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An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes[1–3]

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ABSTRACT

Background: In single-meal studies, dietary protein does not result in an increase in glucose concentrations in persons with or without type 2 diabetes, even though the resulting amino acids can be used for gluconeogenesis.

Objective: The metabolic effects of a high-protein diet were compared with those of the prototypical healthy (control) diet, which is currently recommended by several scientific organizations.

Design: The metabolic effects of both diets, consumed for 5 wk each (separated by a 2–5-wk washout period), were studied in 12 subjects with untreated type 2 diabetes. The ratio of protein to carbohydrate to fat was 30:40:30 in the high-protein diet and 15:55:30 in the control diet. The subjects remained weight-stable during the study.

Results: With the fasting glucose concentration used as a baseline from which to determine the area under the curve, the high-protein diet resulted in a 40% decrease in the mean 24-h integrated glucose area response. Glycated hemoglobin decreased 0.8% and 0.3% after 5 wk of the high-protein and control diets, respectively; the difference was significant ($P < 0.05$). The rate of change over time was also significantly greater after the high-protein diet than after the control diet ($P < 0.001$). Fasting triacylglycerol was significantly lower after the high-protein diet than after the control diet. Insulin, C-peptide, and free fatty acid concentrations were not significantly different after the 2 diets.

Conclusion: A high-protein diet lowers blood glucose postprandially in persons with type 2 diabetes and improves overall glucose control. However, longer-term studies are necessary to determine the total magnitude of response, possible adverse effects, and the long-term acceptability of the diet. *Am J Clin Nutr* 2003;78:734–41.

KEY WORDS Dietary protein, diabetes, diet, insulin, glucagon, glycemic index, triacylglycerol, glycated hemoglobin, Hb A_{1c}

INTRODUCTION

When dietary recommendations for the general population or for persons with diabetes are considered, the focus has been on the relative amounts and types of carbohydrates and fat to include in the diet (1–4). Both the type and amount of protein in the diet has largely been ignored. Generally, protein has only been considered in the context of that necessary for maintenance of lean body mass, ie, that needed to maintain nitrogen balance, whether people have diabetes or not. A role for dietary protein in the management of hyperglycemia either has not been considered or has been considered only in regard to the amount of glucose that theoretically can be derived from the constituent amino acids through gluconeogenesis. However, as early as 1913 (5), it was reported that the ingestion of egg white protein in a single-meal study did not result in an increase in the blood glucose concentration in healthy persons. Subsequently, data from several laboratories (6, 7; reviewed in reference 8)—including our own (9–13)—indicated that the peripheral glucose concentration does not increase after protein ingestion in healthy persons or in persons with type 2 diabetes. In persons with type 2 diabetes, protein ingestion actually results in a small decrease in postprandial glucose concentrations (8, 14). In a single-day study in which mixed meals of various composition were fed to healthy young subjects, the protein content of the meals was calculated to also lower the blood glucose concentration (15). To our knowledge, the effect on glucose metabolism of a high-protein, weight-maintenance diet ingested over a longer period of time has not been reported. In the present study we tested the hypothesis that a 5-wk period of increased dietary protein results in a lower plasma glucose concentration in persons with mild, untreated type 2 diabetes. The responses of glucose, glycated hemoglobin, insulin, C-peptide, glucagon, triacylglycerol, and nonesterified fatty acids to a high-protein diet are included in this report.

SUBJECTS AND METHODS

Twelve subjects (10 men and 2 women) 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 (16). The mean age of the

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subjects was 61 y (range: 39–79 y), and the mean body mass index (in kg/m^2) was 31 (range: 22–37). The mean percent total glycated hemoglobin was 8.0% (range: 7.0–9.3%). 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. Subjects were screened and found not to have hematologic abnormalities, liver disease, kidney disease, macroalbuminuria (> 300 mg/24 h), untreated thyroid disease, congestive heart failure, angina, life-threatening malignancies, proliferative retinopathy, diabetic neuropathy, peripheral vascular disease, serious psychological disorders, or a body weight > 136 kg (300 lb). All subjects were interviewed before the study began to determine their physical activity profile and their food likes and dislikes and to emphasize the commitment that they were about to undertake. The subjects confirmed that they had been weight-stable for ≥ 3 mo and they completed a 3-d food-frequency questionnaire covering the prior 2 wk; one of the days was a Saturday or Sunday. None of the subjects was being treated with oral hypoglycemic agents or insulin.

The control (15% protein) diet was designed according to the recommendations of the American Diabetes Association (2), the American Heart Association (1), the US Department of Agriculture (4), and the American Cancer Society (3) at the time (1997) that the study was conceived. The control diet consisted of 55% carbohydrate (with an emphasis on starch-containing foods), 15% protein, and 30% fat (10% monounsaturated, 10% polyunsaturated, and 10% saturated fat). A second diet—the high-protein diet—was designed to consist of 40% carbohydrate, 30% protein, and 30% fat (10% monounsaturated, 10% polyunsaturated, and 10% saturated fat). Thus, the protein content of the diet was increased at the expense of carbohydrate. The fat and dietary fiber contents of the diets were similar. Examples of each diet are given in **Table 1**. The compositions of the diets are given in **Table 2**.

