Beans, as a Source of Dietary Fiber, Increase Cholecystokinin and Apolipoprotein B48 Response to Test Meals in Men

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ABSTRACT  Dry beans lower plasma cholesterol, an effect that has been associated with both the fiber and the protein content of beans. The objective of this study was to determine the acute hormone and lipid responses to a test meal that contained dry beans as a source of dietary fiber. A crossover design was employed in which men consumed the test meal and a control meal in random order. Both meals contained egg, bread, jelly, orange juice, milk and margarine. The high fiber meal contained white beans, whereas the low fiber (control) meal contained rice and dry milk. The men maintained their normal dietary pattern and fasted overnight before the study days. After a fasting blood sample was drawn, the men consumed the test meal and blood samples were collected over the next 6 h. Blood samples were analyzed for cholecystokinin (CCK), insulin and glucose. Plasma was separated into lipoprotein fractions and the triglyceride, cholesterol, apolipoprotein (apo) B100 and B48 content of triglyceride-rich lipoproteins determined. Insulin and glucose responses did not differ significantly between test meals; however, the CCK response was twice as high after the bean-containing meal than after the low fiber meal (P = 0.03). The increase in apo B48 concentration was significantly higher after the bean meal than after the low fiber meal (P < 0.05). Adding beans to a meal to increase fiber content prolongs the postprandial presence of intestinally derived lipoproteins and augments the CCK response to the meal. J. Nutr. 131: 1485–1490, 2001.

KEY WORDS: • beans • cholecystokinin • triglycerides • lipoproteins • insulin • humans

Sources of viscous polysaccharides lower plasma cholesterol in humans, which contributes to the reduction in risk of cardiovascular disease associated with diets high in fiber-rich foods (1–3). Several mechanisms are responsible for the hypocholesterolemic effects of viscous polysaccharides; these include increasing the fecal excretion of bile acids and sterols, slowing the rate and altering the intestinal site of lipid absorption, and short-chain fatty acid production (4,5). Dry beans lower plasma cholesterol. Several studies have associated the cholesterol-lowering of beans with their content of viscous polysaccharides; however, the protein in beans may also contribute to the response (6–12). The effect of dry beans on bile acid and neutral sterol excretion appears to be variable (6,9–11,13). Thus, other mechanisms of cholesterol lowering, including the effect of beans on the pattern of lipid absorption from the small intestine, may be important in understanding the hypocholesterolemic effects of dry beans.

The purpose of this study was to evaluate the effect of white kidney bean flakes on the alimentary pattern of lipoproteins and hormone response in humans (14,15). Because apolipoprotein (apo) B48 is specifically associated with the intestinal triglyceride-rich lipoproteins (TRL) in humans, measuring the blood plasma concentration of apo B48 allows determination of the time course of intestinal contribution to alimentary lipemia (16). In addition, we determined the alimentary pattern of the gut hormones, insulin and cholecystokinin (CCK), as indicators of gastrointestinal response to two test meals differing in their content of total fiber. The duodenal hormone CCK is released in response to fat or protein in the small intestine, and insulin is released in response to digestible carbohydrate. Thus CCK and insulin responses during the alimentary period provide an indication of the gastrointestinal response to a meal. Our hypothesis is that beans, as a source of viscous polysaccharide, will slow digestion and absorption of the meal and prolong the exposure of the small intestine to the meal contents.

SUBJECTS AND METHODS

Subjects. Healthy men (n = 10) were recruited through advertisements to participate in this study. The men were screened for diabetes, heart disease, medications and unusual food habits. Individuals who exercised >1 h/d were excluded. Fasting cholesterol and
triglyceride (TG) levels were obtained by a finger stick test (Cholestech LDX Lipid Analyzer, Cholestech, Hayward, CA). Individuals with fasting total cholesterol between 4.1 and 6.2 mmol/L, and TG <2.3 mmol/L were admitted into the study. Subject characteristics are shown in Table 1. Subjects were instructed to maintain current dietary and exercise habits for the duration of the study. They kept detailed food records for 7 d before each test meal to ensure compliance with the experimental protocol. Food records were analyzed by a registered dietician using Nutritionist III (N-Squared Computing, San Bruno, CA). The protocol was reviewed and approved by the Human Subjects Review Committee at the University of California at Davis.

