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The metabolic response to a high-protein, low-carbohydrate diet in men with type 2 diabetes mellitus

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Abstract

We recently reported that in subjects with untreated type 2 diabetes mellitus, a 5-week diet of 20:30:50 carbohydrate-protein-fat ratio resulted in a dramatic decrease in 24-hour integrated glucose and total glycohemoglobin compared with a control diet of 55:15:30. Body weight, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and serum ketones were unchanged; insulin and nonesterified fatty acids were decreased. We now present data on other hormones and metabolites considered to be affected by dietary macronutrient changes. The test diet resulted in an elevated fasting plasma total insulin-like growth factor 1, but not growth hormone. Urinary aldosterone was unchanged; free cortisol was increased, although not statistically. Urinary pH and calcium were unchanged. Blood pressure, creatinine clearance, serum vitamin B_{12}, folate, homocysteine, thyroid hormones, and uric acid were unchanged. Serum creatinine was modestly increased. Plasma α-amino nitrogen and urea nitrogen were increased. Urea production rate was increased such that a new steady state was present. The calculated urea production rate accounted for 87% of protein ingested on the control diet, but only 67% on the test diet, suggesting net nitrogen retention on the latter. The lack of negative effects, improved glucose control, and a positive nitrogen balance suggest beneficial effects for subjects with type 2 diabetes mellitus at risk for loss of lean body mass.

1. Introduction

We previously reported that increasing the protein content of the diet from 15% to 30% of total food energy in replacement for carbohydrate, in a 5-week randomized crossover design, resulted in a significant decrease in the percentage of total glycohemoglobin in people with type 2 diabetes mellitus. This was because of a decrease in postprandial glucose without a change in overnight fasting glucose concentration. The 24-hour integrated insulin area response was unchanged [1]. Body weight was stable. In that study, we also reported that the increase in protein content of the diet was associated with an increase in serum insulin-like growth factor 1 (IGF-1) and in 24-hour urinary free cortisol. It also resulted in a net increase in nitrogen retention. A number of other hormones, effectors, metabolic substrates, and products also were measured [2].

More recently, again using a 5-week randomized crossover design protocol, we have reported that a weight-maintenance diet composed of 30% protein, 50% fat, and only 20% carbohydrate, that is, further reduction in carbohydrate content, dramatically decreased the percentage of total glycohemoglobin (from 9.8% to 7.6%) without inducing ketosis. This was the result of a decrease in both overnight fasting and postprandial glucose concentrations. The 24-hour integrated insulin concentration also decreased. There was little change in total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations. The triacylglycerol concentration decreased as expected. We have referred to this diet as the low biologically available glucose (LoBAG) diet [3].

In the present report, we provide data on a number of other determinations done in that study to better characterize

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the metabolic response to the diet. These include, among others, serum IGF-1, growth hormone (GH), urinary free cortisol, and nitrogen balance data.

2. Materials and methods

Eight male subjects with mild untreated type 2 diabetes mellitus were studied in a special diagnostic and treatment unit (SDTU) at the Department of Veterans Affairs Medical Center, Minneapolis, MN. It is similar to a clinical research center. Subjects met the National Diabetes Data Group criteria for the diagnosis of type 2 diabetes mellitus [4]. The patient characteristics were published previously [3]. Briefly, mean age was 63 years (range, 51-82 years). Mean body mass index was 31 kg/m^2 (range, 27-36 kg/m^2). Mean total glycohemoglobin was 9.6% (range, 8.6%-l1.2%). The study was approved by the Department of Veterans Affairs Medical Center and the University of Minnesota Committee on Human Subjects. Written informed consent was obtained from all subjects. Subjects were screened and found not to have hematologic abnormalities, kidney disease, liver disease, macroalbuminuria (>300 mg/24 hours), congestive heart failure, angina, untreated thyroid disease, life-threatening malignancies, proliferative retinopathy, diabetic neuropathy, peripheral vascular disease, serious psychological disorders, or a body weight of more than 136 kg (300 lb). Before the study, all subjects were interviewed to determine their physical activity profile and food aversions. The study process was explained in detail, as was the 10-week commitment. Subjects confirmed they had been weight stable for at least 3 months and completed a 3-day food frequency questionnaire representing the prior 2 weeks, with one of the days being a Saturday or Sunday. If subjects were being treated with oral hypoglycemic agents, treatment was discontinued for at least 3 months before the study was begun. None of the subjects had ever been treated with insulin. Other medications remained unchanged during the study.

The control (15% protein) diet was designed according to the recommendations of the American Heart Association, the United States Department of Agriculture, and the American Cancer Society [5-7]. The 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 20% carbohydrate, 30% protein, and 50% fat (with an emphasis on mono- and polyunsaturated fat and ≤10% saturated fat). It is referred to in the text as the LoBAG or LoBAG$_{20}$. Thus, the protein and fat content of the diet was increased at the expense of carbohydrate. Examples of each diet are given in Table 1.

Subjects were randomized, as determined by a flip of a coin, to begin the study with either the LoBAG or the control diet. Subjects were admitted to the SDTU on the evening before the study. The following day, standardized meals containing 55% carbohydrate, 30% fat, and 15% protein were given. Subjects were asked to remain in the SDTU during the study period with minimal activity.

