Grain Sorghum Lipid Extract Reduces Cholesterol Absorption and Plasma Non-HDL Cholesterol Concentration in Hamsters

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ABSTRACT  Grain sorghum is a rich source of phytochemicals that could potentially benefit human health. In this study, male hamsters were fed AIN-93M diets supplemented with a hexane-extractable lipid fraction from grain sorghum whole kernels. The grain sorghum lipids (GSL) comprised 0.0, 0.5, 1.0, or 5.0% of the diet by weight. After 4 wk, dietary GSL significantly reduced plasma non-HDL cholesterol concentration in a dose-dependent manner with reductions of 18, 36, and 69% in hamsters fed 0.5, 1.0, and 5.0% GSL, respectively, compared with controls. Liver cholesterol ester concentration was significantly reduced by GSL in a dose-dependent manner (r = 0.97, P < 0.05), suggesting that dietary GSL lowers non–HDL cholesterol, at least in part, by inhibiting cholesterol absorption. TLC and GLC analyses of the GSL extract revealed the presence of plant sterols and policosanols at concentrations of 0.35 and 8.0 g/100 g GSL, respectively. Although plant sterols reduce cholesterol absorption, policosanols may inhibit endogenous cholesterol synthesis. The data suggest that these components of GSL extract may work collectively in lowering plasma and liver cholesterol concentrations. Our findings further indicate that grain sorghum contains beneficial components that could be used as food ingredients or dietary supplements to manage cholesterol levels in humans. J. Nutr. 135: 2236–2240, 2005.

KEY WORDS: grain sorghum • plant sterols • policosanols • cholesterol • hamsters
MATERIALS AND METHODS

Animal care. Male F1B Syrian hamsters (Bio Breeders) weighing ~100 g were randomly assigned to treatment groups (n = 7–8/treatment) and housed individually in polycarbonate cages with sawdust bedding. Hamsters were kept in a 25°C room with a 12-h light:dark cycle and had free access to food and water throughout the 4-wk feeding period. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska.

Diets. Hamsters were fed a modified AIN-93M diet (14) supplemented with 0.0, 0.5, 1.0, or 5.0% grain sorghum lipids (GSL) by weight of diet (Table 1). The GSL were prepared from whole kernels obtained from a mixture of commercial red grain sorghum hybrids grown in Nebraska. The kernels were washed with tap water and dried at 45°C for 24 h. The grain sorghum kernels (800 g at ~10% moisture) with 800 mL hexane were refluxed for 30 min in a round-bottomed flask slightly below the boiling point of the solvent. After refluxing, the mixture was filtered through a coffee filter paper lying on top of Whatman No. 2 filter paper. The filtrate was placed in the sample flask of a rotary evaporator. Hexane was evaporated and the sample was collected for storage in a plastic bottle at −18°C until used. The GSL yield was 0.2–0.3% of the whole kernel on a dry weight basis.

GSL analysis. The simple lipid profile of the GSL extract was determined by TLC (Fig. 1). The sample was dissolved in hexane by heating to 75°C, then spotted in 10-µL aliquots onto a 20 × 20 cm silica gel plate (Merck) with a layer thickness of 0.25 mm. Lipid standards (Sigma Chemical) were also spotted onto the TLC plate. The developing solvent was composed of hexane:diethyl ether:acetic acid (85:15:2, by vol). Band intensities of the 3 replicates (Fig. 1) revealed the following lipid composition: 9% hydrocarbons, 10% sterol esters and wax esters, 27% aldehydes, 26% triglycerides, 12% free fatty acids, 8% alcohols, <1% free sterols, 5% diglycerides, and <1% monoglycerides.

The plant sterol concentration in GSL was determined more precisely using GC as previously described (15). The GSL plant sterol concentration was 347 ± 9 mg/100 g GSL (mean ± SD, n = 9). The major sterols were sitosterol (41%), stigmasterol (33%), and campesterol (25%).

We also determined the fatty acid composition of GSL triglyceride collected from HPLC separation. Fatty acids were methylated and quantified as previously described (15). The triglyceride component of the GSL extract contained 17% palmitic, 2% stearic, 35% oleic, 41% linoleic, and 2% linolenic acid.

