Effect of Cocoa Bran on Low-Density Lipoprotein Oxidation and Fecal Bulking

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Background: Legumes have reported benefits in terms of reduced risk for coronary heart disease and of colonic health. A novel legume fiber, cocoa bran, also may have favorable health effects on serum lipid levels, low-density lipoprotein (LDL) cholesterol oxidation, and fecal bulk.

Methods: Twenty-five healthy normolipidemic subjects (13 men and 12 women) (mean±SEM age, 37±2 years; mean±SEM body mass index [calculated as weight in kilograms divided by the square of height in meters], 24.6±0.7) ate cocoa-bran and chocolate-flavored low-fiber breakfast cereals for 2-week periods, with 2-week washout, in a double-blind crossover study. The cocoa-bran cereal provided 25.0 g/d of total dietary fiber (TDF). The low-fiber cereal (5.6 g/d TDF) was of similar appearance and energy value. Fasting blood samples were obtained at the start and end of each period, and 4-day fecal collections were made from days 11 through 14.

Results: High-density lipoprotein (HDL) cholesterol level was higher (7.6%±2.9%; P = .02) and the LDL/HDL cholesterol ratio was lower (6.7%±2.3%; P = .007) for cocoa-bran compared with low-fiber cereal at 2 weeks. No effect was seen on LDL cholesterol oxidation. Mean fecal output was significantly higher for cocoa-bran than for low-fiber cereal (56±14 g/d; P < .001) and equal to the increase seen in the same subjects with wheat fiber in a previous study.

Conclusions: A chocolate-flavored cocoa-bran cereal increased fecal bulk similarly to wheat bran and was associated with a reduction in the LDL/HDL cholesterol ratio. In view of the low-fat, high-fiber nature of the material, these results suggest a possible role for this novel fiber source in the diets of normal, hyperlipidemic, and constipated subjects.

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With the notable exceptions of psyllium seed and oat bran, dietary fiber sources that reduce serum lipid concentrations tend not to increase fecal bulk, and those that increase fecal bulk tend to have no effect on serum lipid levels. In addition, fiber sources that have physiologic effects sometimes lack palatability because of unpleasant taste, grittiness, or high viscosity.

The taste of cocoa and chocolate has broad appeal. However, the energy density of many chocolate-flavored foods has reduced their use in the diets of individuals in whom energy balance is crucial, including overweight and hyperlipidemic subjects and those with type 2 diabetes mellitus.

The cocoa bean, however, is a legume with a bran or seed coat. The seed coat represents approximately 15% of the weight of the cocoa bean and is high in insoluble fiber (44%) and a source of soluble fiber (11%). The bran is present in cocoa powder (1.75% maximum allowed in cocoa) but is largely discarded in chocolate production. The question therefore arose of whether cocoa bran had the properties of lowering cholesterol levels associated with legumes and, more specifically, the antioxidant properties reported for soy and suggested for chocolate. In addition, it was of interest to determine whether cocoa bran possessed the same fecal-bulking properties seen with insoluble fiber-rich cereal brans such as wheat bran. In view of the advantage of cocoa fiber compared with other sources of fiber in terms of flavor, it seemed appropriate to assess its physiologic effects in healthy subjects.

RESULTS

All breakfast cereals during the cocoa-bran and low-fiber control phases were reported as consumed. The diet macronutrient profiles as recorded were similar for both diets, with the exception of an increase in protein of 1% of total energy...
SUBJECTS AND METHODS

Twenty-five healthy subjects (13 men and 12 women) with a mean (±SE) age of 37.2 ± 2 years (range, 22–57 years) and mean body mass index (calculated as weight in kilograms divided by the square of height in meters) of 24.6 ± 0.7 were recruited from university staff and students who had taken part in previous similar studies. They were normcholesterolemic (low-density lipoprotein [LDL] cholesterol, <4.1 mmol/L [160 mg/dL]).14 None had evidence of diabetes or renal or hepatic disease, and none were taking agents that lowered lipid levels or medications that might influence lipid metabolism. Subjects were studied for two 2-week periods with a 2-week washout period between phases. Cocoa-bran and low-fiber control breakfast cereal flakes were taken in random order after a double-blind crossover design. During the cocoa bran 2-week period, cocoa bran (total dietary fiber [TDF], 25.0 g/d) was consumed daily as a flaked cocoa-bran breakfast cereal. A chocolate-flavored low-fiber control breakfast cereal (TDF, 5.6 g/d) was also taken for 2 weeks in the same manner. Because of the volume of both breakfast cereal supplements, subjects were advised to take them in 2 servings daily, ie, morning and evening. The macronutrient profiles of the breakfast cereals are given in Table 1. Diet histories were recorded for the last week of each study period, and subjects were asked to return any uneaten breakfast cereals to assess compliance.