Table 1
Sample menus

Control	LoBAG
Breakfast	Breakfast
57 g (2 oz) total cereal	124 g (4 oz) egg substitute
50 g (2 slices) wheat bread	23 g green pepper
244 g (1 cup) 2% milk	56 g (2 oz) cheddar cheese
10 g (2 tsp) margarine	18 g (1 slice) tomato
10 g (2 tsp) jelly	131 g (1) fresh orange
114g (1) banana	
120 g (4 oz) grape fruit juice	
8 g (2 tsp) sugar	
Lunch	Lunch
50 g (2 slices) wheat bread	226 g (8 oz) roasted ham
85 g (3 oz) lean ham	85 g (3 oz) Swiss cheese
5 g (1 tsp) mustard	90 g (1 small) tomato
56 g (2 oz) lite cheese	28 g (2 tbsp) mayonnaise
10 g (2 tsp) margarine	5 g (1 tsp) mustard
5 g (1) radish	13 g lettuce leaves
36 g (4) carrot sticks	253 g (1 cup) split pea soup
50 g (4) celery sticks	20 g (3) Rye Krisp
166 g (1) fresh pear	
21 g (7) vanilla wafers	
Snack	Snack
72 g (30) grapes	None
58 g (1) banana nut muffin	
5 g (1 tsp) margarine	
Dinner	Dinner
135 g (1 cup) green beans	36 g (l/2 carrot) raw carrot sticks
25 g (1 slice) wheat bread	50 g (l/2 stalk) raw celery sticks
15 g (1 tbsp) margarine	170 g (6 oz) tuna
138 g (1) raw apple	55 g (4 tbsp) mayonnaise
28 g (2) Fig Newtons	80 g (1/2 cup) peas
41 g (3/4 cup) lettuce	138 g (1) raw apple
45 g (l/2 tomato) tomato wedges	28 g (1 slice) whole wheat bread
15 g (1 tbsp) regular Italian dressing	14 g walnuts
113 g (4 oz) lean pork roast	30 g pickle relish
160 g (1 cup) cooked noodles	
Snack	Snack
57 g (2 oz) American cheese	56 g (2 oz) dry-roasted peanuts
17 g (6) saltine crackers	

On the second day in the SDTU, standardized meals again were given (55% carbohydrate, 30% fat, 15% protein) for breakfast, lunch, and dinner, at 8:00 AM, 12:00 PM, and 6:00 PM. This diet is referred to as control/pre and LoBAG/pre in the figures, depending on which study diet followed the inpatient stay. A snack was given at 4:00 and 8:00 PM. Blood was obtained at 7:30, 7:45, and 8:00 AM, every 15 minutes for the first hour after meals, every half hour for the next 2 hours, and then hourly until the next meal when the blood drawing cycle would repeat. Blood was obtained at 46 time points. Subjects were encouraged to drink water to insure adequate urine output. After this 24-hour test period, the subjects were sent home with all the necessary food for the next 2 to 3 days as appropriate for the diet to which they were randomized.

Subjects returned to the SDTU every 2 to 3 days to pick up food and meet with the study dietitian. At that time, they provided a urine specimen for analysis of creatinine and

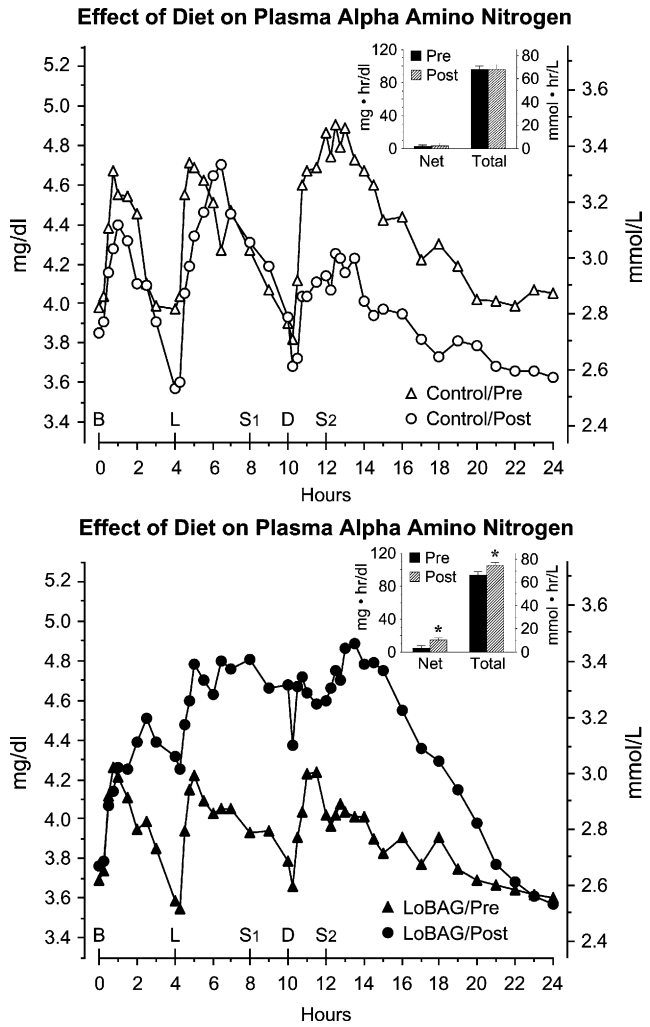

Fig. 1. Top, Mean serum ANN concentration before (open triangles) and after (open circles) 5 weeks on the control diet. Inset, Net and total 24-hour integrated ANN area response. Area response was not significantly different. Bottom, Mean serum ANN concentration before (closed triangles) and after (closed circles) 5 weeks on the LoBAG diet. Inset, Net and total 24-hour integrated ANN area response. Both the net and total area responses were significantly increased after the LoBAG diet ($P \leq .05$).

