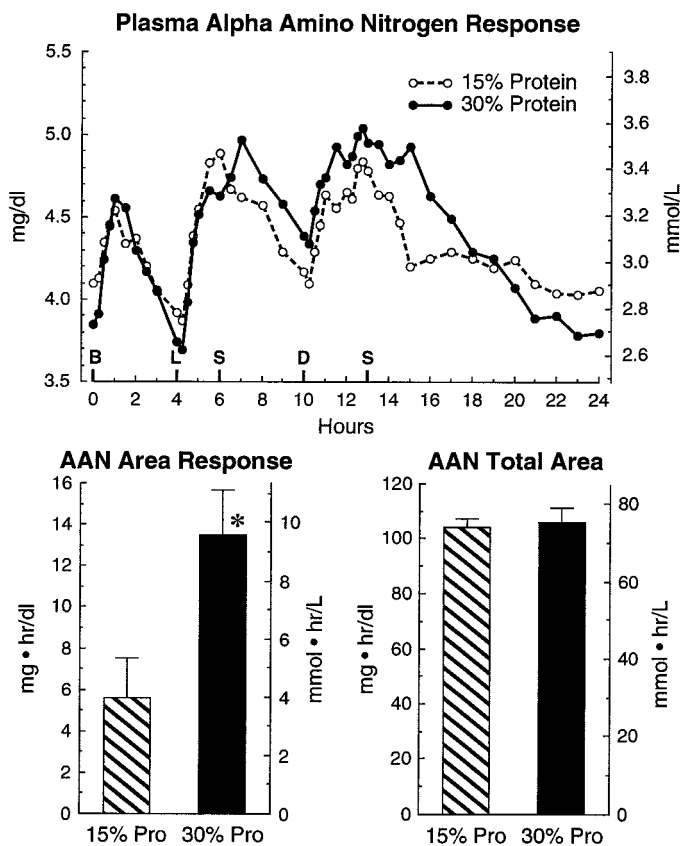


FIG. 1. Top, Twenty-four-hour mean plasma  $\alpha$ -amino nitrogen response to 15% protein (open circles-broken lines) or 30% protein (closed circles-solid lines) diet. Breakfast was given at time 0 (0800 h), lunch at 4 h (1200 h), dinner at 10 h (1800 h), snack 1 at 6 h (1400 h), and snack 2 at 13 h (2100 h). Bottom left, Twenty-four-hour net  $\alpha$ -amino nitrogen area response using the fasting concentration as baseline. \*, Statistical significance ( $P < 0.05$ ). Bottom right, Twenty-four-hour total  $\alpha$ -amino nitrogen area response.

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 gradual further small increase until the 17-h time point. Thereafter, 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 ingestion of the 15% protein meals, 90 g of protein were calculated 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/

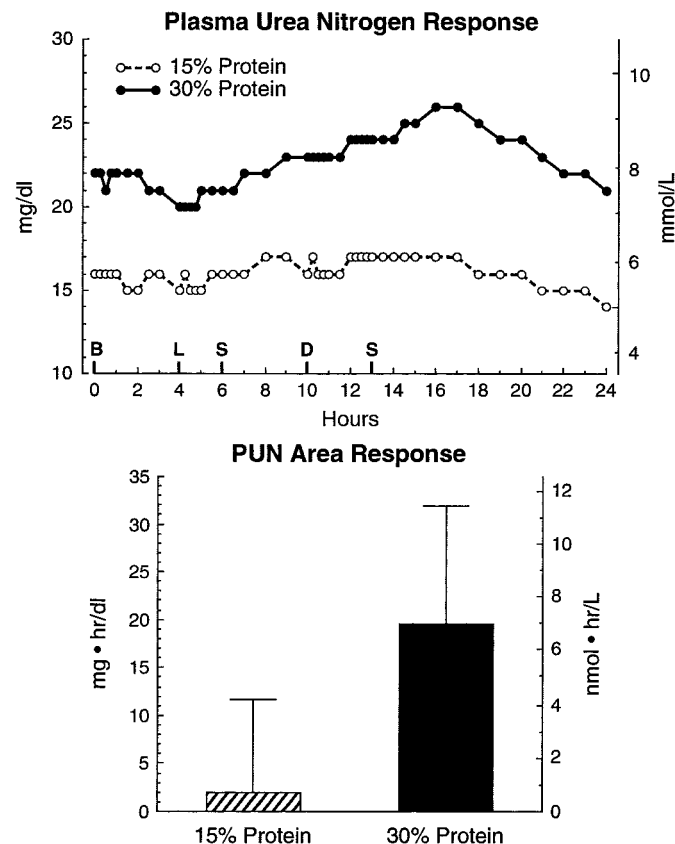


FIG. 2. Top, Twenty-four-hour plasma urea nitrogen response to 15% protein (open circles-broken lines) or 30% protein (closed circles-solid lines) diet. Breakfast was given at time 0 (0800 h), lunch at 4 h (1200 h), dinner at 10 h (1800 h), snack 1 at 6 h (1400 h), and snack 2 at 13 h (2100 h). Bottom, Twenty-four-hour net urea nitrogen area response using the fasting concentration as baseline.