Test meals. The composition of the test meals is shown in Table 2. All foods used were commercially available with the exception of the precooked instant bean flakes, which were a gift from Nestec (Vevey, Switzerland). The preparation method and nutrient content of the bean flakes have been previously described by Tappy et al. (17). Bean flakes (60 g) provided 11.8 g of dietary fiber (3.2 g insoluble fiber). Instant rice and skim milk powder were used in the low fiber meal to balance the protein and carbohydrate of the bean flakes without contributing much additional fiber. The bean flake (BF) meal provided 3060 kJ and the control (CTL) meal provided 3185 kJ. Each test meal provided approximately one third of the daily energy requirements for healthy adult men, and the meals were similar in volume.

Study design. A crossover study design was used in which each subject acted as his own control and consumed both test meals in random order, 1–3 wk apart. Subjects fasted for 12 h before the beginning of the testing period. A catheter was placed in the antecubital vein and a baseline (0-min) blood sample was drawn. The subjects then had 15 min to consume the test meal. Blood samples were drawn at 30, 45, 60, 120, 180, 240, 300 and 360 min after the start of the meal. Subjects refrained from food intake but were allowed free access to water. A continuous slow infusion of sterile saline maintained venous access between blood draws.

Blood was collected into syringes and transferred into Vacutainer tubes containing EDTA (Becton Dickinson, Rutherford, NJ). Blood was centrifuged (20 min, 1200 × g) to separate plasma, which was stored at −20°C until assayed. Lipoproteins were separated from fresh plasma by sequential ultracentrifugation (18) using a fixed angle rotor (TFF 45.6, Du Pont Biomedical Products, Sorvall Instruments, Wilmington, DE) in a Sorvall OTD-65B ultracentrifuge. Fractions were isolated by tube slicing at the following intervals: TRL (chylomicron/LDL), 0.907–1.04 kg/L; IDL (very LDL/LDL), 1.0063–1.04 kg/L; LDL, 1.0063 kg/L; and HDL, 1.063–1.21 kg/L. Fractions were frozen until assayed. For CCK analysis, fresh plasma was used and the CCK extracted by applying 2 mL plasma to a preconditioned C-18 Sep-Pak cartridge (Waters, Milford, MA), which was then rinsed with 20 mL distilled, deionized water. The loaded cartridges were stored at −70°C until assayed.

Analytical procedures. Plasma glucose was determined by the glucose oxidase method (Kit # 315, Sigma Chemical, St. Louis, MO) and insulin by RIA according to Yalow and Bennett (19) with a modified precipitation method (20). Plasma CCK was eluted from the Sep-Pak cartridges and assayed by RIA (21).

Total and nonesterified plasma and lipoprotein cholesterol were measured by the cholesterol oxidase method (22,23). Plasma and lipoprotein TG were determined by a colorimetric enzymatic method (24) that measures TG by glycerol release (Kit # 336, Sigma Chemical). Apo B100 and B48 were determined in the TRL as described previously (16,25).

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of men studied</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>38.1 ± 2.8</td>
</tr>
<tr>
<td>Ht, cm</td>
<td>178.3 ± 1.7</td>
</tr>
<tr>
<td>Wt, kg</td>
<td>81.6 ± 2.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6 ± 0.8</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, and range, n = 8, BMI, body mass index.

### Table 2

<table>
<thead>
<tr>
<th>Food content and composition of test meals</th>
<th>High fiber meal</th>
<th>Low fiber meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g</strong></td>
<td><strong>g</strong></td>
<td><strong>g</strong></td>
</tr>
<tr>
<td>Egg</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>White bread</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Strawberry jelly</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Orange juice</td>
<td>249.0</td>
<td>249.0</td>
</tr>
<tr>
<td>2% milk</td>
<td>244.0</td>
<td>244.0</td>
</tr>
<tr>
<td>Margarine</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>Instant rice</td>
<td>—</td>
<td>132.0</td>
</tr>
<tr>
<td>Nonfat milk powder</td>
<td>—</td>
<td>34.0</td>
</tr>
<tr>
<td>Bean flakes</td>
<td>60.0</td>
<td>—</td>
</tr>
<tr>
<td>Total energy from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>%</strong></td>
<td><strong>%</strong></td>
<td><strong>%</strong></td>
</tr>
<tr>
<td>Protein</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>Fat</td>
<td>23</td>
<td>22</td>
</tr>
</tbody>
</table>