urea to determine dietary compliance; a qualitative test for acetoacetate also was done. They were weighed, and had blood pressure, total glycohemoglobin, and glucose measured. In addition, they were interviewed regarding dietary compliance, questions or concerns about the study, and so on. At the end of the 5-week period, the subjects again were admitted to the SDTU, and blood was drawn as described above. At this time, the control or LoBAG meals (breakfast, lunch, dinner, and snacks) were given, as appropriate. The distribution of total food energy intake for the control diet was 24% for breakfast, 27% for lunch, 9% for the 4:00 PM snack, 32% for supper, and 8% for the 8:00 PM snack. For the LoBAG diet, the distribution was 17% for breakfast, 38% for lunch, 32% for supper, and 12% for the 8:00 PM snack. The amount of carbohydrate in the meals and snacks for the control diet was approximately 113 g for breakfast, 79 g for lunch, 38 g for the 4:00 PM snack, 109 g for dinner, and 34 g for the 8:00 pm snack (total of 373 g carbohydrate); for the LoBAG diet, it was approximately 25 g for breakfast, 53 g for lunch, 42 g for dinner, and 21 g for the 9:00 PM snack (total of 141 g carbohydrate). After the first 5-week study period, the subjects were sent home to resume a weight-maintaining, ad libitum diet (washout period). After 5 weeks, the subject returned to the SDTU, and blood was drawn again at 46 time points over a 24-hour period. After this test period, subjects were sent home with all the necessary food for the next 2 to 3 days, as appropriate for the second arm of the study.

After a 2-lb weight loss or gain, sustained for a l-week period, the energy content of the diet was increased or

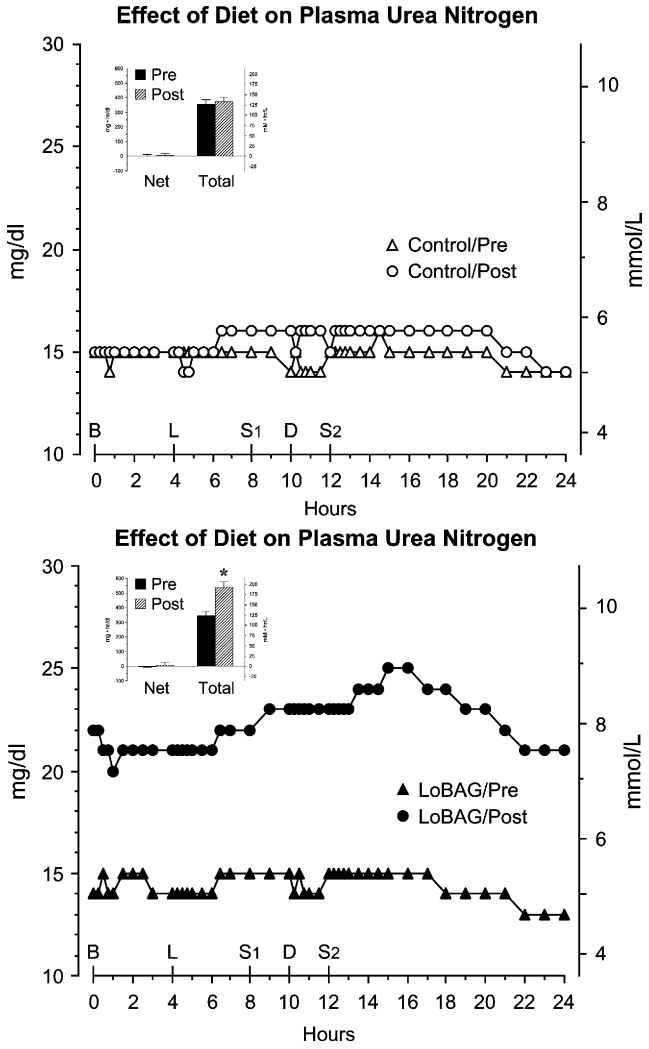

Fig. 2. Top, Mean plasma urea nitrogen concentration before (open triangles) and after (open circles) 5 weeks on the control diet. Inset, Net and total 24-hour integrated urea nitrogen area response. Area response was not significantly different. Bottom, Mean plasma urea nitrogen concentration before (closed triangles) and after (closed circles) 5 weeks on the LoBAG diet. Inset, Net and total 24-hour integrated urea nitrogen area response. The total area response was significantly increased after the LoBAG diet ($P \leq .05$).

