High-protein diets in hyperlipidemia: effect of wheat gluten on serum lipids, uric acid, and renal function

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ABSTRACT
Background: The metabolic effects of diets high in vegetable protein have not been assessed despite much recent interest in the effect of soy proteins in reducing serum cholesterol.

Objective: We assessed the metabolic effects of diets high in vegetable protein (specifically, wheat gluten) on serum lipids, uric acid concentrations, and renal function.

Design: Twenty hyperlipidemic men and women consumed isonenergetic test (high-protein) and control metabolic diets for 1 mo in a randomized crossover design. In the high-protein diet, 11% of the total dietary energy from starch in the control bread was replaced by vegetable protein (wheat gluten), resulting in 27% of total energy from protein compared with 16% in the control diet. In other respects, the 2 diets were identical.

Results: Compared with the control, the high-protein diet resulted in lower serum concentrations of triacylglycerol (by 19.2 ± 5.6%; P = 0.003), uric acid (by 12.7 ± 2.0%; P < 0.001), and creatinine (by 2.5 ± 1.1%; P = 0.035) and higher serum concentrations of urea (by 42.2 ± 5.8%; P < 0.001) and a higher 24-h urinary urea output (by 99.2 ± 17.2%; P < 0.001). No significant differences were detected in total or HDL cholesterol or in the renal clearance of creatinine. LDL oxidation, assessed as the ratio of conjugated dienes to LDL cholesterol in the LDL fraction, was lower with the high-protein diet (by 20.6 ± 3.6%; P = 0.009).

Conclusions: High intakes of vegetable protein from gluten may have beneficial effects on cardiovascular disease risk by reducing oxidized LDL, serum triacylglycerol, and uric acid. Further studies are required to assess the longer-term effects on renal function. Am J Clin Nutr 2001;74:57–63.

KEY WORDS High-protein diets, vegetable protein, wheat gluten, functional foods, oxidized LDL cholesterol, triacylglycerol, hyperlipidemia, uric acid, cardiovascular disease, creatinine, urea, renal function

INTRODUCTION

High-protein diets, with protein intakes ranging from 25% to 38% of dietary energy, are promoted for weight loss (1). These percentages of protein, however, are considerably higher than the 10–20% advised by most government and health-related agencies (2–7). Some investigators have expressed concern that diets high in animal-protein foods and low in fiber may increase cardiovascular disease risk (1), and that increased protein intakes, by promoting renal hyperfiltration, may lead to renal damage in susceptible individuals (8).

Much of the debate concerning protein has focused on the issue of protein restriction in the preservation of renal function in high-risk subjects, including persons with preexisting renal disease and persons with diabetes (9–14). The concept of restricting protein to slow the progression of chronic renal disease and reduce the symptoms of renal failure dates to Bright (13) and Beale (15) in the mid-19th century. At present, there is agreement that lower protein intakes, by reducing phosphates, sodium, and acid metabolites, improve some of the complications of renal failure, including renal osteodystrophy, hypertension, electrolyte disturbances, and metabolic acidosis (12). However, opinion is divided over the extent to which protein restriction can slow the progression of renal insufficiency and the extent to which higher protein intakes cause damage (9, 11, 12). Much of the concern relates to the interpretation over the past 5 y of the results of the Modification of Diet in Renal Disease (MDRD) Study (16). The MDRD Study was the most extensive study devoted to the protein issue but was criticized for having too short a duration (mean 2.2 y of follow-up) and for having inadequate compliance (9, 11, 12). Nevertheless, the most recent assessments of the MDRD data suggest a slower rate of reduction...
in the glomerular filtration rate and a prolongation of time to dialysis with lower protein intakes (12, 17).