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 difference 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 Student's *t* test ( $P = 0.06$ ) ( $P < 0.02$  Wilcoxon's sign-rank test)

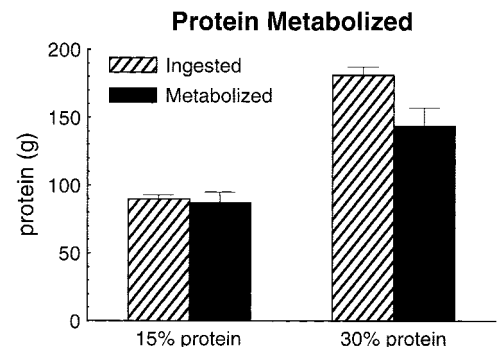
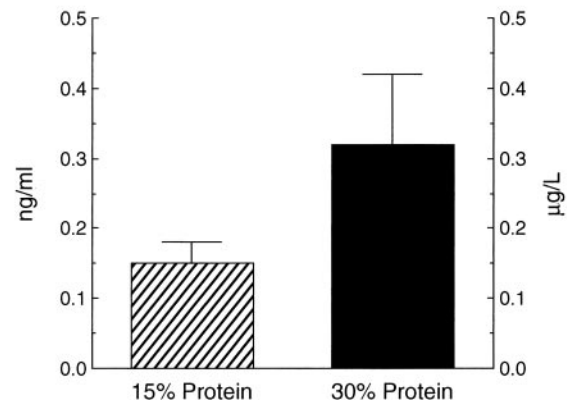


FIG. 3. Amount of protein ingested and metabolized.

**Effect of Diet on Growth Hormone Concentration**



**Effect of Diet on IGF-1 Concentration**

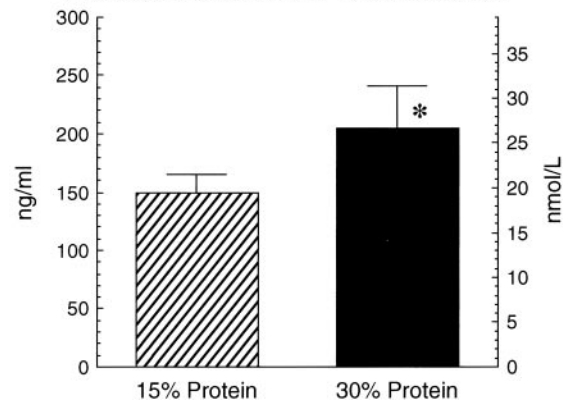


FIG. 4. Top, Plasma GH and (bottom) IGF-I concentrations. \*, Statistical significance ( $P < 0.05$ ).

(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 ( $P < 0.05$ ).



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

TABLE 1. Hormone and metabolite data

Test	15% Protein		30% Protein	
	Common	SI	Common	SI
TSH	1.45 ± 0.02 µIU/ml	1.45 ± 0.02 mU/liter	1.49 ± 0.03 µIU/ml	1.49 ± 0.03 mU/liter
Total T <sub>3</sub>	76 ± 0.4 ng/dl	1.2 ± 0.01 nmol/liter	78 ± 0.4 ng/dl	1.2 ± 0.1 nmol/liter
Free T <sub>4</sub>	0.91 ± 0.001 ng/dl	11.7 ± 0.01 pmol/liter	0.92 ± 0.001 ng/dl	11.8 ± 0.01 pmol/liter
B 12	449 ± 46 pg/ml	331 ± 34 pmol/liter	463 ± 55 pg/ml	342 ± 41 pmol/liter
Folate	21.7 ± 2.1 ng/ml	49 ± 4.8 nmol/liter	18.7 ± 1.9 ng/ml	42 ± 4.3 nmol/liter
Homocysteine	120 ± 6.8 µg/dl	8.9 ± 0.5 µmol/liter	124 ± 9.5 µg/dl	9.2 ± 0.7 µmol/liter
Uric acid	6.3 ± 0.6 mg/dl	375 ± 36 µmol/liter	6.2 ± 0.6 mg/dl	369 ± 36 µmol/liter
Creatinine	1.0 ± 0.05 mg/dl	88.4 ± 4.4 µmol/liter	1.0 ± 0.05 mg/dl	88.4 ± 4.4 µmol/liter

SI, Systeme Internationale.

