Biochemical and Molecular Roles of Nutrients

Fecal Losses of Sterols and Bile Acids Induced by Feeding Rats Guar Gum Are Due to Greater Pool Size and Liver Bile Acid Secretion

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ABSTRACT The effect of dietary guar gum (GG, 7.5%) on lipid metabolism and on bile acid secretion and reabsorption was investigated in rats adapted to cholesterol-free or 0.3% cholesterol diets. Compared with controls (fiber-free/cholesterol-free), rats fed cholesterol had significantly elevated plasma and liver cholesterol and triglyceride. In these rats, GG had a potent plasma cholesterol-lowering effect and also counteracted the liver accumulation of triglyceride and cholesterol esters. Fecal excretion of steroids, the major route of cholesterol elimination, was markedly enhanced by GG, especially in rats fed the cholesterol-containing diet (P < 0.001). The biliary bile acid flux into the small intestine was enhanced by dietary cholesterol (+30%) or GG (+52%) or both (P < 0.001). The fecal excretion of bile acids was significantly elevated by GG alone (+74%) and by dietary cholesterol (+190%). Small intestine reabsorption of bile acids appears to be significantly enhanced by GG, which also enhanced the transfer of bile acids into the large intestine, hence a greater fecal loss of steroids, although bile acid reabsorption was very effective in the cecum. GG feeding induced liver hydroxymethyl-glutaryl coenzyme A (HMG CoA) reductase, even in cholesterol-fed rats, as well as cholesterol 7a-hydroxylase (P < 0.001). The cholesterol-lowering effect of GG thus appears to be mediated by an accelerated fecal excretion of steroids and a rise in the intestinal pool and biliary production of bile acids. Although liver HMG CoA reductase and cholesterol 7a-hydroxylase are induced in parallel, this is not sufficient to compensate for fecal steroid losses. J. Nutr. 127: 1068–1076, 1997.

KEY WORDS: • rats • cholesterol • bile acids • guar gum • biliary secretion

Dietary fiber and related compounds such as oligosaccharides and resistant starches have received considerable attention for their plasma cholesterol-lowering effect. One of these compounds, guar gum (GG),1 a gel-forming galactomannan obtained from Cyamopsis tetragonoloba, received particular attention because of its consistent cholesterol-lowering and glucostatic effects (Gatenby 1990, Todd et al. 1990). The mechanisms underlying these effects of GG are not fully understood, but one common proposal is that guar gum interferes with the intestinal absorption of steroids, because of its viscosity or its binding properties. GG alters emulsification of dietary fat and lipolysis under conditions prevailing in the upper part of the digestive tract (Pasquier et al. 1996) and delays gastric emptying in dogs (Bueno et al. 1981). In the small intestine, GG also delays lipid dispersion and the rate of absorption of lipolysis end-products, but in rats it is still uncertain whether GG actually affects lipase activity (Ikegami et al. 1990, Poksay and Schneeman 1983). In fact, even if GG does not affect the overall digestibility of glycerides (which remains very high), its presence prolongs the duration of lipid digestion and displaces lipid absorption to a more distal portion of the small intestine (Mazur et al. 1990, Redard et al. 1992). This could alter the structure of triglyceride-rich lipoproteins (TGRLP) released by the digestive tract and their further metabolism, hence their potential atherogenicity (Sethi et al. 1993). The enterohepatic cycling of steroids, especially bile acids, is considered to be a process particularly prone to interfering effects of sequestrants and polysaccharides such as GG (Stedronsky 1994). Besides inhibition of cholesterol absorption in the upper small intestine, GG could also impair the ileal absorption of bile acids, thus promoting their transfer into the large intestine and their fecal excretion. This spillover of the bile acid pool is liable in turn to elicit an up-regulation of their hepatic synthesis at the expense of the body cholesterol pool. In the rat, the accelerated oxidation of cholesterol into bile acids may be coupled to an induction of the apolipoprotein B/E (apo B/E) receptor (Mazur et al. 1990), as well as of the microsomal hydroxymethylglutaryl (HMG) CoA reductase activity in the liver (Favier et al. 1995, Moundras et al. 1994). This last response seems to be a good reflection of fiber’s capacity to depress plasma cholesterol in rats fed cholesterol-free diets, but it remains to be established whether this mechanism is still operative when a cholesterol-containing diet is fed and HMG CoA reductase activity is repressed.

The aim of the present study, therefore, was to further
document the lipid-lowering effect of GG in rats, in the absence or the presence of a moderate level of dietary cholesterol. The present work was more specifically focused on the effects of GG on the enterohepatic cycling of bile acids and on the role of the large intestine in this process.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (IFFA-CREDO, L’Arbresle, France) were fed a commercial pelleted diet (AO3 pellets, U.A.R., Villemoisson, Orge, France) until their body weight reached ~150 g. Rats were then fed the semipurified diets (distributed as a moistened powder for 21 d) in which 7.5% GG was included in place of wheat starch or which were supplemented with 0.3% cholesterol (Table 1). The rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in temperature-controlled rooms (22°C) with the dark period from 2000 to 0800 h. For each cage, the feces were collected over three consecutive days. The animals were maintained and handled according to the recommendations of the Institutional Ethics Committee.