Subjects were randomly assigned to begin the study with the high-protein or the control diet, as determined by the flip of a coin. The subjects were admitted to the SDTU on the evening before the study began. On the following day, the subjects were fed standardized meals containing 55% carbohydrate, 30% fat, and 15% protein. After the subjects had fasted overnight for 12 h, blood was drawn for lipid studies.

On the second day in the STDU, standardized meals (55% carbohydrate, 30% fat, and 15% protein) were again fed for breakfast, lunch, and dinner at 0800, 1200, and 1800, respectively. Snacks were given at 1400 (snack 1) and 2100 (snack 2). Blood was obtained at 0730, 0745 and 0800; one-half hour after each meal; and hourly throughout the remainder of the 24-h period of the study, for a total of 46 time points. Subjects were encouraged to drink water to ensure adequate urine output. After this 24-h test period, the subjects were sent home with food for the next 2–3 d, as appropriate for the diet to which they were randomly assigned. All of the food to be eaten was supplied to the subjects.

The subjects returned to the SDTU every 2–3 d to pick up food. At that time, they provided a urine specimen (for measurement of creatinine and urea to determine dietary compliance), were weighed, and had their blood pressure and total percent glycated hemoglobin measured. In addition, the subjects were interviewed regarding dietary compliance and about any concerns that they had about the study.

At the end of the 5-wk period, the subjects again were admitted to the SDTU, and blood was drawn as described above. At this time the control or high-protein diet (breakfast, lunch, dinner, and 2 snacks) was given, as appropriate. The distribution of calories was as follows: 21% for breakfast, 27% for lunch, 13% for snack 1, 34% for dinner, and 5% for snack 2. The amount of carbohydrate in the meals and snacks for the control diet was ≈82 g for breakfast, 69 g for lunch, 36 g for snack 1, 79 g for dinner, and 33 g for snack 2; the respective amounts for the high-protein diet were ≈65, 49, 22, 67, and 20 g.

The plasma glucose concentration was determined with the use of a glucose oxidase method (Beckman glucose analyzer with an oxygen electrode; Beckman Instruments, Fullerton, CA). Total glycated hemoglobin was measured by boronate affinity HPLC. Serum immunoreactive insulin was measured with the use of a standard double-antibody radioimmunoassay method with kits produced by Incstar (Stillwater, MN). Glucagon was determined with the use of a radioimmunoassay kit purchased from Linco Research (St Louis). C-peptide was measured with the use of a radioimmunoassay kit manufactured by Diasorin (Stillwater, MN). Nonesterified fatty acids (NEFAs) were determined enzymatically with the use of a kit manufactured by Wako Chemicals USA, Inc (Richmond, VA). Creatinine, urea nitrogen, total cholesterol, HDL cholesterol, and triacylglycerol were measured with the use of an automated method on an Ortho-Clinical Diagnostics Vitros 950 analyzer (Raritan, NJ). LDL cholesterol was calculated with the Friedewald formula. Microalbumin was determined by using a Beckman-Coulter Array 360 analyzer. Weight was determined with a digital scale (Scaletronix, White Plains, NY) while the subjects were wearing street clothes and no shoes. Blood pressure was determined by using a Dinamap instrument (Critikon/Mediq, Pennsauken, NJ) with a digital readout.

The net 24-h area responses were calculated by using a computer program based on the trapezoid rule (17). Statistics were determined by using Student's *t* test for paired variates with the STATVIEW 512+ program (Brain Power, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA). A P value < 0.05 was the criterion for significance. Data are presented as means ± SEMs.

RESULTS

The average body weight of the subjects was 96 kg (211 lb). Body weight remained stable with both diets (**Figure 1**). The large SEM was due to the variation between subjects. None of the subjects lost or gained > 1 kg (2 lb) with either diet.

The ratios of urine urea to creatinine with the high-protein and control diets were ≈14 ± 1.3 and ≈7 ± 0.7 at week 1 and remained relatively stable throughout the 5-wk intervention period. In other words, the ratio with the high-protein diet was double that with the control diet, as would be expected with excellent compliance.