In several studies, investigators focused on replacing animal proteins with vegetable proteins in the treatment of disease states including hyperlipidemia, hepatic encephalopathy, and renal disease (10, 13, 18–25). In most of these studies, soy was used as the vegetable-protein source (13–21), and few studies in humans have assessed the effects of other vegetable proteins. We therefore examined the effect of increased intake of a commonly eaten vegetable protein (wheat gluten) to determine whether the beneficial effects on serum lipids were similar to those of soy and whether high intakes of vegetable protein compromise renal function.

SUBJECTS AND METHODS

Subjects

Twenty subjects (15 men and 5 women) aged (x ± SE) 55.6 ± 1.9 y (range: 35–71 y) with a mean body mass index (BMI; in kg/m²) of 26.0 ± 0.7 (range: 20.3–31.2) completed a crossover study involving high-vegetable-protein and control metabolic diets, each lasting 1 mo. This duration was selected because in previous studies of soy and dietary fiber we saw maximum decreases in serum lipids between 2 and 4 wk (26, 27). Ten subjects started the control diet in the first phase of the study and 10 subjects started the high-protein diet first. All subjects had been shown previously to have elevated LDL-cholesterol concentrations (>4.1 mmol/L) (6) and had been instructed about a National Cholesterol Education Program Step II diet (total fat <30% of energy, saturated fat <7% of energy, and dietary cholesterol <200 mg/d) ≥2 mo before starting the study. At the start of the study, 4 subjects had both increased serum LDL-cholesterol and increased serum triacylglycerol concentrations (>2.3 mmol/L), 8 had increased LDL cholesterol alone, 5 had increased triacylglycerol alone, and 3 were normolipidemic. Subjects had no clinical evidence of cardiovascular disease, diabetes, liver disease, or renal disease. None were taking lipid-lowering medications, hormone replacement therapy, or L-thyroxine. One subject was taking a β-blocking agent and 2 were taking angiotensin-converting enzyme inhibitors. All medications were held constant throughout the study periods. Subjects were also asked to maintain the same level of physical activity throughout the study.

During the metabolic periods, the subjects were provided with all food to be consumed; the food was prepackaged and was delivered by a courier weekly. Also at weekly intervals, the subjects came fasting to the Clinical Nutrition Center, where they were weighed, had their blood pressure measured by the same observer after being seated for 15 min, and had their dietary compliance assessed on the basis of returned uneaten items and menu plans (on which the subjects recorded the amount of all items eaten). When necessary, diets were adjusted to avoid changes in body weight. Fasting blood samples were collected at baseline and at the end of weeks 2 and 4 of each metabolic phase; also at the end of week 4, each subject collected his or her 24-h urinary output in a 4-L plastic container to which no preservatives had been added.

The study was approved by the Ethics Committee of the University of Toronto. Informed consent was obtained from all subjects.

Diet

The diets in both phases were identical and consisted of the same foods, apart from the bread, which constituted ≈18% of the total energy intake of each subject. The macronutrient composition of the control bread as a percentage of energy was 1.3% protein, 8.1% fat, and 90.7% available carbohydrate, with 1.55 g fiber/MJ (6.5 g fiber/1000 kcal). The composition of the test (high-protein) bread was 50.4% protein, 7.0% fat, and 42.6% available carbohydrate, with 1.24 g fiber/MJ (5.2 g fiber/1000 kcal). Because the breads represented the only difference between the 2 diets, they were also analyzed for calcium, magnesium, sodium, and potassium contents. In the control diet, the bread contributed 22 mg Ca/d, 13 mg Mg/d, 174 mg Na/d, and 83 mg K/d. The test bread contributed 47 mg Ca/d, 48 mg Mg/d, 1434 mg Na/d, and 117 mg K/d. Only for sodium was the bread mineral contribution reflected in a significant increase in total dietary intake. This difference was the result of the very low sodium content of the control bread. The sodium content of the high-protein bread was similar to that of breads made from regular enriched wheat flour (28).