One overnight fasting blood sample was taken in the morning at the start and end of each study period, and blood pressure was measured in the left arm with the subject seated as the mean of 2 successive readings. Four-day fecal collections were obtained on days 11 through 14 during both phases of the study. Collections were made on an outpatient basis. Participants were provided with under-seat lavatory frames on which to attach plastic collection bags. After use, bags were sealed, labeled, and placed on frozen carbon dioxide in a polystyrene container. At the end of day 4, these containers were returned to the laboratory, where samples were weighed. The fecal data from our study were also compared with wheat-bran data from studies performed 1 and 2 years previously. 

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intake for the cocoa-bran diet (Table 2). No difference in body weight was seen between treatments (Table 3).

Serum lipid data are presented in Table 3. For the cocoa-bran diet, no change in serum lipid levels was seen across the dietary period. However, in the low-fiber control diet, the HDL cholesterol level fell and the LDL/HDL cholesterol ratio rose. Compared with the low-fiber control diet at 2 weeks, the cocoa-bran diet resulted in a significantly higher HDL cholesterol concentration (7.6%±2.9%; P=.02), lower total/HDL (6.7%±1.9%; P=.002) and LDL/HDL cholesterol ratios, and a reduced apo B/A-I ratio (4.3%±2.1%; P=.06) that approached significance. The significance level of the treatment effect for HDL cholesterol level, total/HDL and LDL/HDL cholesterol ratios, and apo B/A-I ratio was confirmed by the General Linear Model procedure (P=.04, P=.006, P=.02, and P=.06, respectively).

No significant changes were seen across either diet or between treatments in oxidized LDL cholesterol or the ratio of conjugated dienes to cholesterol in the LDL fraction (Table 3).

Significantly lower systolic and diastolic blood pressures were seen at the end of the low-fiber control diet compared with the cocoa-bran diet (3.7%±1.5% [P=.02] vs 3.9%±1.4% [P=.01]) (Table 3). Only the rise in diastolic blood pressure for the cocoa-bran diet was significant (4.3%±1.8%; P=.02).

The mean fecal output for the cocoa-bran diet was 191±16 g/d and, for the low-fiber control diet, 135±10 g/d (Table 4). The difference between treatments was significant (56%±14%; P<.001) and represented a 2.9-g increase in fecal weight per gram of additional fiber from the cocoa-bran cereal supplement. Nineteen subjects had taken part in previous studies of wheat bran at a similar level of fiber intake.15,16 The increases in fecal weight during the cocoa- and wheat-bran diets relative to their respective low-fiber control diets were comparable at 66±15 and 83±12 g/d, respectively (P=.43), or a 3.4- and 4.2-g increase in fecal weight per gram of additional fiber from the respective cereal supplement. The mean absolute fecal weights resulting from cocoa- and wheat-bran cereals were also not significantly different (198±19 vs 224±15 g/d; P=.21) (Figure). In these 19 subjects, the

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### Table 1. Daily Contribution and Composition of Cereals as Analyzed*

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Low-Fiber†</th>
<th>Cocoa-Bran†</th>
<th>Low-Fiber, % of Energy</th>
<th>Cocoa-Bran, % of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily supplement</td>
<td>159</td>
<td>183</td>
<td>. . . . . . . . . . .</td>
<td>. . . . . . . . . . .</td>
</tr>
<tr>
<td>Energy, MJ (kcal)</td>
<td>2.50 (598)</td>
<td>2.50 (598)</td>
<td>. . . . . . . . . . .</td>
<td>. . . . . . . . . . .</td>
</tr>
<tr>
<td>Protein</td>
<td>7.0</td>
<td>14.0</td>
<td>4.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Fat</td>
<td>2.4</td>
<td>2.6</td>
<td>3.6</td>
<td>3.9</td>
</tr>
<tr>
<td>SFA</td>
<td>1.7</td>
<td>1.1</td>
<td>2.6</td>
<td>1.7</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.2</td>
<td>0.7</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Available carbohydrate</td>
<td>137.0</td>
<td>130.0</td>
<td>91.6</td>
<td>87.0</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>5.6</td>
<td>25.0</td>
<td>2.24 (9.4)‡</td>
<td>10.00 (41.82)‡</td>
</tr>
</tbody>
</table>

* SFA indicates saturated fatty acids; MUFA, monosaturated fatty acids; and PUFA, polyunsaturated fatty acids.
† All data are given as grams per day unless otherwise indicated.
‡ Data are given as grams per megajoule (grams per 1000 kcal).