TABLE 1
Composition of control diet fed to hamsters1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
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</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>465.2</td>
</tr>
<tr>
<td>Dextrinized cornstarch2</td>
<td>155.0</td>
</tr>
<tr>
<td>Casein</td>
<td>140.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
</tr>
<tr>
<td>Palm oil</td>
<td>30.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>50.0</td>
</tr>
<tr>
<td>Fiber3</td>
<td>35.0</td>
</tr>
<tr>
<td>AIN-93 mineral mix</td>
<td>10.0</td>
</tr>
<tr>
<td>AIN-93 vitamin mix</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.6</td>
</tr>
</tbody>
</table>

1 Control diet contained 0% GSL. Treatment diets contained 0.5, 1.0 and 5.0% GSL and were added at the expense of cornstarch.

2 Dyetrose, Dyets.

3 Soluta-Floc® cellulose, International Fiber Corporation.

FIGURE 1. TLC analysis of hexane-extractable grain sorghum lipids fed to hamsters. Lipids were developed with a solvent system of hexane:diethyl ether:acetic acid (85:15:2, by vol). Band intensities of the 3 replicates had the following lipid composition: 9% hydrocarbons, 10% sterol esters and wax esters, 27% aldehydes, 26% triglycerides, 12% free fatty acids, 8% alcohols, <1% free sterols, 5% diglycerides, and <1% monoglycerides.

A normal phase HPLC method was used to measure the tocopherol composition of GSL in which 99.5% hexane and 0.5% isopropanol served as the mobile phase. The stationary phase was a 3.9 × 300 mm μPorasil column (Waters). Samples were dissolved in hexane, heated to 40°C, filtered with 0.2-µm syringe filters (Pall), and analyzed using an injection volume of 20 µL. Data acquisition and analysis were performed with a Chrom Perfect workstation using tocopherol standards (Sigma). The only isomer detected was β-tocopherol at a concentration of 4 mg/100 g GSL.

Cholesterol absorption. Cholesterol absorption efficiency was measured during wk 3 by simultaneous oral administration of [3H]-sitostanol and [14C]-cholesterol as previously described (15). Radioisotopes were purchased from American Radiolabeled Chemicals. Sitostanol was shown to be essentially unabsorbed in the intestinal tract of hamsters (16), thus serving as a reference compound for quantification of cholesterol absorption. Briefly, feces were finely ground and saponified, and total radioactivity was quantified by scintillation spectrometry. Cholesterol absorption efficiency was calculated as a percentage from the ratio of the 2 radiolabels in the dose and feces using the following equation: % cholesterol absorption = [(14C/3H in dose − 14C/3H in feces)/(14C/3H in dose)] × 100.

Fecal sterol analysis. Nonradioactive feces were collected during wk 4 for quantification of cholesterol-derived neutral sterols and bile acids. The following neutral steroids were quantified and represented fecal cholesterol and its metabolites: cholesterol, coprostanol, dihydrocholesterol, epicholesterol, coprostanone, coprostanol, and cholestanone. Ground feces (~100 mg) were acidified by adding 0.2 mL of 0.5 mol/L HCl. Lipids were then extracted into chloroform:methanol (2:1, v/v) (17) containing 10 mg/L 5α-cholestane as an internal standard. The lower phase solvent was evaporated and the samples saponified in 2 mL of 1 mol/L methanolic KOH for 1 h at 50°C. After the addition of 2 mL deionized water, the fecal steroids were extracted into 5 mL hexane. The hexane was evaporated under nitrogen and the steroids derivatized and analyzed by GC as described above for GSL sterols.
Fecal bile acid concentration was quantified enzymatically as reported previously (15). Samples were read at 340 nm before and after the addition of 3α-hydroxysteroid dehydrogenase to account for background absorbance. The concentration of total bile acids was calculated by the difference of the 2 absorbance readings, compared against a calibration curve using cholic acid standards.