The macronutrient composition of the total control and high-protein diets is given in Table 1. For the metabolic diets, energy intake was assessed for weight maintenance by using standard tables (29), with adjustment for each subject’s physical activity and prediet 7-d diet history. Diets were devised and dietary intakes calculated by using a database in which most of the foods

### Table 1

Calculated macronutrient intakes with the control and high-protein metabolic diets

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>High-protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ/d)</td>
<td>11.86 ± 0.58</td>
<td>11.56 ± 0.77</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2835 ± 139</td>
<td>2764 ± 183</td>
</tr>
<tr>
<td>Total protein (g/d)</td>
<td>111 ± 6</td>
<td>189 ± 12</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>15.6 ± 0.3</td>
<td>27.4 ± 0.3</td>
</tr>
<tr>
<td>Vegetable protein (g/d)</td>
<td>59 ± 4</td>
<td>139 ± 10</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>8.2 ± 0.3</td>
<td>20.1 ± 0.4</td>
</tr>
<tr>
<td>Available carbohydrate (g/d)</td>
<td>415 ± 20</td>
<td>323 ± 22</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>58.6 ± 0.5</td>
<td>46.7 ± 0.4</td>
</tr>
<tr>
<td>Total fiber (g/d)</td>
<td>40 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>80 ± 4</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>25.5 ± 0.5</td>
<td>25.6 ± 0.4</td>
</tr>
<tr>
<td>SFA (g/d)</td>
<td>16 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>4.9 ± 0.1</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>35 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>11.2 ± 0.3</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>23 ± 1</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>7.2 ± 0.2</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Dietary cholesterol (mg/d)</td>
<td>38 ± 2</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
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*SEM; ± indicates standard error of the mean; n = 20. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
had been analyzed in the laboratory with use of Association of Official Analytical Chemists methods for fat, protein (30), and fiber (31), with available carbohydrate calculated by difference. The fatty acid composition was determined by gas chromatography (32). The food-composition tables of the US Department of Agriculture (33) and food labels were used for foods that had not been analyzed directly.

Analyses

Serum stored at \(-70^\circ\mathrm{C}\) was analyzed in a single batch according to the Lipid Research Clinics (34) protocol for total cholesterol, triacylglycerols, and HDL cholesterol after dextran sulfate–magnesium chloride precipitation (35). LDL cholesterol was calculated (36) for all but one subject who previously had serum triacylglycerol concentrations >4.0 mmol/L. In this subject, LDL cholesterol was assessed after ultracentrifugation of fresh plasma into fractions with densities >1.006 or <1.006 g/L (34). Non-HDL cholesterol was also calculated (as total cholesterol minus HDL cholesterol). The CVs for these analyses were as follows: total cholesterol, 1.5% (range: 0.8–3.2%); HDL cholesterol, 3.2% (range: 1.6–5.5%); and triacylglycerols, 3.0% (range: 1.9–5.0%) (37). Oxidized LDL was also assessed as conjugated dienes in the LDL fraction (38–40).

Serum and urine samples stored at \(-70^\circ\mathrm{C}\) were also analyzed for each subject in a single batch in the routine clinical chemistry laboratory by standard methods for urea, creatinine (Kodak Ektachem analyzers; Eastman Kodak, Rochester, NY), and uric acid (41). Urine samples from the first 12 subjects enrolled were analyzed for urinary C-peptide by radioimmunoassay (42). Urinary C-peptide was selected as a measure of 24-h insulin secretion (43–46), recognizing that a significant effect may be seen only with relatively large differences in insulin secretion (43).

Statistical analyses

The results are expressed as means ± SEMs. Treatment differences were assessed by analysis of covariance with the general linear model procedure (PROC GLM/SAS) with end-of-treatment value as the response variable and the following main effects as covariates: diet, sex, treatment order (sequence), sex-by-sequence interaction, and baseline value (47). Paired two-tailed Student’s \(t\) tests were used to assess the significance of the percentage difference between end values for the 2 treatments.