The mean fasting glucose concentration was not significantly different after 5 wk of either diet, 6.3 ± 0.3 mmol/L, or 114 ± 6 mg/dL (**Figure 2**). After ingestion of the control diet, the mean glucose concentration increased to a peak of 11.4 ± 0.8 mmol/L (205 ± 14 mg/dL) after the breakfast meal, 8.4 ± 0.7 mmol/L (151 ± 13 mg/dL) after the lunch meal, 9.6 ± 0.6 mmol/L (172 ± 11 mg/dL) after the dinner meal, and 9.5 ± 0.6 mmol/L (171 ± 11 mg/dL) after snack 2. After ingestion of the high-protein diet, the mean peak glucose concentration was 10.9 ± 0.7 mmol/L (196 ± 12 mg/dL) after the breakfast meal, 7.9 ± 0.6 mmol/L (142 ± 10 mg/dL) after

TABLE 1
Sample menus

	Control (15% protein) diet		High-protein (30% protein) diet
Breakfast	244 g (8 oz) 2%-fat milk	Breakfast	245 g (8 oz) skim milk
	21 g (1 slice) cracked-wheat toast		24 g (1 slice) whole-wheat toast
	9 g (2 tsp) soft margarine		5 g (1 tsp) soft margarine
	14 g (2 tsp) jelly		7 g (1 tsp) jelly
	28 g (1 oz) Raisin Bran[1]		28 g (1 oz) Raisin Bran
	44 g (1) egg		227 g (8 oz) low-fat yogurt
	226 g (8 oz) grapefruit juice		
	4 g (1 tsp) sugar		
Lunch	36 g carrot and 48 g celery sticks	Lunch	36 g carrot and 48 g celery sticks
	57 g (2 oz) hamburger on 40-g bun		113 g (4 oz) beef and 28 g (1 oz) low-fat cheese[2] on 40-g bun
	28 g (1 oz) potato chips		28 g (1 oz) potato chips
	138 g apple		138 g apple
	15 g catsup, 5 g mustard, 21 g onion, 7 g lettuce, and 7 g (0.5 Tbsp) mayonnaise		15 g catsup, 5 g mustard, and 4 g (1 tsp) soft margarine
			245 g (8 oz) skim milk
Snack	43 g (6) graham crackers	Snack	85 g (3 oz) low-fat cottage cheese
			13 g (2) Rye Krisp[3]
Supper	85 g (3 oz) baked chicken	Supper	255 g (9 oz) baked chicken
	122 g baked potato		61 g baked potato
	184 g broccoli		138 g broccoli
	55 g lettuce salad/16 g diet French dressing		55 g lettuce salad/16 g diet French dressing
	13 g (1 Tbsp) soft margarine		13 g (1 Tbsp) soft margarine
	42 g (2 slices) cracked-wheat toast		245 g (8 oz) skim milk
	154 g peaches (3 halves)		
Snack	65 g bagel	Snack	245 g (8 oz) skim milk
	28 g (1 oz) cream cheese		6 g (2) Saltines[4]
	60 g (25) grapes		

[1] Kellogg Co, Battle Creek, MI.
[2] Kraft Cheese-Lite; Kraft, Glenview, IL.
[3] Bemmer, Inc, Princeton, KY.
[4] Keebler, Elmhurst, IL.

the lunch meal, 9.2 ± 0.8 mmol/L (165 ± 14 mg/dL) after the dinner meal, and 8.6 ± 0.3 mmol/L (154 ± 6 mg/dL) after snack 2. Thus, the glucose concentration was consistently lower after the high-protein diet, particularly in the evening.

The mean 24-h integrated glucose area responses, with the fasting glucose concentration as baseline, were 34.1 ± 7.2 mmol · h/L (614 ± 130 mg · h/dL) and 21.0 ± 4.2 mmol · h/L (378 ± 75 mg · h/dL) after the control and high-protein diets, respectively. The difference was statistically significant ($P < 0.02$).

The mean total percent glycated hemoglobin decreased from 8.0 ± 0.2% to 7.7 ± 0.3% after 5 wk of the control diet (**Figure 3**). After 5 wk of the high-protein diet, the total percent glycated hemoglobin decreased from 8.1 ± 0.3% to 7.3 ± 0.2%. The decrease was statistically significant after 4 and 5 wk of the high-protein diet

TABLE 2
Composition of diets[1]

	Control (15% protein) diet	High-protein (30% protein) diet
Energy (kcal)	2266	2235
Protein (g)	84	166
Carbohydrate (g)	302	223
Monosaccharides (g)	47	33
Disaccharides (g)	44	42
Fat (g)	86	75
Monounsaturated fat (g)	32	41
Polyunsaturated fat (g)	15	16
Saturated fat (g)	28	33
Cholesterol (mg)	298	337
Dietary fiber (g)	26	29

[1] Values are averages of 6-d menus. Analysis performed by using NUTRITIONIST PRO version 2.0 (FIRSTDATABANK; The Hearst Corporation, San Bruno, CA).