### Statistical analysis

Two-factor repeated-measures ANOVA (diet, time, diet × time) was used to analyze data. One-factor ANOVA was used to compare responses based on diet at specific time points. Differences between postprandial time points and baseline for each diet period were determined using a Dunnett test. Glucose, insulin, cholecystokinin and plasma TG values were converted to increments by subtracting baseline values from each time point. Values are reported as means ± SEM. A probability value of P < 0.05 was considered significant.

### RESULTS

Eight subjects completed the study because two subjects did not fast before one of the test meals and were dropped due to noncompliance with the experimental protocol. As assessed by their food records and subject interviews, subjects’ diet and exercise habits were stable during the experimental period. For the 7 d before each test meal, there were no differences in dietary pattern; subjects consumed, on the basis of energy, 16.9% protein, 50.5% carbohydrate and 30% fat; their fiber intake was 17.8 g/d.

**Glucose and insulin response.** Postprandial glucose response peaked at 30 min for each meal (BF, 7.7 ± 0.2 mmol/L; CTL, 8.1 ± 0.3 mmol/L) (Fig. 1). There were no significant differences in postprandial glucose responses between meals over the postprandial period. After the BF meal, glucose remained significantly above baseline for 1 h; after the CTL meal, glucose remained significantly above baseline for 3 h. The areas under the incremental glucose response curves (AUC) were calculated for 0–6 h and were not significantly different between the two test meals [BF, 3.9 ± 0.8 mmol·L·h; CTL, 5.1 ± 0.7 mmol·L·h; P = 0.29]. Both meals resulted in significant increases in insulin concentrations from baseline, but there were no significant differences in postprandial insulin responses between meals (Fig. 1). The peak postprandial insulin response for the BF meal was 446.1 ± 62.3 pmol/L at 45 min, whereas the peak for the CTL meal was 497.1 ± 49.6 pmol/L at 30 min. After the BF meal, insulin was significantly higher than baseline for 4 h; after the CTL meal, insulin was significantly above baseline for 5 h. The incremental AUC for glucose response were not significantly different [BF, 798 ± 143 pmol·L·h; CTL, 1027 ± 144 pmol·L·h; P = 0.28].

**Cholecystokinin (CCK).** In response to both test meals, CCK concentrations increased significantly from baseline
The postprandial incremental CCK response was significantly higher after the BF meal than after the CTL meal. After the BF meal, CCK remained consistently higher than baseline for 4 h; however, after the CTL meal, CCK was higher than baseline at 30, 120 and 180 min and did not differ significantly from baseline at 45, 60, 240 and 360 min. The BF meal produced almost twice the CCK response, measured by AUC, as the CTL meal (BF, 25 ± 6 pmol/L·h; CTL, 14 ± 2 pmol/L·h; P = 0.03).

Lipid and lipoprotein. Both meals increased plasma TG postprandially, and concentrations were significantly higher than baseline at 2, 4 and 6 h (Fig. 3). The differences between the BF and CTL meals were not significant. The differences in the maximum TG increment (BF, 3.08 ± 0.43; CTL, 2.14 ± 0.28 pmol/L) and the sum of increments above fasting (BF, 7.07 ± 1.10; CTL, 4.89 ± 0.73 pmol/L) were significantly higher after the BF meal than after the CTL meal.

FIGURE 2 Plasma cholecystokinin (CCK) response to test meals in men fed a low fiber, control (CTL) meal or a bean flake (BF)-containing meal. Values (means ± SEM, n = 8) are reported as the increment in concentration above the fasting baseline value. Fasting CCK concentrations were 6.3 ± 0.9 and 4.1 ± 0.5 pmol/L for the CTL and BF meals, respectively. A solid, filled symbol indicates that the value at that time point is significantly different from the concentration at 0 h, P < 0.05. The CCK response to the BF meal is significantly higher than the response to the CTL meal, P < 0.05.