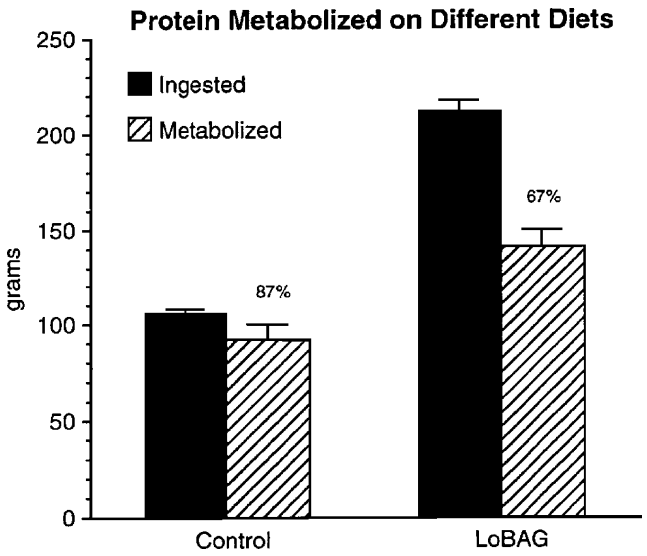

Fig. 3. Mean protein ingested and metabolized. The grams of protein ingested (solid bars) are compared with the grams of protein metabolized (hatched bars) on the control (left) and LoBAG (right) diets.

decreased, as appropriate, in an attempt to keep body weight stable. However, in the present study, the mean body weight decreased by 4 lb (1.8 kg) during the 5-week study period, regardless of diet.

The total α-amino nitrogen (AAN) concentration was determined by the method of Goodwin, which is a measure of the total amino acid concentration. The plasma thyroid-stimulating hormone (TSH; Abbott Architect, Abbott Park, IL), GH (Quest, New Brighton, MN), vitamin B_{12}, and folate (Diagnostic Products, Los Angeles, CA) were determined by chemiluminescence. Total T_3 and free T_4 were determined by Chemiflex (Abbott Architect). IGF-1 was determined by radioimmunoassay (Quest). Homocysteine was measured by high-performance liquid chromatography (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 Vitros 950 analyzer (Raritan, NJ). Microalbumin was determined using a Beckman-Coulter array 360 analyzer (Fullerton, CA). Urinary free cortisol was determined in the laboratory of Dr B Pearson-Murphy using a high-performance liquid chromatography purification step followed by a cortisol-binding assay. Urinary aldosterone was determined by radioimmunoassay (Diagnostic Products). Urinary calcium and magnesium were measured colorimetrically on a J&J Vitros Instrument (J&J Engineering, Poulsbo, WA). Qualitative urinary ketones were measured with a Ketostix (Bayer, Elkhart, IN).

The total amount of protein oxidized was determined by quantifying the urine urea nitrogen excreted over the 24 hours 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-hour 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. Total body water as a percentage of body weight was calculated using the equation of Watson et al [8]. The overall assumption is that a change in plasma urea concentration is indicative of a corresponding change in total body water urea concentration. In this 24-hour study, the beginning and ending urea nitrogen concentrations were essentially identical, indicating no retention of urea. 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 [9].

The 24-hour area responses were calculated using a computer program based on the trapezoid rule. Net area response was calculated using the fasting value as baseline. Total area response was calculated using 0 as baseline. Statistics were determined using Student *t* test for paired variates, or analysis of variance, as appropriate, with the StatView 512+ program (Brain Power, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA).

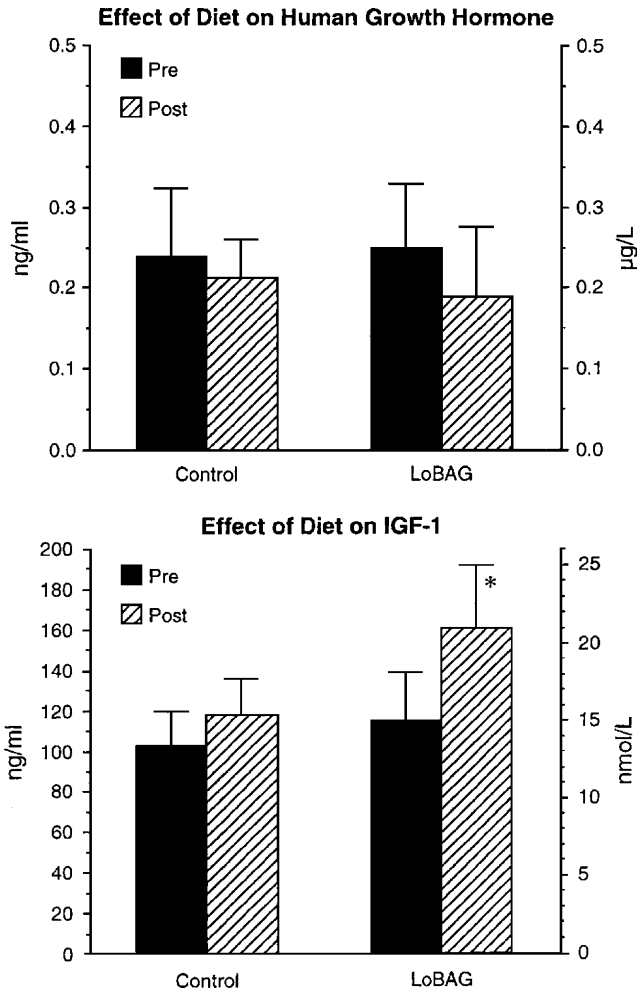

Fig. 4. Top, Mean fasting serum GH concentration before (solid bars) and after (hatched bars) 5 weeks on the control (left) or LoBAG (right) diet. Bottom, Mean fasting serum IGF-1 concentration before (solid bars) and after (hatched bars) 5 weeks on the control (left) or LoBAG (right) diet. *$P \leq .05$, statistically different from the concentration before the diet (pre).