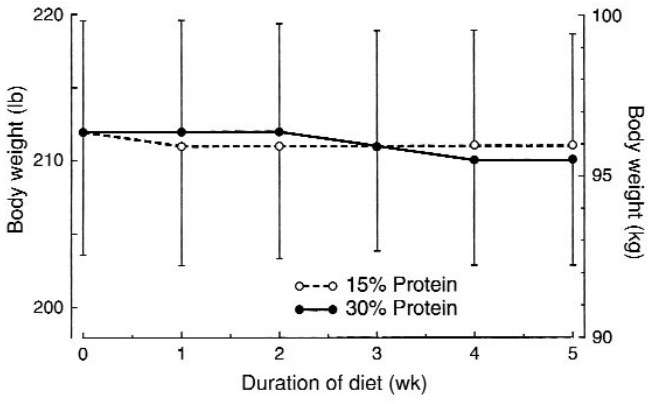

FIGURE 1. Mean (± SEM) body weight of the subjects during the 5-wk control (15% protein) and high-protein (30% protein) diets. $n = 12$.

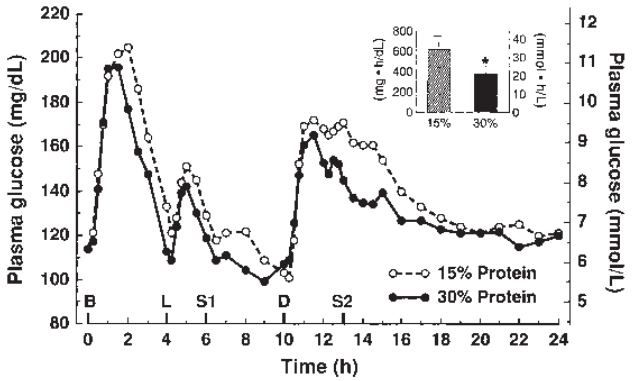

FIGURE 2. Twenty-four–hour plasma glucose response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). $n = 12$. Inset: mean (±SEM) 24-h net glucose area response, with the fasting glucose concentration as baseline. *Significantly different from the control diet, $P < 0.05$ (Student's t test for paired variates). $n = 12$.

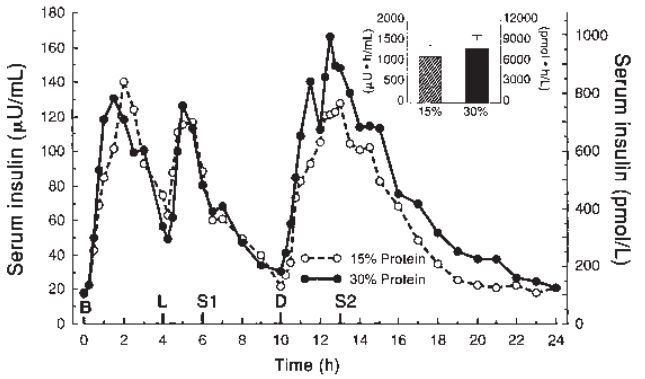

FIGURE 4. Twenty-four–hour serum insulin response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). $n = 12$. Inset: mean (±SEM) 24-h net insulin area response, with the fasting insulin concentration as baseline. $n = 12$.

($P < 0.05$) but not after the control diet. The rate of decline also was significantly greater after the high-protein diet ($P < 0.001$), which was determined by using Student's paired t test of the decrement in slope over 5 wk.

The mean fasting serum insulin concentrations were 104 ± 18 pmol/L (17.3 ± 3.0 μU/mL) and 110 ± 21 pmol/L (18.4 ± 3.5 μU/mL) after the control and high-protein diets, respectively (**Figure 4**). The insulin concentrations increased rapidly after the meals, as expected. The insulin excursion was not significantly different with either diet after breakfast and lunch but was modestly greater after dinner with the high-protein diet.

The mean 24-h integrated insulin area response, with the fasting value as baseline, was 6720 ± 1710 pmol · h/L (1120 ± 285 μU · h/mL) after the high-protein diet. After the high-protein diet, the insulin area response was 7962 ± 1890 pmol · h/L (1327 ± 315 μU · h/mL), or 18% greater than that after the control diet. This difference in area response was not statistically significant ($P = 0.25$).

The mean fasting C-peptide concentration was 0.27 ± 0.03 nmol/L (0.82 ± 0.08 pg/mL) after both the control and the high-protein diets. The C-peptide response was similar to the insulin response (**Figure 5**).

The mean 24-h integrated C-peptide area response was 5.5 ± 0.8 nmol · h/L (16.4 ± 2.4 pg · h/mL) with the control diet and 6.9 ± 1.0 nmol · h/L (20.7 ± 3 pg · h/mL) with the high-protein diet, ie, 26% greater than that produced by the control diet. This difference in area response was not statistically significant ($P = 0.07$).