RESULTS

Compliance with both treatments was satisfactory. The subjects consumed 96.4 ± 0.9% of the dietary energy provided for the control diet and 93.4 ± 1.7% for the high-protein diet. There were no significant differences in weight change between treatments (Table 2). At the end of the metabolic study, mean body weights were 75.9 ± 3.1 kg with the control diet and 75.9 ± 3.0 kg with the high-protein diet. There was also no treatment difference in blood pressure.

Serum lipids

Serum total and LDL-cholesterol concentrations decreased with both treatments: by 10.6 ± 2.3% \((P < 0.001)\) and 17.2 ± 4.7% \((P = 0.002)\), respectively, with the control diet and by 11.1 ± 2.6% \((P < 0.001)\) and 10.9 ± 3.3% \((P = 0.004)\), respectively, with the high-protein diet. A direct comparison of the percentage differences between treatments showed that serum LDL cholesterol tended to be higher after the high-protein diet than after the control diet (by 6.9 ± 3.7%; NS), whereas serum triacylglycerol was 19.2 ± 5.6% \((P = 0.003)\) lower after the high-protein diet than after the control diet (Table 2). A similar treatment difference in triacylglycerol was seen by 2 wk with the high-protein diet (17.1 ± 7.9%; \(P = 0.044)\). The significance of the LDL-cholesterol and triacylglycerol differences was confirmed by using absolute values in the general linear models procedure \((P = 0.047 \text{ and } P = 0.006, \text{ respectively})\). LDL oxidation, as assessed by the ratio of conjugated dienes in the LDL fraction, was lower after the high-protein diet than after the control diet (by 10.6 ± 3.6%; \(P = 0.009)\). In addition, the non-HDL-cholesterol concentration tended to be lower after the high-protein diet (NS). No other significant treatment differences were seen in blood lipids or lipoprotein ratios.

Serum urea, creatinine, and uric acid

As shown in Table 2, blood urea concentrations were 42.2 ± 5.8% higher after the high-protein diet than after the control diet, whereas serum uric acid and serum creatinine were lower (by 12.7 ± 2.0% and 2.5 ± 1.1%, respectively). The significance levels for these comparisons were confirmed for the absolute concentrations by using the general linear models procedure \((P < 0.001, P < 0.001, \text{ and } P = 0.055, \text{ respectively})\).

Urinary measurements

Urine volumes were similar over 24 h for both treatments, as were 24-h urinary creatinine and uric acid outputs (Table 2). However, urinary urea excretion was higher by 99.2 ± 17.2% \((P < 0.001)\) after the high-protein diet. Apparent urea clearance was also higher by 39.3 ± 12.3% \((P = 0.005)\). No significant difference was seen between treatments in creatinine clearance or uric acid clearance. Twenty-four-hour urinary C-peptide excretion was almost identical with each treatment.

DISCUSSION

A high-vegetable-protein diet resulted in significant reductions in serum concentrations of triacylglycerol and uric acid and in the proportion of oxidized LDL cholesterol, effects that may reduce cardiovascular disease risk, particularly in persons with diabetes (48–56). Large increases were seen in serum urea, although serum creatinine was reduced and there was no apparent change in creatinine clearance. The unchanged creatinine clearance suggests that the vegetable protein (gluten) had no major adverse effects on renal function in the short term, although the long-term effects are unknown.

Previous studies showed that substituting monounsaturated fat for carbohydrate reduces serum triacylglycerol concentrations (57–60). The present study is the first study we know of in hyperlipidemic subjects to find a similar reduction in serum triacylglycerol with protein substitution. Previous reports on soy noted a cholesterol-lowering effect and only in a meta-analysis was the triacylglycerol effect significant (61). However, a study of soy in patients with renal disease noted significant decreases in serum triacylglycerol (13). The factors in soy responsible for the cholesterol-lowering effect include the soy-protein-associated isoflavones (62), the relatively high arginine content and low lysine and methionine contents of the protein (19, 20), and the presence of a 7S globulin fraction (63). No such explanations exist for gluten,
which is relatively low in arginine. Nevertheless, there are reports that if dietary fatty acids and cholesterol are held constant in self-selected diets, then high-protein diets from any source may result in lower LDL-cholesterol concentrations (64, 65). In addition, higher-protein diets were associated in large cohort studies with a reduced risk of cardiovascular disease (66). The use of these diets has also been justified from an evolutionary perspective (67).