**Plasma, liver, and gallbladder lipids.** On d 28, hamsters were killed by CO₂ asphyxiation. Blood was collected by cardiac puncture using 10-mL syringes containing 10 mg EDTA as an anticoagulant. RBC were removed by centrifuging the blood at 1000 x g for 30 min at 4°C; ~2–4 mL plasma was recovered from each hamster. Aprotinin (1 mg/mL) and phenylmethylsulfonyl fluoride (80 mg/L) were added to the plasma as preservatives. Plasma total cholesterol concentration was determined enzymatically using our microplate method (18). Plasma HDL cholesterol concentration was measured after apolipoprotein B precipitation, and non-HDL cholesterol (VLDL + LDL) was calculated by difference. Previous hamster studies in our laboratory showed that the non-HDL plasma fraction contains >90% LDL cholesterol (19).

Livers were perfused with saline through the portal vein to eliminate residual blood before being excised. Gallbladder bile was collected using a preservative 31-gauge syringe, weighed on an analytical balance, and volume estimated assuming a density of 1 kg/L. Aliquots of frozen liver were minced and lipids extracted into chloroform:methanol (2:1, v:v) (17). Total cholesterol, free cholesterol, triglyceride, and phospholipid were quantified enzymatically (18), and liver esterified cholesterol was calculated as the difference between total and free cholesterol.

**Statistics.** All data are expressed as means ± SEM. Treatment differences were determined by one-way ANOVA, followed by Fisher’s multiple comparison procedure to identify differences in treatment means. Differences were considered significant at P < 0.05. Data were analyzed using SigmaStat 3.0 (SPSS).

**RESULTS**

Hamster body weight measured at the end of the 4 wk study did not differ among treatment groups and was 115 ± 1 g. Cumulative body weight gain (0.49 ± 0.03 g/d) and food intake (6.9 ± 0.1 g) measured over the 4-wk study were also unaffected by dietary treatment, indicating that GSL consumed up to 5% of the diet was palatable and did not adversely affect animal growth.

Plasma HDL cholesterol was not affected by the consumption of GSL during the 4-wk study (Table 2), although there was a trend for HDL cholesterol to increase with GSL intake (P = 0.07). In contrast, the plasma non-HDL cholesterol fraction, containing mainly LDL cholesterol, decreased significantly as GSL was increased incrementally in the diet (Table 2). Note that the percentage of plasma cholesterol in hamsters fed purified diets is typically carried in the HDL fraction (21), whereas LDL cholesterol is the predominant fraction in humans. Therefore, we expressed the data as the percentage of change relative to controls (Fig. 2). Plasma HDL cholesterol in hamsters fed 5.0% GSL increased 19% (P < 0.05), whereas non-HDL cholesterol decreased 36% and 69% (P < 0.05) in hamsters fed 1.0 and 5.0% GSL, respectively.

The hepatic concentrations of phospholipid and free cholesterol were not altered by dietary GSL (Table 2). Liver weight was also not affected by dietary treatment (data not shown). In contrast, liver esterified cholesterol was significantly reduced by dietary GSL; a maximum reduction was apparently achieved with the diet containing 1.0% GSL. Liver triglyceride concentration was significantly increased in hamsters fed 5.0% GSL compared with the other treatment groups.

**TABLE 2**

<table>
<thead>
<tr>
<th>Dietary GSL, %</th>
<th>Plasma HDL cholesterol</th>
<th>Plasma non-HDL cholesterol</th>
<th>Liver Phospholipid</th>
<th>Liver Free cholesterol</th>
<th>Liver Esterified cholesterol</th>
<th>Liver Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>µmol/g wet weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>1.93 ± 0.13</td>
<td>0.87 ± 0.13</td>
<td>16.0 ± 0.4</td>
<td>3.81 ± 0.24</td>
<td>12.95 ± 1.12</td>
<td>3.12 ± 0.41</td>
</tr>
<tr>
<td>0.5</td>
<td>1.98 ± 0.12</td>
<td>0.71 ± 0.14</td>
<td>16.7 ± 1.2</td>
<td>3.57 ± 0.25</td>
<td>8.52 ± 1.08</td>
<td>2.88 ± 0.29</td>
</tr>
<tr>
<td>1.0</td>
<td>2.00 ± 0.08</td>
<td>0.56 ± 0.08</td>
<td>16.2 ± 1.0</td>
<td>3.41 ± 0.22</td>
<td>4.54 ± 0.72</td>
<td>3.16 ± 0.50</td>
</tr>
<tr>
<td>5.0</td>
<td>2.29 ± 0.09</td>
<td>0.27 ± 0.05</td>
<td>16.2 ± 0.6</td>
<td>3.43 ± 0.11</td>
<td>4.35 ± 0.58</td>
<td>5.37 ± 1.07</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7–8. * Means in a column with superscripts without a common letter differ, P < 0.05.
factors. There were no treatment differences for any of the major biliary lipids. The overall biliary cholesterol concentration for all hamsters was 1.1 ± 0.2 mmol/L, phospholipid concentration was 3.7 ± 0.3 mmol/L, and bile acid concentration was 37 ± 3 mmol/L. Consequently, the CSI was not affected by dietary GSL.