The mean fasting glucagon concentrations were not significantly different after the control and high-protein diets: 78 ±

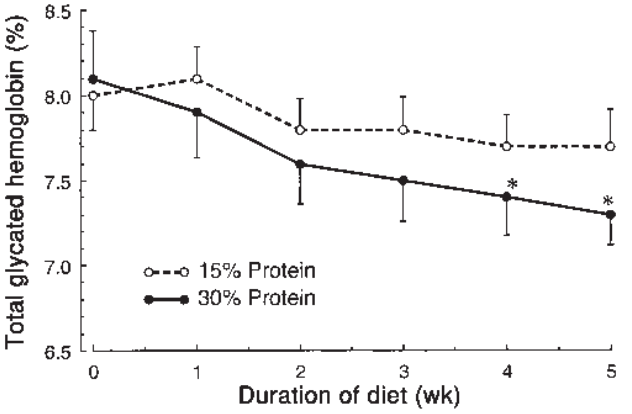

FIGURE 3. Mean (±SEM) total glycated hemoglobin response of subjects to the control (15% protein) and high-protein (30% protein) diets over the 5-wk study period. *Significantly different from the control diet, $P < 0.05$ (Student's t test for paired variates). The rate of decline was also significantly greater after the high-protein diet, $P < 0.001$ (Student's paired t test of the decrement in slope over 5 wk). $n = 12$.

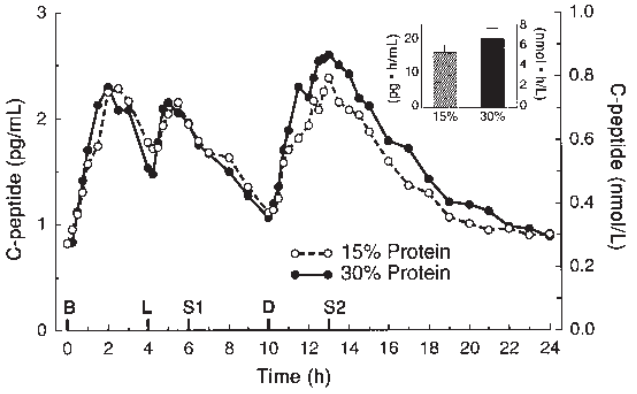

FIGURE 5. Twenty-four–hour C-peptide response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). $n = 12$. Inset: mean (±SEM) 24-h net C-peptide area response, with the fasting C-peptide concentration used as baseline. $n = 12$.

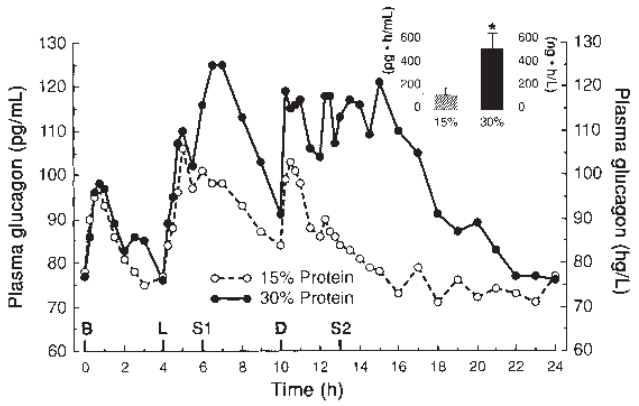

FIGURE 6. Twenty-four–hour glucagon response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). $n = 12$. Inset: mean (±SEM) 24-h net glucagon area response, with the fasting glucagon concentration used as baseline. *Significantly different from the control diet, $P < 0.01$ (Student's t test for paired variates). $n = 12$.

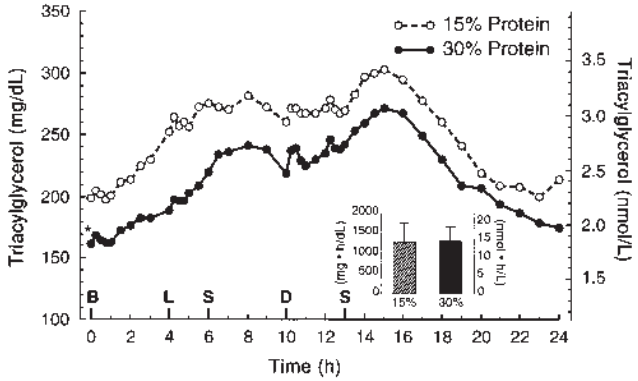

FIGURE 8. Twenty-four–hour triacylglycerol response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). *Significantly different from the fasting control value, $P < 0.03$ (Student's t test for paired variates). $n = 12$. Inset: mean (±SEM) 24-h net triacylglycerol area response, with the fasting triacylglycerol concentration as baseline. $n = 12$.

8.9 and 77 ± 10.5 ng/L (78 ± 8.9 and 77 ± 10.5 pg/mL), respectively. The glucagon response was not significantly different for the first 2 h after breakfast and lunch. However, from 1400 onward (6 h), the glucagon concentration was higher with the high-protein diet. After dinner, the glucagon concentration remained higher at all time points, until 0800 the next morning (**Figure 6**).

The mean 24-h integrated glucagon area response was 127 ± 63 and 525 ± 136 ng · h/L (127 ± 63 and 525 ± 136 pg · h/mL) as a consequence of the control and high-protein diets, respectively, which represented a 4-fold difference ($P < 0.01$).