## Discussion

We have previously reported that increasing the protein content of the diet from 15–30% of total food energy, as a replacement for the same amount of carbohydrate, resulted in a decrease in total glycohemoglobin. It also resulted in a decrease in postprandial glucose concentrations but not in the fasting glucose concentration. The glucagon concentration was increased and there was a small increase in the 24-h integrated insulin concentration. Total cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol were little changed, but the fasting and 24-h integrated triglyceride concentration was decreased.

It has been suggested that there is an inverse relationship between fat content and the fasting triglyceride concentration, *i.e.* it is the fat content of the diet that determines the fasting triglyceride concentration (13). In the present study, the fat content of the diet was held constant, indicating it is the carbohydrate content of the diet that regulates the fasting triglyceride concentration. Body weight was stable (1).

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

**TABLE 2.** Twenty-four-hour urine data

Test	15% Protein			30% Protein		
	Intake <sup>a</sup>	Output		Intake <sup>a</sup>	Output	
		Common	SI		Common	SI
Volume			3304 ± 496 ml			2554 ± 462 ml
pH			6.2 ± 0.1			5.8 ± 0.1 <sup>b</sup>
Sodium	3353 mg	4531 ± 322 mg	197 ± 14 mmol	3774 mg	5704 ± 506 mg <sup>c</sup>	248 ± 22 <sup>c</sup> mmol
Potassium	3102 mg	3237 ± 273 mg	83 ± 7 mmol	3832 mg	3471 ± 156 mg	89 ± 4 mmol
Calcium	980 mg	184 ± 28 mg	4.7 ± 0.7 mmol	1380 mg	210 ± 26	5.3 ± 0.7 mmol
Phosphorus	1362 mg	952 ± 67 mg	30.7 ± 2.2 mmol	2172 mg	1422 ± 89 mg	45.9 ± 2.9 mmol
Magnesium	292 mg	109 ± 7 mg	4.5 ± 0.3 mmol	390 mg	108 ± 9 mg	4.5 ± 0.4 mmol
Glucose		1073 ± 627 mg	6.0 ± 3.5 mmol		1018 ± 456 mg	5.7 ± 2.5 mmol
Creatinine		1735 ± 140 mg	15.4 ± 1.2 mmol		1984 ± 266 mg	17.6 ± 2.4 mmol
Urea		13.0 ± 0.1 g	0.22 ± 0.02 mol		20.1 ± 1.6 g <sup>b</sup>	0.34 ± 0.03 mol <sup>b</sup>
Uric acid		668 ± 48 mg	4.0 ± 0.3 mmol		897 ± 66 mg <sup>b</sup>	5.4 ± 0.4 mol <sup>b</sup>
μAlbumin		7.8 ± 1.17 mg			7.0 ± 0.81	

n = 12 subjects. SI, Systeme Internationale.

<sup>a</sup> Calculated average for 6-d rotating menu. <sup>b</sup> *P* < 0.05.

<sup>c</sup> *P* = 0.054.

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 was not assessed. The present data indicate that increasing the protein content from 15–30% of food energy indeed increases the 24-h urinary free cortisol, an index of cortisol production. The mean increase was 39% (Fig. 5). It should be emphasized that data were obtained using a method that is specific for cortisol (8). This 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 is more stable (23) and generally is considered to be an index of the 24-h integrated GH secretion when the diet is stable. Thus, the current GH and IGF-I data indicate that the higher protein diet most likely stimulated a significant increase in integrated GH secretion (Fig. 4). However, 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 T<sub>4</sub>, or total T<sub>3</sub> 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 diet generally results in a net increase in nitrogen balance as noted by others (21, 37–39) and in the present study (Fig. 3).

Of some interest was the apparent accelerated removal of amino acids and urea from the circulation when the subjects were on the 30% protein diet (Fig. 1). This suggests that an increased protein diet results in an adaptation in which the rate of amino acid metabolism in general is increased, and correspondingly the rate at which the resulting urea produced from amino acid deamination is increased. However, the partitioning of absorbed amino acids between protein synthesis and amino acid deamination and ultimate oxidation was not addressed. It is generally considered that the rate of oxidation of amino acids increases with an increase in circulating amino acid concentration (40) as does the rate of urea formation (41). An increased capacity to oxidize amino acids and to synthesize urea has been demonstrated in animals given a high-protein diet (42). Others also reported an increased urea synthesis rate that was independent of the amino acid availability in human subjects on an increased protein intake (43). This has been attributed to an increased glucagon concentration (44).