Cholesterol absorption efficiency was significantly reduced at each level of GSL intake (Table 3). A significant positive correlation was observed between cholesterol absorption and the plasma non-HDL cholesterol concentration ($r = 0.97$, $P = 0.034$) (Fig. 3). Cholesterol absorption was also positively correlated with liver esterified cholesterol concentration ($r = 0.97$, $P = 0.035$). As expected, fecal neutral sterol excretion increased significantly in hamsters fed 5.0% GSL (Table 3). Fecal bile acid excretion was only about one fourth the rate of neutral sterol excretion and was not affected by dietary GSL.

**DISCUSSION**

The present study demonstrates that a hexane-extractable lipid fraction from grain sorghum whole kernels significantly lowers plasma and liver cholesterol when fed to male Syrian hamsters. The dietary GSL fraction appears to exert its cholesterol lowering abilities in a dose-dependent manner. The data also indicate that a primary mechanism of action is reduced cholesterol absorption and a concomitant increase in fecal sterol excretion.

One of the components of GSL most likely to inhibit cholesterol absorption is plant sterols. Studies in humans and animals clearly documented the ability of dietary plant sterols to inhibit cholesterol absorption and reduce plasma LDL cholesterol concentration (6,22,23). In fact, the National Cholesterol Education Program now recommends a diet that contains 2 g/d of plant sterols as an effective cholesterol-lowering therapy (24). Grain sorghum is a relatively rich source of plant sterols compared with fruits, vegetables, and other cereal grains commonly found in the food supply (25). Singh et al. (26) recently reported that the sterol content of grain sorghum, measured in ground kernels, is ~50 mg/100 g. The hexane-extracted lipid fraction used in the present study was from intact whole kernels and contained 347 mg sterol/100 g total lipid. Because the mean food intake for hamsters during the 4 wk study was 6.9 g/d and their mean body weight was 115 g, then the estimated plant sterol intake was 1.2, and 10 mg/(kg·d) for hamsters fed 0.5, 1.0, and 5.0% GSL, respectively. Typical human consumption of plant sterols is 2 g/d as an effective cholesterol-lowering therapy (27). Although human intake is comparable to the level of intake observed in the present study, the magnitude of cholesterol-lowering in the hamsters was greater than what would be expected at this modest level of plant sterol intake. We previously reported that plant sterol intake of 150 mg/(kg·d) was required to achieve similar reductions in liver and plasma cholesterol concentration in hamsters (28), suggesting that other components in the GSL fraction also contributed to its cholesterol-lowering abilities.