Significance was represented by a P value of less than .05. Data are presented as the mean ± SEM.

3. Results

3.1. Plasma α-amino nitrogen

The AAN concentration increased after meals, as expected (Fig. 1). When the meals contained 15% protein, the AAN concentration increased with each meal, but decreased to near basal levels between meals. However, when the diet contained 30% protein (LoBAG diet), only modest decreases were measured after breakfast and lunch (Fig. 1, bottom). The AAN concentration did return to the fasting concentration overnight in all cases. The increase in AAN after the dinner meal in the control/pre is unexplained.

The net area responses integrated over 24 hours using the fasting value as baseline were 2.6, 3.6, 4.8, and 15 (mg · h)/dL in the control/pre, control/post, LoBAG/pre, and LoBAG/post diets, respectively (Fig. 1, insets). Thus, the area response was approximately 3-fold greater after ingestion of the LoBAG diet, which contained twice as much protein ($P < .05$). When the total area was calculated, using 0 as a baseline, the response to the LoBAG diet again was significantly greater ($P < .05$) (Fig. 1, bottom, right inset).

3.2. Plasma urea nitrogen

The fasting urea nitrogen was 14 to 15 mg/dL before and after the control diet and before instituting the LoBAG diet. At the end of the 5-week period on the LoBAG diet, it had increased to 22 mg/dL (Fig. 2, bottom). Thus, the LoBAG (30% protein) diet resulted in a 57% increase in fasting plasma urea nitrogen. A gradual further small increase in urea nitrogen occurred throughout the day while ingesting the LoBAG diet, until the 17-hour time point, after which the concentration decreased to 21 mg/dL by the following morning. This late evening increase in concentration was nearly identical to that we reported previously in subjects who ingested a 30% protein, 40% carbohydrate, and 30% fat diet [2]. The total urea nitrogen area response, using 0 as baseline, was 45% greater ($P < .05$) after ingestion of the 30% protein/LoBAG diet (Fig. 2, bottom, left inset).

Table 2
Blood pressure, plasma/serum hormones, vitamins, and metabolites

	Control/pre	Control/post	LoBAG/pre	LoBAG/post
Blood pressure (mm Hg)	133/77	127/72	146/76	133/74
Serum creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.05	0.9 ± 0.05	1.0 ± 0.05*
Renin (ng/mL)	0.64 ± 0.3	1.03 ± 0.3	0.69 ± 0.1	0.47 ± 0.1
Serum uric acid (mg/dL)	4.9 ± 0.2	5.5 ± 0.03	5.3 ± 0.3	5.8 ± 0.3
TSH (μIU/mL)	1.60 ± 0.22	1.49 ± 0.16	1.50 ± 0.3	1.39 ± 0.16
Total T_3 (ng/dL)	83.3 ± 8.5	79.6 ± 7.3	86.9 ± 7.9	81.9 ± 6.9
Free T_4 (ng/dL)	0.90 ± 0.04	0.85 ± 0.02	0.98 ± 0.05	1.04 ± 0.03
Folate (ng/mL)	16.5 ± 2.3	20.2 ± 1.3	18.0 ± 2.1	15.8 ± 2.2
Homocysteine (μg/dL)	8.1 ± 0.7	8.1 ± 0.8	8.9 ± 1.1	7.8 ± 2.1
Vitamin B_{12} (pg/mL)	524 ± 119	496 ± 99	557 ± 120	475 ± 108

Values are expressed as mean ± SEM.
* $P < .02$ compared with LoBAG/pre.