DISCUSSION

CCK concentrations differed markedly in men after consumption of the two test meals. The fact that both peak concentrations and total AUC were higher and the duration of response was longer resulted in a 100% higher CCK response after the BF meal than after the CTL meal. Differences in CCK response were not due to differences in the amount of fat and protein in the two test meals. Factors in beans that could contribute to the difference in the CCK response include the type of fiber in beans, the low level of trypsin inhibitor (TI) or the type of protein in beans. The fiber may act by slowing the disappearance of the meal from the stomach and small intestine, thus prolonging the period of CCK release. An additional factor that may contribute to the higher CCK response after the BF meal is that the white bean flakes...
logic responses to diets containing beans. CCK is a regulator of meal and high fiber meal containing beans observed in the present study. The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK (32). The elevation of CCK above the fasting concentration after a meal that contains a previous study, we demonstrated that consumption of barley protein in enhancing the CCK response to beans (27–31). In and is likely to be more important than the overall type of carbohydrate plus residual TI) activity may augment the CCK (32). The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK (32). The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK (32). The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK (32). The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK (32).

FIGURE 3 Plasma triglyceride and apolipoprotein (apo) B48 response to test meals in men fed a low fiber, control (CTL) meal or a bean flour (BF)-containing meal. Values (means ± SEM, n = 8) are reported as the increment in concentration above the fasting baseline value. Fasting triglyceride concentrations were 1.13 ± 0.19 and 1.02 ± 0.14 mmol/L for the CTL and BF meals, respectively, and fasting apo B48 concentrations were 0.96 ± 0.47 and 0.36 ± 0.24 nmol/L for the CTL and BF meals, respectively. A solid, filled symbol indicates that the value at that time point is significantly different from the concentration at 0 h, \( P < 0.05 \). The apo B48 response is significantly higher after the BF meal than after the CTL meal, \( P < 0.05 \). The triglycerides did not differ between test meals, \( P = 0.05 \).

TABLE 3 Plasma cholesterol and concentration of cholesterol, triglyceride, and apolipoprotein (apo) B100 in triglyceride-rich lipoproteins (TRL) in men after consuming a low fiber, control meal or a meal containing bean flakes

<table>
<thead>
<tr>
<th>Time postmeal, h</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>0.28 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.18 ± 0.04</td>
</tr>
</tbody>
</table>

* Indicates the value is significantly different from time 0 h, \( P < 0.05 \). The apo B48 response is significantly higher after the BF meal than after the CTL meal, \( P < 0.05 \). The triglycerides did not differ between test meals, \( P = 0.05 \).

The enhanced CCK response to a bean-containing meal compared with a low fiber meal may help explain the physiologic responses to diets containing beans. CCK is a regulator of gastric emptying, and thus of the rate of digestion and absorption. The postprandial rise in CCK has been associated with reductions of plasma glucose and insulin concentrations in diabetic patients (31,33,34). Schwartz et al. (31) demonstrated in noninsulin-dependent diabetic (NIDDM) or type II diabetic patients that an oral protease inhibitor, which stimulates CCK release, delays gastric emptying and decreases insulin and glucose responses to a solution containing glucose and protein. Patients with NIDDM have reduced plasma concentrations of CCK and more rapid gastric emptying times than nondiabetic subjects (35–37). Our study was conducted in nondiabetic subjects; however, in NIDDM patients, the ability of beans to enhance postprandial CCK concentration may reduce plasma insulin and glucose responses (31,35,37).

CCK release has been associated with satiety in humans and animal models (38–42). Although measures of satiety were not included in the current experimental design, Leathwood et al. (43) reported previously that the bean puree used in the present study, compared with potato puree, delays the return of hunger and desire to eat a snack. Duodenal infusion of TI into rats resulted in early termination of feeding without shortening the intermeal interval (28). Inclusion of TI in the duodenal infusate allowed a low fat/high carbohydrate infusion to mimic the greater satiating effect of a high fat/low carbohydrate infusate (28). Marciani et al. (44) reported that increased meal viscosity is associated with greater feelings of satiety in humans. Given our results, the higher CCK response to the BF meal may account for the previously reported greater feelings of satiety associated with bean flakes. Such an effect suggests that foods such as beans may be associated with a greater feeling of fullness and satiety when these foods are consumed in conjunction with low fat diets.