Policosanols, at doses of 10–20 mg/d, were shown in many clinical studies to lower LDL cholesterol while raising HDL cholesterol [reviewed in (8,29)]. These studies, conducted mainly by one research group in Cuba, included several distinct populations such as postmenopausal women and patients with type II hypercholesterolemia and type 2 diabetes. Policosanols appear to elicit their cholesterol-lowering effect by reducing the activity of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaric acid Coenzyme A (HMG-CoA) reductase, and by increasing LDL receptor activity (30–33). Cho et al. (34) reported that sorghum hexane extracts inhibited HMG-CoA reductase in a dose-dependent manner. But unlike statin drugs, policosanols apparently do not inhibit HMG-CoA reductase by competitive inhibition. Although the precise mechanisms are not known, policosanols may regulate the synthesis and degradation of the enzyme (31,33). We estimated that the policosanol content of the GSL fed to hamsters was 8% of total extractable lipid (Fig. 1). Therefore, the policosanol intake in hamsters consuming 0.5, 1.0, and 5.0% GSL (assuming a food intake of 6.9 g/d and 115 g body weight) was 24, 48, and 240 mg/(kg·d), respectively. Contrary to our findings, Wang et al. (35,36) found that policosanols fed to hamsters at a level of 25 mg/(kg·d) significantly increased HDL cholesterol, but non-HDL cholesterol concentration was not affected. A major difference between our study and those of Wang et al. (35,36) was the amount of dietary cholesterol. Hamsters in the present study were fed 0.05% cholesterol by weight of the diet, whereas Wang et al. (35,36) fed 0.25% cholesterol. We demonstrated previously that cholesterol synthesis can be completely shut down in hamsters consuming cholesterol in excess of 0.05% of the diet (37). At 0.25% dietary cholesterol, hamsters also exhibit significant reductions in LDL receptor activity (38). Feeding 0.25% dietary cholesterol to hamsters is likely to obscure any regulatory effect of

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**TABLE 3**

**Cholesterol absorption and fecal sterol excretion in hamsters fed GSL for 4 wk**

<table>
<thead>
<tr>
<th>Dietary GSL</th>
<th>Cholesterol absorption</th>
<th>Fecal neutral sterol excretion</th>
<th>Fecal bile acid excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>[ μmol/(d · 100 g body wt)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>67.2 ± 1.8c</td>
<td>2.68 ± 0.21a</td>
<td>0.61 ± 0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>62.5 ± 1.1b</td>
<td>2.87 ± 0.57a</td>
<td>0.87 ± 0.13</td>
</tr>
<tr>
<td>1.0</td>
<td>59.7 ± 2.1ab</td>
<td>2.93 ± 0.22a</td>
<td>0.86 ± 0.13</td>
</tr>
<tr>
<td>5.0</td>
<td>56.6 ± 0.7a</td>
<td>3.95 ± 0.24a</td>
<td>0.81 ± 0.18</td>
</tr>
</tbody>
</table>

1. Values are means ± SEM, $n = 7$–8. a,b Means in a column with superscripts without a common letter differ, $P < 0.05$. 

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**FIGURE 3** Association between cholesterol absorption and plasma non-HDL cholesterol concentration in hamsters fed 0.0, 0.5, 1.0 or 5.0% grain sorghum lipids. The correlation coefficient is $r = 0.97$ ($P < 0.05$).
policosanols. Moreover, relatively small amounts of policosanols given to human subjects at 10–20 mg/d, ~0.14–0.28 mg/(kg·d), produced significant reductions in LDL cholesterol concentration (8,29). It is possible that the significant reduction in non-HDL cholesterol concentration observed in the present study was due to the relatively high dietary intake of policosanols or to the combined effects of plant sterols and policosanols in GSL. Because plant sterols inhibit cholesterol absorption and promote cholesterol excretion, there is a concomitant increase in cholesterol synthesis (28,39). Thus, the components of GSL may play an additive role in cholesterol-lowering by reducing both cholesterol absorption and synthesis.

Dietary soluble fiber also reduces plasma LDL cholesterol (40). However, the GSL preparation used in the current study is unlikely to contain fiber because negligible fiber was extracted with hexane from maize, rice bran, and soybeans (41–43). The cholesterol-lowering effect of GSL observed in the present study was not likely due to soluble fiber. Similarly, grain sorghum contains a number of phenolic compounds that are beneficial to cardiovascular health (12,44). In addition to their ability to reduce inflammation, reduce LDL oxidation, improve endothelial function, and inhibit platelet aggregation (10), phenolic compounds have demonstrated a modest cholesterol-lowering ability in some studies (45,46). The majority of studies, however, did not show a significant cholesterol-lowering effect of phenolic compounds (47). Additional studies are underway in our laboratory to isolate the effects of lipid-soluble grain sorghum phytochemicals on lipoprotein metabolism and biomarkers of atherogenesis.

LITERATURE CITED