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### Table 2. Calculated Dietary Intakes for Week 2 of Treatment Periods*

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Low-Fiber</th>
<th>Cocoa-Bran</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ/d (kcal/d)</td>
<td>8.45 ± 0.27 (2020 ± 64)</td>
<td>9.05 ± 0.28 (2164 ± 90)</td>
<td>.09 (0.09)</td>
</tr>
<tr>
<td>Total protein, g/d (%)</td>
<td>71 ± 4 (14 ± 1)</td>
<td>82 ± 5 (15 ± 1)</td>
<td>.02 (0.03)</td>
</tr>
<tr>
<td>Available carbohydrate, g/d (%)</td>
<td>314 ± 9 (62 ± 1)</td>
<td>335 ± 14 (62 ± 1)</td>
<td>.10 (0.03)</td>
</tr>
<tr>
<td>Total dietary fiber, g/d</td>
<td>17 ± 1</td>
<td>39 ± 1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>g/MJ (g/1000 kcal)</td>
<td>2.1 ± 0.2 (8.7 ± 0.4)</td>
<td>4.3 ± 0.3 (18.6 ± 0.6)</td>
<td>&lt;.001 (&lt;.001)</td>
</tr>
<tr>
<td>Soluble fiber, g/d</td>
<td>4 ± 0</td>
<td>9 ± 0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>g/MJ (g/1000 kcal)</td>
<td>0.51 ± 0.03 (2.2 ± 0.1)</td>
<td>1.05 ± 0.04 (4.5 ± 0.2)</td>
<td>&lt;.001 (&lt;.001)</td>
</tr>
<tr>
<td>Total fat, g/d (%)</td>
<td>48 ± 2 (21 ± 1)</td>
<td>50 ± 3 (21 ± 1)</td>
<td>.19 (0.94)</td>
</tr>
<tr>
<td>SFA, g/d (%)</td>
<td>17 ± 1 (8 ± 0)</td>
<td>18 ± 1 (8 ± 0)</td>
<td>.18 (0.86)</td>
</tr>
<tr>
<td>MUFA, g/d (%)</td>
<td>17 ± 1 (7 ± 0)</td>
<td>18 ± 1 (7 ± 0)</td>
<td>.12 (0.47)</td>
</tr>
<tr>
<td>PUFA, g/d (%)</td>
<td>10 ± 1 (4 ± 0)</td>
<td>10 ± 1 (4 ± 0)</td>
<td>.49 (0.98)</td>
</tr>
<tr>
<td>Dietary cholesterol, mg/d</td>
<td>183 ± 14</td>
<td>200 ± 13</td>
<td>.79</td>
</tr>
<tr>
<td>mg/MJ (mg/1000 kcal)</td>
<td>22 ± 2 (91 ± 7)</td>
<td>22 ± 1 (95 ± 6)</td>
<td>.11 (0.11)</td>
</tr>
<tr>
<td>Alcohol, g/d (%)</td>
<td>7 ± 2 (2 ± 1)</td>
<td>6 ± 2 (2 ± 1)</td>
<td>.31 (0.42)</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SE (n = 25). SFA indicates saturated fatty acids; MUFA, monosaturated fatty acids; and PUFA, polyunsaturated fatty acids.
The cocoa-bran diet appeared to prevent the fall in HDL cholesterol levels seen with the control phase and resulted in a significantly lower LDL/HDL cholesterol ratio at 2 weeks for the test vs the control breakfast cereal. In addition, the results demonstrate that cocoa bran has a fecal bulking effect similar to that of coarse wheat bran. This was achieved despite the fine particle size to which the cocoa bran had been milled. However, the chocolate-flavored control and the cocoa-fiber cereals did not alter the concentration of oxidized LDL cholesterol, assessed as conjugated dienes in the LDL fraction, although it is possible that longer studies would be required to detect a modest effect.