In summary increasing the protein content of the diet from 15–30% of food energy in people with untreated, type 2 diabetes raised the plasma urea nitrogen and increased urea nitrogen excretion as expected. The urea concentration correlated with an increased total amino acid concentration during the day (Figs. 1 and 2). The urinary free cortisol was

increased; aldosterone was unchanged (Fig. 5), as was the 24-h calcium excretion (Table 2). The serum TSH, free T<sub>4</sub>, and total T<sub>3</sub> were unchanged. The serum IGF-I was increased (Fig. 4). The serum mean GH also was increased, but this was not statistically significant.

As we reported previously, 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.

### Acknowledgments

Received March 13, 2003. Accepted May 5, 2003.

Address all correspondence and requests for reprints to: Frank Q. Nuttall, M.D., Ph.D., Chief, Endocrinology, Metabolism & Nutrition Section, One Veterans Drive (111G), Department of Veterans Affairs Medical Center, Minneapolis, Minnesota 55417.

This work is supported by grants from the American Diabetes Association and the Minnesota Beef Council, NE Beef Council, CO Beef Council, and Merit Review Funds from the Medical Research Service, Department of Veterans Affairs. A.S. is a Fellow in Endocrinology. K.J. is a Graduate Student.

### References

- Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H, An increase in dietary protein improved the blood glucose response in people with type 2 diabetes. *Amer J Clin Nutr*, in press
- American Heart Association 1986 Dietary guidelines for healthy American adults. A statement for physicians and health professionals by the Nutrition Committee. *Circulation* 74:1465A–1468A
- American Diabetes Association 1987 Nutritional recommendations and principles for individuals with diabetes mellitus: 1986. *Diabetes Care* 10:126–132
- World Cancer Research Fund/American Institute for Cancer Research 1997 Food, nutrition, and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research
- National Diabetes Data Group 1979 Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057
- Gannon MC, Nuttall FQ 2002 Effect of a 30% protein diet on blood glucose control in people with untreated type 2 diabetes. *Diabetes* 51:A400 (Abstract 1641-P)
- Goodwin JF 1968 The colorimetric estimation of plasma amino nitrogen with DFNB. *Clin Chem* 14:1080–1090
- Murphy BEP 2002 Urinary free cortisol determinations: what they measure. *The Endocrinologist* 12:143–150
- Gannon MC, Nuttall JA, Damberg G, Gupta V, Nuttall FQ 2001 Effect of protein ingestion on the glucose appearance rate in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 86:1040–1047
- Watson PE, Watson ID, Batt RD 1980 Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr* 33:27–39
- Hamberg O, Vilstrup H 1994 Effects of insulin and glucose on urea synthesis in normal man, independent of pancreatic hormone secretion. *J Hepatol* 21: 381–387
- Fuller G, Parker RM 1964 Approximate integration. *Applications* 13–16. In: *Analytical geometry and calculus*. Princeton, NJ: Van Nostrand Publ; 367–368
- Willett W, Stampfer M, Chu NF, Spiegelman D, Holmes M, Rimm E 2001 Assessment of questionnaire validity for measuring total fat intake using plasma lipid levels as criteria. *Am J Epidemiol* 154:1107–1112
- Larsen PR, Ingbar SH 1992 The thyroid gland. In: *Williams textbook of endocrinology*. 8th ed. Philadelphia: W. B. Saunders Co.; 382–383
- Barzel US, Massey LK 1998 Excess dietary protein can adversely affect bone. *J Nutr* 128:1051–1053
- Heaney RP 1998 Excess dietary protein may not adversely affect bone. *J Nutr* 128:1054–1057
- Weaver CM, Proulx WR, Heaney R 1999 Choices for achieving adequate dietary calcium with a vegetarian diet. *Am J Clin Nutr* 70(Suppl):543S–548S
- Kim Y, Linkswiler HM 1979 Effect of level of protein intake on calcium metabolism and on parathyroid and renal function in the adult human male. *J Nutr* 109:1399–1404
- Promislow JH, Goodman-Gruen D, Slymen DJ, Barrett-Connor E 2002 Pro-