The mean fasting NEFA concentrations were 623 ± 53 μmol/L (623 ± 53 μEq/L) and 659 ± 80 μmol/L (659 ± 80 μEq/L) after 5 wk of the control and high-protein diets, respectively

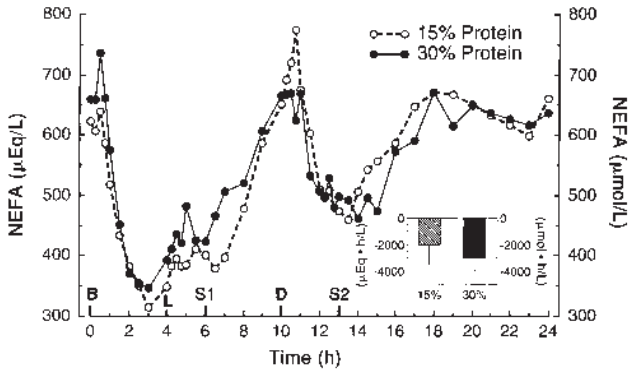

FIGURE 7. Twenty-four–hour nonesterified fatty acid (NEFA) response of subjects to the control (15% protein) and high-protein (30% protein) diets. Breakfast (B) was given at time 0 (0800), lunch (L) at 4 h (1200), dinner (D) at 10 h (1800), snack 1 (S1) at 6 h (1400), and snack 2 (S2) at 13 h (2100). $n = 12$. Inset: mean (±SEM) 24-h net NEFA area response, with the fasting NEFA concentration used as baseline. $n = 12$.

(**Figure 7**). After ingestion of the low-protein diet, the NEFA concentration changed little for 30 min. The concentration then decreased progressively for 3 h after breakfast. It subsequently increased and reached a peak of 774 ± 132 μmol/L (774 ± 132 μEq/L) at 45 min after dinner, declined until 3 h after dinner, and then increased again. The NEFA concentration increased 30 min after breakfast with the high-protein diet. Thereafter, the NEFA response to the high-protein diet was not significantly different from the low-protein diet, except that the peak was reached 30 min after breakfast (736 ± 59 μmol/L, or 736 ± 59 μEq/L), and the concentration at dinner was not significantly different from the fasting concentration.

The mean 24-h integrated NEFA area response was −2027 ± 1496 μmol · h/L (−2027 ± 1496 μEq · h/L) after the control diet (Figure 7). After the high-protein diet, the NEFA area response was −3008 ± 988 μmol · h/L (−3008 ± 988 μEq · h/L), or a reduction of ≈50% more than that after the control diet. There was considerable variability between subjects, and the differences in response were not statistically significant ($P = 0.44$).

The mean fasting triacylglycerol concentrations were 2.2 ± 0.2 mmol/L (199 ± 20 mg/dL) after 5 wk of the control diet and 1.8 ± 0.26 mmol/L (161 ± 23 mg/dL) after 5 wk of the high-protein diet (**Figure 8**). The difference was statistically significant ($P < 0.03$). After ingestion of either diet, the triacylglycerol concentration increased until ≈1600, remained stable until ≈2000, and then increased again until 0200. It then decreased toward baseline by 0800 the following morning.

The mean 24-h integrated triacylglycerol area response, above the fasting concentration, was 14.3 ± 5.2 mmol · h/L (1278 ± 462 mg · h/dL) and 16.4 ± 3.9 mmol · h/L (1304 ± 352 mg · h/dL) after the control and high-protein diets, respectively. Thus, although the fasting triacylglycerol concentration was lower after the high-protein diet than after the control diet ($P < 0.03$), the net triacylglycerol area response after meals was not significantly different.

Total cholesterol concentrations were 4.7 ± 0.4 and 4.4 ± 0.3 mmol/L (181 ± 15 and 171 ± 12 mg/dL) after 5 wk of the

control and high-protein diets, respectively. HDL-cholesterol concentrations were 1.0 ± 0.08 and 1.0 ± 0.08 mmol/L (38 ± 3 and 39 ± 3 mg/dL), and LDL-cholesterol concentrations were 2.6 ± 0.3 and 2.6 ± 0.3 mmol/L (100 ± 12 and 101 ± 12 mg/dL) with the control and high-protein diets, respectively. These differences were not statistically significant.

The urinary creatinine clearance was 122 ± 11 and 113 ± 27 mL/min after 5 wk of the control and high-protein diets, respectively. The urinary microalbumin value was 7.75 ± 1.71 mg after 5 wk of the control diet and 7.01 ± 0.81 mg after 5 wk of the high-protein diet. None of these differences were statistically significant.

The average blood pressure was 132 (systolic) and 74 (diastolic) mm Hg before the 5 wk of the control and high-protein diets and remained stable throughout the study (data not shown).