The effect on serum lipid levels was unexpected. Viscous legume fibers, such as the galactomannans of guar and locust bean, have long been known to reduce serum total and LDL cholesterol levels. In general, however, viscous fiber sources have been associated with no
change in LDL/HDL cholesterol ratio, even if no significant reduction in HDL cholesterol level was recorded.\textsuperscript{37-39} Many dietary maneuvers aimed at reducing cholesterol concentrations, such as high-carbohydrate diets and increased intake of polyunsaturated fat, tend to lower LDL and HDL cholesterol levels and therefore do not improve the LDL/HDL cholesterol ratio.\textsuperscript{40} More recently, monounsaturated fats and soy protein have attracted attention, specifically because they appear to improve the LDL/HDL cholesterol ratio by reducing LDL while preserving HDL cholesterol concentrations.\textsuperscript{41-44} The effect seen herein of an apparent increase in HDL cholesterol level with cocoa bran and a corresponding reduction in the LDL/HDL cholesterol ratio is therefore unusual. This pattern of blood lipid level change has not been reported previously for legume fiber\textsuperscript{45} or whole legumes.\textsuperscript{46} No explanation can be offered yet. There was no apparent change in the total fat level or the nature of the fatty acids between the test and control diets. The small increase in protein intake resulting from the cocoa protein associated with the cocoa bran seems an unlikely candidate. However, if these results are confirmed in larger studies of longer duration, the effects of cocoa-bran protein and associated substances (flavonoids, lignans, etc) will have to be explored. It is also possible that the relatively high-carbohydrate diets eaten by our subjects may have tended to lower HDL cholesterol and raise triglyceride concentrations, and that the presence of the fiber and associated substances in the cocoa-bran cereal may have minimized this effect. Fecal sterol measurements might have helped define the effect of fiber, but these measurements were not made.

A further surprise was the significant rise in blood pressure seen with cocoa fiber. Beverages such as coffee and tea are recognized to contain methylxanthines (caffeine, theophylline, and theobromine), which may raise blood pressure,\textsuperscript{30,31} and the same may be true for cocoa. The effect was not large, with no change for the low-fiber control diet and mean rises of 1.4 and 2.9 mm Hg in systolic and diastolic blood pressure, respectively, for the cocoa-bran diet. To detect as significant the same treatment difference we observed in systolic blood pressure at the 5% level 80% of the time (\(\alpha = .05; \beta = .80\)), a sample size of at least 40 subjects would be required, assuming also the same standard deviation. Therefore, in many studies of this nature, blood pressure changes may not be detected because of the large numbers of subjects required.

An increase in fecal bulk seen with the cocoa-bran diet was not unexpected in view of the insoluble fiber content of the bran (44% insoluble fiber). What was unexpected was the magnitude of the increase, especially in view of the fine mean particle size of the bran. The cocoa bran had been milled to a very fine homogeneous powder (mean particle size, <40 \(\mu\)m). When the particle size of wheat bran has been reduced to less than 500 to 700 \(\mu\)m, a significant reduction in fecal bulking activity has been reported.\textsuperscript{32} Agencies concerned with health have therefore advised that coarse-particle bran be used, especially where a laxative effect is required.\textsuperscript{33}

Previous reports have indicated that chocolate may have antioxidant properties.\textsuperscript{11,12} Our interest was whether the antioxidant activity was associated with the chocolate flavor. Flavonoids in teas, fruit, and vegetables have attracted attention as antioxidants, and their consumption has been associated with a reduction in risk of cardiovascular disease.\textsuperscript{34,35} Legumes in the form of soy and their associated isoflavones are also recognized as antioxidants\textsuperscript{9} and have been shown to reduce LDL cholesterol oxidation.\textsuperscript{10} Of direct relevance to our study, consumption of isoflavone-rich soy protein reduced conjugated dienes in circulating LDL cholesterol.\textsuperscript{30,32} The lack of effect with the chocolate-flavored cocoa-fiber and low-fiber cereals suggests that the antioxidant activity is not associated with the flavor but may be more related to the protein, as in soy, or possibly the lipid fractions of cocoa.

CONCLUSIONS

A chocolate-flavored low-fat, high-fiber source, cocoa bran, has a beneficial effect on laxation and a potentially interesting action in maintaining HDL cholesterol levels compared with low-fiber chocolate-flavored cereal flakes used as a control. The effect on blood pressure, although small, requires confirmation and explanation of mechanism. Overall, cocoa bran warrants further study in view of its potential health benefits.

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