The test meals used in the present study were low in total fat (23% of energy) relative to other alimentary studies that used in this study contain a low activity of TI (25). Langelkilde et al. (26) reported that the product contains 4.2 TI units/mg and that in ileostomy patients, protein excretion was slightly higher in those fed the beans than in those fed potatoes. TI, found in most legumes, is a potent stimulator of CCK release and is likely to be more important than the overall type of protein in enhancing the CCK response to beans (27–31). In a previous study, we demonstrated that consumption of barley that contains β-glucan, a viscous polysaccharide, prolongs the elevation of CCK above the fasting concentration after a meal (32). The combination of factors in beans (viscous polysaccharides plus residual TI) activity may augment the CCK response, resulting in the difference between the low fiber meal and high fiber meal containing beans observed in the present study.

The enhanced CCK response to a bean-containing meal compared with a low fiber meal may help explain the physiologic responses to diets containing beans. CCK is a regulator of...
use a bolus of fat (>40% energy) to stimulate responses, yet both meals resulted in increased concentrations of plasma TG and apo B48 as well as CCK after the meal. The increment in plasma TG concentration did not differ between the dietary treatments, as reported in other similar studies (32,45). Plasma cholesterol concentrations did not change during the alimentary period. Although the concentration of TG in the TRL fraction appeared to be higher after the bean meal, the differences were not significant due in part to the variability in TG response. Cholesterol concentrations in the TRL increased after the meal. Although the difference in cholesterol concentration after consumption of the test meals was not significant, the elevation in TRL cholesterol after the bean meal occurred at 2, 4 and 6 h, whereas it occurred only at 4 and 6 h after the low fiber meal. The higher increment in apo B48 concentration after the BF than the CTL meal indicates a higher concentration of chylomicrons after the bean-containing meal. This higher concentration could be due to delayed clearance of chylomicrons, prolonged appearance from the intestine or more effective chylomicron synthesis because less fat is taken up through the portal vein. Increasing total fat content or variations in fatty acid composition cause delays in the clearance of TRL (25). However, the fat content and fatty acid composition of the test meals used in this study were similar and would not account for the differences in apo B48. The appearance of apo B48–containing TRL may be prolonged after consumption of the bean-containing diet. This interpretation would be consistent with the known effects of CCK in delaying gastric emptying, potentially delaying the time period for lipid absorption after the meal. Prolonged lipid absorption might contribute to directing more lipid absorption through chylomicron synthesis rather than through direct portal uptake. Further research is required to determine whether the difference in the increment of apo B48 lipoproteins after the BF and CTL meals is associated with chylomicrons or chylomicron remnants, which will clarify whether differences in B48 are due to the rate of clearance or appearance of chylomicrons. Beans are considered a low glycemic food; however, we did not observe a difference between the BF and CTL meal in men’s glucose or insulin responses (15,46). Others have reported that incorporation of low glycemic foods into a meal that contains protein and fat can obscure differences in plasma glucose response observed when individual foods are tested (32,47,48). Although glucose and insulin concentrations did not differ after the men consumed the two diets, both insulin and glucose remained above the baseline concentration for a longer time after the low fiber meal (CTL) than after the BF meal. The prolonged elevation in insulin and glucose above fasting concentrations suggests an overall higher glycemic response to the low fiber meal than the meal containing bean flakes. This study showed that diets rich in fiber from beans can enhance the CCK response to a meal and prolong the presence of apo B48–TRL in plasma. The studies suggest new areas of research to determine whether the presence of beans and viscous polysaccharides in low fat diets can affect the subsequent metabolism and clearance of TRL or enhance the satiating effect of meals and prolong the feelings of fullness and reduced hunger after a low fat meal.

LITERATURE CITED