DISCUSSION

Several years ago we reported that protein, on a weight basis, is just as potent as is glucose in stimulating insulin secretion in persons with type 2 diabetes (14). When protein was given with glucose, a synergistic effect on insulin was observed. As a result, the glucose area response was significantly less after ingestion of protein plus glucose than after ingestion of glucose alone. The insulin response was linearly related to the amount of protein ingested. These data were obtained by using very lean beef protein. Subsequently, we reported that 7 different protein sources were effective in stimulating an increase in circulating insulin concentrations in persons with type 2 diabetes, although they varied in potency (18). These highly significant insulin responses to different proteins resulted in either no change or a modest decrease in glucose concentration. This suggested that an increase in protein content in the diet—particularly if associated with a decrease in carbohydrate content—would result in a decrease in the integrated glucose concentration. Such a diet could be useful for controlling blood glucose in persons with type 2 diabetes, provided it does not result in any adverse effects. The present data indicate that an increase in dietary protein can improve the 24-h integrated glucose concentration and result in a significant decrease in glycated hemoglobin over a 5-wk period. This time frame was chosen because it represents the time required for glycated hemoglobin to decrease by 50% of its ultimate value ($t_{1/2}$ = 33 d) (19). In addition, with the method used to measure total glycated hemoglobin, each 1% represents ≈18 mg glucose/dL integrated over the life span of a red blood cell (20). In the present study the decrease in total glycated hemoglobin was 0.8%, or ≈14.5 mg/dL glucose. Thus, the final integrated glucose response would be expected to be a decrease of ≈29 mg/dL.

Doubling the protein content of the diet resulted in a doubling of the urea-creatinine ratio by week 1. The ratio remained stable over the 5 wk of the study. Thus, compliance was excellent. The order in which the diets was given did not significantly affect the results. Weight stability also was excellent (Figure 1). We considered this a critical aspect of the study design because our primary goal was to determine the effect of the diet per se on total glycated hemoglobin, without the confounding effect of weight loss (or gain) or a reduced (or increased) food-energy intake. Blood pressure also remained stable throughout the study. On the basis of weight stability and dietary compliance, the metabolic changes that occurred after 5 wk of the high-protein diet were considered to be attributed to the increase in protein or decrease in carbohydrate content of the diet, or both, rather than other confounding factors.

The mean fasting glucose concentration at the time the subjects were recruited and enrolled in the study was 148 ± 8 mg/dL (8.2 ± 1 mmol/L) (total glycated hemoglobin = 8.1%). However, at the beginning of the first 5-wk study period, the glucose concentration had decreased to 114 mg/dL, which is lower than ideal for a study designed to show a decrease in glucose concentration. In our experience, even though we asked the volunteers not to change their diets or eating patterns before beginning the study, it is not uncommon for subjects to report for study with a fasting glucose concentration lower than when they were recruited (13, 21). The reason for the lower concentration is unknown; there was no change in body weight. However, we suspect that subtle, unintentional changes in diet or activity occur because of the increased attention paid to the subject and his or her disease. This effect has been referred to as the Hawthorn, or intervention, effect (22). These changes indicate the need for all studies in which the subjects have diabetes to be carefully controlled and not merely to be observational in nature. The change in fasting glucose also represents a potential limitation in interpretation of the data obtained in the present study.

The high-protein diet resulted in a glucose concentration that was modestly lower after each of the meals. This was most apparent after the dinner meal, at which time both the insulin and glucagon concentrations were also greater. The net result was a statistically significant decrease in the 24-h integrated glucose area response, with the fasting value as baseline (Figure 2).

Glucose excursion was lower after the midday meal, even though the carbohydrate content of the lunch meals was only modestly decreased (82 compared with 69 g for the control diet and 65 compared with 49 g for the high-protein diet). This indicates an increase in insulin sensitivity with a second meal, which may represent the so-called Staub-Traugott phenomenon (23, 24).

The prelunch and predinner glucose concentrations were also lower than the overnight fasting value; this finding was reported previously in persons with type 2 diabetes (25–27). Lower midday glucose concentrations also have been observed when persons were fasting (28), but the decrease in concentration was less in the persons without diabetes (29, 30). To our knowledge, the reason for this circadian variation in glucose has not been determined.

The postmeal insulin area responses were modestly but not statistically significantly increased with the high-protein diet. These increases were less than we anticipated on the basis of single-meal and single-day studies. The reason for this is unclear but it may have been due to a corresponding decrease in carbohydrate in the diet.