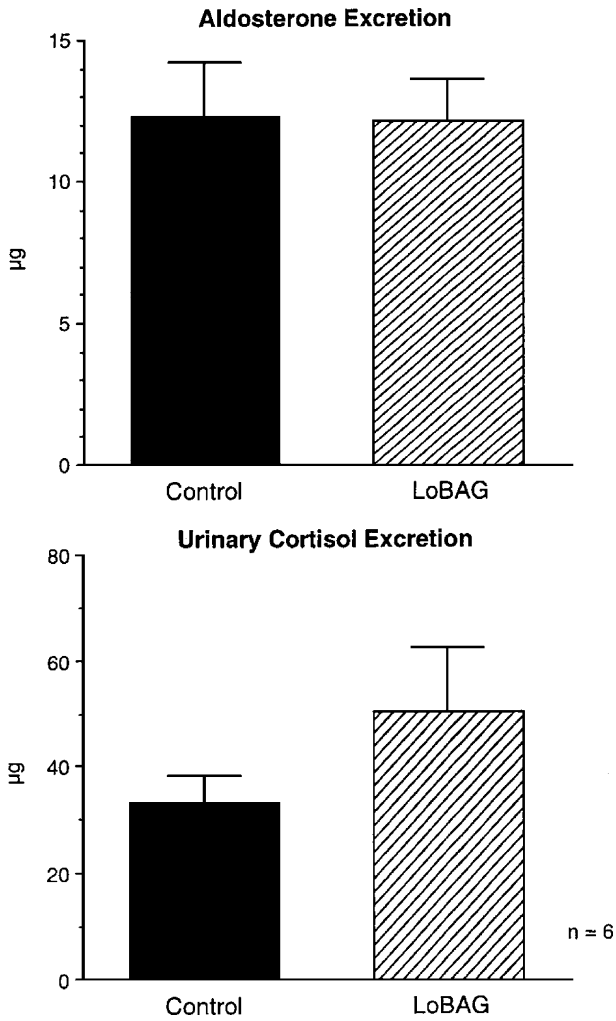

Fig. 5. Mean urinary 24-hour aldosterone excretion (top) and cortisol excretion (bottom) after the control (left bar) and LoBAG (right bar) diets. The differences were not statistically significant.

3.3. Protein metabolized

The calculated total amount of protein ingested during the 24-hour study period was compared with the total protein metabolized.

After ingestion of the 15% protein meals (control), 106 g of protein was calculated to have been ingested and 92 g was calculated to have been metabolized (87%) (Fig. 3). After ingestion of the 30% protein meals (LoBAG), 212 g of protein was calculated to have been ingested and 142 g was calculated to have been metabolized (67%). This difference was statistically significant ($P < .03$).

3.4. Serum growth hormone and insulin-like growth factor 1

Serum GH concentrations did not differ significantly between treatments (Fig. 4, top). The serum IGF-1

concentration was similar before and after ingestion of the control diet and before the LoBAG diet. However, it increased significantly from a mean of 115 to 161 ng/mL after 5 weeks on the LoBAG diet ($P < .01$) (Fig. 4, bottom).

3.5. Plasma renin, urinary aldosterone, and urinary free cortisol

Plasma renin activity was determined in 7 subjects. There was a mean increase when the subjects ingested the control diet. After institution of the LoBAG diet, it decreased (Table 2). These differences were not statistically significantly different ($P = .13$ and .20, respectively).

Mean 24-hour urinary aldosterone excretion was not different between diets (Fig. 5). The mean urinary free cortisol was obtained in only 6 subjects. It increased by 44% consequent to the ingestion of the LoBAG diet (Fig. 5), but this was not statistically significant ($P = .17$).

3.6. Serum thyroid-stimulating hormone, serum free levorotatory thyroxine, and serum total triiodothyronine

Serum TSH, free levorotatory thyroxine, and total triiodothyronine were not significantly affected by ingestion of the LoBAG diet, although it contained much less carbohydrate than the control diet (Table 2).

3.7. Other determinations

Blood pressure remained unchanged. Serum homocysteine, folate, and vitamin B_{12} also remained unchanged (Table 2). Urinary β-hydroxybutyrate excretion did not increase nor did the urinary pH change when the subjects ingested the LoBAG diet (Table 3). The creatinine clearance and microalbumin excretion also did not change. Sodium excretion was increased. The 24-hour urinary urea nitrogen increased when the subjects ingested the LoBAG diet. However, the mean increase was only approximately 60% and not 2-fold, as would be expected with a doubling of the protein content of the diet.

Table 3
Urine data

	Control/pre	Control/post	LoBAG/pre	LoBAG/post
Volume (mL)	4129 ± 707	3961 ± 691	4366 ± 502	4127 ± 558
Glucose (g)	22 ± 8	14 ± 4	17 ± 9	0.3 ± 0.3
Potassium (mg)	3315 ± 254	3471 ± 312	3471 ± 250	3081 ± 156
Sodium (mg)	5451 ± 276	5451 ± 713	4692 ± 253	6923 ± 759*
Urea (g)	12.2 ± 0.9	13.3 ± 1.0	12.8 ± 0.9	20.6 ± 1.4*
Uric acid (g)	0.84 ± 0.12	0.72 ± 0.06	0.78 ± 0.11	0.90 ± 0.09†
Microalbumin (mg)	NA	9.7 ± 1.7	NA	8.3 ± 1.1
β-Hydroxybutyrate (μmol/L)	187 ± 7	203 ± 10	196 ± 8	196 ± 8
Calcium (g)	220 ± 52	217 ± 62	221 ± 62	214 ± 64
pH	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.1
Creatinine (mg)	1.8 ± 0.15	1.7 ± 0.13	1.8 ± 0.13	1.8 ± 0.15
Creatinine clearance (mL/min)	143 ± 15	127 ± 13	144 ± 17	137 ± 10

Values are expressed as mean ± SEM. NA indicates not assessed.
* $P < .05$ compared with LoBAG/pre.
† $P = .06$ compared with LoBAG/pre.

4. Discussion

As indicated in our previous publication of data obtained in the present study [3], the LoBAG diet resulted in a major decrease in fasting and postprandial glucose concentration and in the percentage of total glycohemoglobin. This was associated with a decrease in insulin. As indicated in the present article, the serum β-hydroxybutyrate concentration remained unchanged. The urinary β-hydroxybutyrate excretion also was unchanged. Thus, the diet is not ketogenic.