There is increasing interest in serum triacylglycerol as a possible risk factor for cardiovascular disease in susceptible individuals, including those with diabetes, low HDL-cholesterol concentrations, or elevated apolipoprotein B concentrations (68, 69). A recent meta-analysis of 17 prospective studies, in which changes in HDL were controlled for, concluded that an 88-mg/dL (1-mmol/L) difference in fasting triacylglycerol would result in a 76% reduction in risk of cardiovascular disease (70). This estimate would translate into a 55% decrease in the present study if a 76% reduction in risk of cardiovascular disease (70). This estimate would translate into a 55% decrease in the present study if dietary fatty acids and cholesterol are held constant in self-selected diets, then high-protein diets from any source may result in lower LDL-cholesterol concentrations (64, 65). In addition, higher-protein diets were associated in large cohort studies with a reduced risk of cardiovascular disease (66). The use of these diets has also been justified from an evolutionary perspective (67).

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those with preexisting renal disease and those with diabetes who might benefit from a reduction in refined carbohydrate intake. Increasing the proportion of vegetable protein in the diet was shown in at least one study to improve renal function in persons with type 1 diabetes by reducing the glomerular filtration rate and the fractional clearance of albumin (10), assuming that these changes represent reduced glomerular hypertension and hyperfiltration (8). In this respect, acute meal feeding studies showed that soy has the least stimulatory effect postprandially on the glomerular filtration rate compared with other protein sources, including beef, chicken, and fish (77–79). No studies have assessed the effect of gluten.

In the present study, the high-vegetable-protein diet raised serum urea concentrations into the clinically abnormal range. Apparent urea clearance also increased significantly but this was likely because—unlike for serum creatinine—significant postprandial rises occur in serum urea. With a creatinine-free diet, the fasting serum creatinine concentration represents the mean 24-h serum creatinine concentration, which, together with the 24-h urine collection, is used to calculate clearance. This is not so for urea, for which high protein intakes accentuate the postprandial rises, preventing the fasting sample from representing the mean 24-h serum concentration. The difference in urea clearance is therefore likely to be an artifact related to the timing of the serum sample. More importantly, the renal clearance of creatinine remained unchanged. Although creatinine clearance is not as precise as are isotopic studies of the glomerular filtration rate, it may go some way toward addressing the original concern that increased protein loads would result in renal hyperfiltration and, in the long-term, renal damage (8). Furthermore, there are no studies documenting deleterious effects of modestly elevated serum urea concentrations outside the normal range in otherwise healthy subjects. The present study addressed only the absence of a deleterious effect of vegetable protein (gluten). Our findings may not apply to the more essential-amino-acid-rich animal proteins, which promote increased intrarenal pressure and also result in a higher renal filtration (8). In this respect, acute meal feeding studies showed that soy has the least stimulatory effect postprandially on the glomerular filtration rate compared with other protein sources, including beef, chicken, and fish (77–79).

We conclude that a high intake of vegetable protein in the form of added wheat gluten may have benefits similar to the ingestion of monounsaturated fat in reducing serum triacylglycerol (56–59). Possibly of greater significance, this dietary change was associated with a reduction in oxidized LDL. In addition, uric acid concentrations were reduced, an additional factor associated with cardiovascular disease risk reduction. Despite the lack of effect on creatinine clearance, the chronic effects of high protein intakes on renal function require further assessment before the widespread adoption of total protein intakes above the 15–20% of energy currently recommended.

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