The glucagon area response to the high-protein diet was robust, ie, 4-fold greater than with the control diet, with most of the increase occurring later in the day (Figure 6). This finding is similar to data obtained in healthy subjects (31). This highly significant increase in glucagon would be expected to result in a stimulation of gluconeogenesis and glycogenolysis and a subsequent increase in circulating plasma glucose concentrations. However, the glucose response was less with the high-protein diet. This raises the issue of the role of glucagon in the regulation of hepatic glucose production, both after an overnight fast and after meals, as we pointed out previously (13). Tachyphylaxis has been reported to occur during a 45-min infusion of glucagon (32). Tachyphylaxis may explain

the lack of increase in glucose; however, it needs to be documented by determining the endogenous glucose production rate. Note that in a single-meal study, ingestion of 50 g beef protein resulted in only a very modest increase in the glucose appearance rate, even though the glucagon concentration increased briskly (13).

The 24-h NEFA profile was interesting. The NEFA concentration rapidly decreased after the breakfast meal and then gradually increased throughout the day, with a sharp peak occurring after the dinner meal. These increases occurred even though the insulin concentration was elevated over most of this time period. The reason for this also is not clear and would appear to be paradoxical. Insulin is known to lower the NEFA concentration in a very sensitive fashion (33). The decrease in NEFA concentration could have been due to hydrolysis of the ingested triacylglycerols by capillary bed lipoprotein lipase activity in response to the higher insulin concentrations (34). If so, this phenomenon exceeded the known effect of insulin to suppress triacylglycerol hydrolysis in fat cells and to stimulate triacylglycerol synthesis and storage in the same cells (35).

It is also interesting that a transient increase in the NEFA concentration occurred after the high-protein breakfast meal. This finding was observed previously in single-meal studies of the ingestion of protein or protein plus glucose (10, 11, 13, 18, 36). However, it has not been a consistent finding (9, 37), and the mechanism is unknown.

Over the duration of the present study, no adverse effects were noted. However, longer-term studies will be required to properly investigate this issue.

The creatinine clearance was modestly and nonstatistically significantly decreased when the subjects ingested the high-protein diet. An increase in dietary protein has been associated with an increase in creatinine clearance in subjects with normal kidney function. This has been reported in acute (38–42) as well as in longer-term (5 d to 3 wk) studies (43–47); however, this is not a consistent finding. In a population-based study (48) and in a 6-mo outpatient study (49), an increased dietary protein intake was not associated with an increase in creatinine clearance. It may be that the kidney eventually adapts to an increase in dietary protein; however, this possibility requires confirmation in long-term, controlled, intervention studies.

Total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations were essentially unchanged with the high-protein diet. However, the fasting triacylglycerol concentration decreased by 20% after 5 wk of the high-protein diet (Figure 8). This finding is similar to the decrease reported in a 1-mo study of hyperlipidemic people without diabetes who consumed a 27%-protein diet in which 11% of total dietary energy from starch was replaced with vegetable protein (50). These decreases in triacylglycerol were likely due to decreases in dietary carbohydrate (51). Several studies reported an increase in triacylglycerol concentrations when dietary fat is replaced with carbohydrate. However, the replacement of fat with protein has been reported to not affect triacylglycerol concentrations (*see* reference 51 for a review).

During the review process, it was requested that we reanalyze the data to exclude the independent variables of age, body mass index, total glycated hemoglobin, duration of diabetes, weight change, and treatment with β blockers or diuretics. None of these independent variables had an effect on the total percent glycated hemoglobin, glucose area, or fasting triacylglycerol concentration—the primary outcome variables.

In summary, an increase in the protein content and a decrease in the carbohydrate content of the diet for 5 wk resulted in an improvement in the 24-h integrated net blood glucose area response and a statistically significant decrease in glycated hemoglobin in subjects with untreated type 2 diabetes. These improvements in glucose control occurred without a change in body weight. In addition, microalbumin and creatinine clearance, indicators of kidney function, were unchanged. Furthermore, the triacylglycerol concentration decreased, and the total cholesterol and HDL- and LDL-cholesterol fractions were unchanged; however, these data must be interpreted with caution because some subjects were taking medications for lipid control. Overall, the data suggest that a high-protein diet may improve blood glucose control in persons with type 2 diabetes. Nevertheless, longer-term studies are necessary to determine potential adverse effects of such a diet, if any. The long-term acceptability of such a diet should also be determined. Data for plasma urea nitrogen, creatinine, α amino nitrogen, uric acid, growth hormone, and insulin-like growth factor I and for urinary urea, aldosterone, and cortisol is published separately (52).

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MCG and FQN were responsible for designing the experiment, evaluating the statistics, interpreting the data, writing the manuscript, and organizing the figures and tables. AS was responsible for recruiting the patients and providing medical care during the study. KJ was responsible for collecting and managing the data, analyzing the laboratory data, and managing the patients and samples during the study. HH was responsible for designing the diets, interviewing the patients biweekly, and managing the diet during the study. MCG, FQN, and HH are full-time employees of the Department of Veterans Affairs, Minneapolis. MCG and FQN are members of the American Diabetes Association Professional Society. The authors had no affiliation with the Boards of Directors of the American Diabetes Association or the Minnesota Beef Council, the Colorado Beef Council, or the Nebraska Beef Council.

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