Ingestion of the LoBAG diet for 5 weeks did not affect the blood pressure (Table 2), the urinary microalbumin excretion (Table 3), or the serum homocysteine concentration (Table 2). As indicated previously [3], it did not significantly affect the total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations. The triacylglycerol concentration decreased. Thus, the diet did not adversely affect these purported risk factors for cardiovascular disease.

4.1. Homocysteine

In people with type 2 diabetes mellitus, a direct relationship between blood glucose control, as indicated by the fasting glucose concentration and the percentage of HbA_{1c}, and the serum homocysteine concentration has been reported [10]. In addition, both folate and vitamin B_{12} as well as pyridoxine are important determinants of homocysteine production, and an inverse correlation between vitamin B_{12} and folate and homocysteine concentration has been reported [11]. Thus, a rise in homocysteine is expected if any of these cofactors is deficient. An elevated homocysteine concentration is considered to be a risk factor for cardiovascular disease [11].

In the present study, even with a decrease in 24-hour integrated glucose concentration and percentage of glycohemoglobin, the homocysteine concentration remained unchanged. The serum folate and vitamin B_{12} concentrations also were unchanged. Actually, an increase in serum B_{12} might have been anticipated because of the increase in meat consumption when the subjects ingested the 30% protein diet. Because flour is now being supplemented with folate [12], a decrease in folate values might also have been anticipated because of the decreased consumption of flour products. This also was not observed (Table 2).

4.2. Insulin-like growth factor 1 and growth hormone

We previously reported that increasing the protein content of the diet from 15% to 30% of total food energy in exchange for a 15% reduction of carbohydrate (55%-40%) resulted in an increase in mean overnight fasted serum IGF-1 concentration [1]. In that study, there also was an increase in mean GH concentration. However, the increase was not statistically significant. In the present study, the protein content was again 30%. However, the carbohydrate content was further reduced to approximately 20% of food energy (~62% reduction). Therefore, we were

particularly interested in determining whether the increase in IGF-1 and possibly GH was the result of the increase in protein content or the reduction in carbohydrate content. The present data indicate that the increase in IGF-1 is the result of the increase in protein content. The further decrease in carbohydrate did not result in a further increase in IGF-1. In fact, the increase was approximately the same (138% and 136%, respectively).

The increase in GH noted in the previous study was not observed in the present study, indicating that the increase noted previously was merely fortuitous and due to the known fluctuation of GH concentrations throughout the day [13].

The present data do not rule out a GH-mediated increase in IGF-1 because most of the hormone is secreted early after the initiation of sleep [13]. Thus, it will be of interest in the future to determine the effect of these diets on the 24-hour integrated GH concentration as well as a potential effect on the ghrelin concentration, an independent stimulator of GH secretion [14,15].

4.3. Cortisol

Some years ago, we reported that high-protein meals (40% of food energy) resulted in an increased postprandial cortisol and corticotropin concentration [16]. In our more recent study [2], when the protein content of the diet was increased from 15% to 30%, an increased 24-hour urinary free cortisol excretion also was present. A similar increase was noted in the present study (43% and 44% increase, respectively). However, data were only obtained in 6 subjects, and the increase was not statistically significant in this smaller group of subjects. The mechanism, as well as the metabolic consequences of this increase, remains to be determined.

4.4. Renin and aldosterone

A direct correlation between the protein content of the diet and plasma renin activity has been reported, as well as an increase in serum aldosterone in a short-term study (see Reference [2] for a review). Indeed, it has been suggested that dietary protein content may have a role in regulating the entire renin-angiotensin system [17]. However, in our previous study [2] as well as in the current study, we did not observe an increase in 24-hour urinary aldosterone with an increase in protein content of the diet. In the present study, we determined the plasma renin activity as well. This also was not increased when the subjects ingested the LoBAG diet; rather a decrease was present, although this was not statistically significant (Table 2). (The increase in renin with the control and the decrease with the LoBAG diet are significantly different using Wilcoxon signed rank test.) Of some interest, the 24-hour urinary sodium excretion was increased when the diet contained 30% protein, both in the previous and current studies when compared with when the subjects ingested a 15% protein diet. The calculated sodium content of the diet was 4.7 and 5 g for the control and LoBAG diet, respectively; that is, the sodium content was similar. Thus, our long-term data indicate that high-protein diets do not stimulate the renin-angiotensin system or cause sodium retention, at least in subjects with type 2 diabetes mellitus. In this regard, the blood pressure in our subjects also remained unchanged (Table 2) as did the creatinine clearance.

4.5. Serum thyroid-stimulating hormone, serum free levorotatory thyroxine, and serum total triiodothyronine

Serum TSH, free T_4, and total T_3 remained unchanged after the LoBAG diet, similar to the findings in our previous 30% protein diet study. Thus, neither an increase in protein from 15% to 30% nor a decrease in carbohydrate from 55% to 20% results in a change in these thyroid function tests.

We reported previously [2] that the 24-hour integrated postprandial plasma amino acid concentration increased 2.4-fold when the subjects ingested the 30% protein diet. In the present study, when compared with the control diet, the postprandial response increased 4.2-fold. The modestly greater fold increase (4.2-fold) with the LoBAG diet when compared with the previous 30% protein diet (2.4-fold) was actually due to a difference in the control diets, that is, the denominator. The incremental increase with both the 30% protein diet and the LoBAG diet was nearly identical ($3.9 \pm 0.2 \rightarrow 5.0 \pm 0.3$ and $3.8 \pm 0.2 \rightarrow 4.9 \pm 0.2$ mg/dL). Thus, an increase in protein content from 15% to 30% of total food energy in both studies resulted in a similar net increase in amino acid response.