- tein consumption and bone mineral density in the elderly: the Rancho Bernardo Study. *Am J Epidemiol* 155:636–644
20. **Slag MF, Ahmed M, Gannon MC, Nuttall FQ** 1981 Meal stimulation of cortisol secretion: a protein induced effect. *Metabolism* 30:1104–1108
  21. **Oddoye EA, Margen S** 1979 Nitrogen balance studies in humans: long-term effect of high nitrogen intake on nitrogen accretion. *J Nutr* 109:363–377
  22. **Daniels BS, Hostetter TH** 1990 Effects of dietary protein intake on vasoactive hormones. *Am J Physiol* 258:R1095–R1100
  23. **Thissen JP, Ketelslegers JM, Underwood LE** 1994 Nutritional regulation of the insulin-like growth factors. *Endocr Rev* 15:80–101
  24. **Yakar S, Wu Y, Le Roith D** 2002 Systemic versus local IGF-I production in normal development and disease. In: Kleinberg DL, Clemmons DR, eds. *Central and peripheral mechanisms in pituitary disease*. Bristol, UK: BioScientifica Ltd.; 177–181
  25. **Takahashi Y, Kipnis DM, Daughaday WH** 1968 Growth hormone secretion during sleep. *J Clin Invest* 47:2079–2090
  26. **Holmes MD, Pollak MN, Willett WC, Hankinson SE** 2002 Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev* 11:852–861
  27. **Knopf RF** 1965 Plasma growth hormone response to intravenous administration of amino acids. *J Clin Endocrinol Metab* 25:1140–1144
  28. **Isidori A, Lo Monaco A, Cappa M** 1981 A study of growth hormone release in man after oral administration of amino acids. *Curr Med Res Opin* 7:475–481
  29. **Rabinowitz D, Merimee TJ, Mofessoli R, Burgess JA** 1986 Patterns of hormonal release after glucose, protein and glucose plus protein. *Lancet* ii: 454–457
  30. **Pallotta JA, Kennedy PJ** 1968 Response of plasma insulin and growth hormone to carbohydrate and protein feeding. *Metabolism* 17:901–908
  31. **Merimee TJ, Lillicrap DA, Rabinowitz D** 1965 Effect of arginine on serum-levels of human growth-hormone. *Lancet* 2:668–670
  32. **Kontessis P, Jones S, Dodds R, Trevisan R, Nosadiini R, Fioretto P, Borsata M, Scaerdoti D, Viberti GC** 1990 Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney Int* 38:136–144
  33. **Clemmons DR, O'Connell T, Zheng B, Busby W** 2002 Mediation of growth hormone and IGF-I actions through autocrine/paracrine and endocrine mechanisms in humans. In: Kleinberg DL, Clemmons DR, eds. *Central and Peripheral Mechanisms in Pituitary Disease*. Bristol, UK: BioScientifica Ltd.; 199–210
  34. **Underwood L, Backeljauw P, the GHIS Collaborative Group** 2002 Growth hormone resistance as a model for understanding IGF-I actions. In: Kleinberg DL, Clemmons DR, eds. *Central and peripheral mechanisms in pituitary disease*. Bristol, UK: BioScientifica Ltd.; 213–222
  35. **Liu Z, Barrett EJ** 2002 Human protein metabolism: its measurement and regulation. *Am J Physiol Endocrinol Metab* 283:E1105–E1112
  36. **Horber FF, Haymond MW** 1990 Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J Clin Invest* 86:265–272
  37. **Young VR, Wayler A, Garza C, Steinke FH, Murray E, Rand WM, Schrimshaw NS** 1984 A long-term metabolic balance study in young men to assess the nutritional quality of an isolated soy protein and beef proteins. *Am J Clin Nutr* 39:8–15
  38. **Hegsted DM** 1976 Balance studies. *J Nutr* 106:307–311
  39. **Rand WM, Pellett PL, Young VR** 2003 Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* 77:109–127
  40. **Dangin M, Boirie Y, Guillet C, Beaufrere B** 2002 Influence of the protein digestion rate on protein turnover in young and elderly subjects. *J Nutr* 132:3228S–3233S
  41. **Rafoth RJ, Onstad GR** 1975 Urea synthesis after oral protein ingestion in man. *J Clin Invest* 56:1170–1174
  42. **Das TK, Waterlow JC** 1974 The rate of adaptation of urea cycle enzymes, aminotransferases and glutamic dehydrogenase to changes in dietary protein intake. *Br J Nutr* 32:353–373
  43. **Hamberg O, Nielsen K, Vilstrup H** 1992 Effects of an increase in protein intake on hepatic efficacy for urea synthesis in healthy subjects and in patients with cirrhosis. *J Hepatol* 14:237–243
  44. **Hamberg O, Andersen V, Sonne J, Larsen S, Vilstrup H** 2001 Urea synthesis in patients with chronic pancreatitis: relation to glucagon secretion and dietary protein intake. *Clin Nutr* 20:493–501