Although the net area responses were 2- to 4-fold greater when 30%, rather than 15%, of the food energy was protein, the amino acid concentration still returned back to the fasting value at 24 hours, that is, an accelerated removal rate was present. The 2 studies [2] resulted in essentially identical 24-hour integrated total AAN areas.

The observation that the integrated AAN concentrations were similar although the integrated insulin concentration decreased by approximately 40% in the present study [3], whereas it was increased by approximately 20% in the previous study [2], was surprising and is of considerable interest. Insulin is considered to be the major hormone regulating the rate of endogenous proteolysis. It also may facilitate an amino acid–stimulated synthesis of protein [18].

Although the removal rate of amino acids in total was independent of the insulin concentrations, the rate of accumulation of body proteins, as well as the rate of amino acid oxidation, could have been affected differentially by the major difference in insulin response between the 2 diets. If so, the results are the opposite of those expected with a lower insulin concentration.

The urine urea nitrogen excreted was 13.3 and 20.6 g during the control and LoBAG diets, respectively. This was essentially identical to that in our previous study [2] (13.0 vs 20.1 g, respectively) when the patients were on a 15% vs a 30% protein diet, but in which the carbohydrate content was greater. In the present study, the calculated protein intake

was 212 g compared with 181 g in our previous study [2], that is, a 31-g greater amount.

The calculated protein balance was positive in both studies (37 ± 10.3 and 70 ± 5.6 g/d for the previous and current studies, respectively; $P < .03$). However, if it is assumed that both diets contain the same amount of protein, that is, 181 g, then the calculated protein balance when the subjects were on the LoBAG diet would be a mean of 39 g/d, that is, essentially the same as when the subjects were on the previous 30% protein diet, but with a higher carbohydrate content. We suspect that this actually was the case. However, a direct dietary analysis will be required to resolve this issue. In any regard, the calculated protein retention was the same or even greater with the LoBAG diet, although the insulin concentration was much smaller. Overall, our data in subjects with type 2 diabetes mellitus on 2 different 30% protein diets cannot be explained solely by an increase in total amino acid concentration or a quantitative difference in insulin response. Thus, the long-term role of insulin as well as other factors in regulation of protein balance remains uncertain and in need of further study, at least in subjects with type 2 diabetes mellitus. An alternative explanation for the net positive protein balance with both diets containing 30% protein as calculated could be a differential loss of nitrogen in the feces. In this regard, the loss has been reported to vary directly with the nitrogen content of the diet, but also to be increased by an increased carbohydrate content of the diet [18]. In the present study, a constant fraction was assumed to be lost in the feces. A correction for a carbohydrate effect on the calculated nitrogen balance was not made. The decrease in dietary carbohydrate compared with control was 62% in the present study compared with 27% in our previous study. Thus, a greater loss would have been expected in the previous study. This is the opposite of the calculated relative nitrogen retention in the 2 studies.

Quantification of urea metabolism also has been reported to be confounded by the cycling of urea in the gut back into the circulation. To our knowledge, the amount of recycling is controversial [18], but must have been similar in our 2 studies as evidenced by the nearly identical ratio of nitrogen retained to plasma amino acid area responses in the respective 30% protein diets.

4.6. Potential limitations of the study

Only males were studied. The number of patients proposed for the study was determined by the mathematical analysis for predicting the number [19] based on the difference between means and sample variance of the percentage of total glycohemoglobin, our primary outcome for the entire study. To show a change of 1.5 in the percentage of total glycohemoglobin, using .05 as the level of significance, setting the type II error at 0.2 (80% power), with σ_x of 0.9, assuming a mean total glycohemoglobin of 9%, solving for the number indicated that 3 subjects would be required. Because we intended to perform multiple analyses, that number was increased by 2.7. However, a power calculation was not done for each of the parameters measured. Therefore, it is possible that a type II error could be present (ie, failure to identify differences).

As indicated previously [3], 3 of the subjects who started on the control diet did not complete the study for personal reasons (death of spouse, move across country, chose not to finish). Because the results obtained were not affected by order of diet received, we do not think that the loss of these 3 subjects affected the results. Data are reported for only those subjects who completed both arms of the study.

We were confident that a 5-week washout period was adequate for this study based on previous data from our laboratory. In this study, the mean the percentage of total glycohemoglobin was identical at the beginning of each diet period, despite its decrease from 9.8% to 7.6% after 5 weeks on the $LoBAG_{20}$ diet. Thus, during the 5-week washout period, the percentage of total glycohemoglobin increased back to 9.8%. Furthermore, glucose, insulin, glucagon, triglyceride, AAN, urea nitrogen, GH, and IGF-I were also nearly identical at the beginning of each diet period.

In summary, an increase in protein content of the diet results in several metabolic adaptations, some of which are not well understood. However, in general, the amount of carbohydrate in the diet relative to the fat content does not appear to have a major effect on the metabolic response, although the insulin and glucose concentrations are greatly different.

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