Sampling procedures. Rats were killed at the end of the dark period, a time at which cecal fermentations are still very active. They were anesthesized with sodium pentobarbital (40 mg/kg) and maintained on a hot plate at 37°C. Blood was drawn into heparinized syringes from the cecal vein (~0.8 mL), then from the abdominal aorta (~4 mL). For blood flow measurement, bromosulfo-phtalein in normal saline (5 mmol/L) was infused at a rate of 50 μL/min into the small afferent vein on the internal curvature of the cecum; dilution of the marker in the vein draining the whole cecum allows determination of the cecal blood flow. Blood from each rat was placed in a plastic tube containing heparin and centrifuged at 10,000 × g for 5 min. After centrifugation, plasma was removed and kept at 4°C until measurement of enzyme activities. Protein content of the preparation was determined using the Pierce BCA Reagent kit (Interchim, Montluçon, France).

Analytical procedures. Plasma lipoproteins were separated by ultracentrifugation on a density gradient, as described by Sérougne et al. (1987). Because of the relatively low level of LDL and the partial overlapping of HDL1 and HDL2 fractions in the rat, only two fractions were analyzed: the d < 1.040 kg/L fraction (chiefly TGRLP) and the d > 1.040 kg/L fraction (HDL). The collected fractions were kept at 4°C for lipid analysis. For each diet, analyses were carried out in triplicate on a pool of plasma from eight rats.

Short-chain fatty acids (SCFA) were measured by gas-liquid chromatography, after ethanolic extraction of plasma samples (Rémesy and Demigné 1974), and on supernatants of cecal contents (40,000 × g for 15 min at 4°C). Bile acids were quantified by an enzymatic procedure, using the reaction catalyzed by 3 α-hydroxysteroid dehydrogenase (EC 1.1.1.50, Sigma Chemical, St. Louis, MO). The enzymatic determination was effected either on undiluted plasma or diluted bile (1/100 in normal saline) samples, or after extraction from digestive content samples (small intestine, cecum) or feces by 10 volumes of ethanolic KOH 0.5 mol/L (90 min at 60°C). Neutral steroids were extracted three times with 1 mL hexane from a 100-μL aliquot of the alkaline ethanolic extract after addition of 5α-cholestane as an internal standard. The extracts were centrifuged for 5 min at 3000 × g; the solvent was evaporated under a stream of N2, and the residue dissolved in hexane. Portions (0.5 μL) of this extract were injected into a gas chromatograph (Delsi 330, Paris, France) which was equipped with a 12 m × 0.25 mm (i.d.) fused silica capillary column (BP10, SGE, Villeneuve-St-Georges, France) and a flame-ionization detector. Helium was used as a carrier gas at a pressure of 40 kPa, and the steroids were separated isothermally at 260°C. Sterols were calculated from the peak areas relative to the peak area of the internal standard. Differences in detector response among the various compounds were corrected on the basis of the response factors calculated from a mixture of pure steroids with known molar composition.

### Table 1

Composition of the diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>0.3% Cholesterol</th>
<th>7.5% Guar gum</th>
<th>0.3% Cholesterol/7.5% Guar gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>690</td>
<td>687</td>
<td>615</td>
<td>612</td>
</tr>
<tr>
<td>Guar gum1</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Cholesterol2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin mixture3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture4</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

1. Guar gum was kindly supplied by TICGums (Belcamp, MD).
2. Sigma Chemical (St. Louis, MO).
3. Minerals supplied (kg of diet): CaHPO4, 15 g; K2HPO4, 2.5 g; KCl, 5 g; NaCl, 5 g; MgCl2, 2.5 g; Fe2O3, 2.5 mg; MnSO4, 125 mg; CuSO4·5H2O, 0.2 mg; ZnSO4·7H2O, 100 mg; KI, 0.4 mg. Purchased from U.A.R. (Villemoisson, Epinary-sur-Orge, France).
4. Vitamins supplied, per kg of diet (except as noted): thiamin, 20 mg; riboflavin, 15 mg; pyridoxin, 10 mg; nicotinamide, 100 mg; calcium pantothenate, 70 mg; folic acid, 5 mg; biotin, 0.3 mg; cyanoocobalamin, 0.05 mg; retinyl palmitate, 1.5 mg; α-tocopherol acetate, 125 mg; cholecalciferol, 0.15 mg; menadione, 1.5 mg; ascorbic acid, 50 mg; myo-inositol, 100 mg; choline 1.36 g. Purchased from U.A.R.

### Table 1 Continued

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>0.3% Cholesterol</th>
<th>7.5% Guar gum</th>
<th>0.3% Cholesterol/7.5% Guar gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>180</td>
<td>180</td>
<td>180</td>
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</tr>
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<td>Groundnut oil</td>
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<tr>
<td>Wheat starch</td>
<td>690</td>
<td>687</td>
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<td>Guar gum1</td>
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<tr>
<td>Cholesterol2</td>
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<td>Vitamin mixture3</td>
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<td>10</td>
</tr>
<tr>
<td>Mineral mixture4</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

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Triglycerides (Biotrol) and total cholesterol (BioMérieux, Charbonnières-les-Bains, France) were measured in plasma and lipoprotein fractions by enzymatic procedures. Triglycerides were determined with lipoprotein lipase/glycerokinase/glycerophosphate oxidase and total cholesterol with cholesterol esterase/cholesterol oxidase; in both cases, 

$H_2O_2$ formed was reacted with chloro-4 phenol/amin-4 antipyrine in the presence of peroxidase to form a pink chromogen (500 nm). Liver lipids were extracted and analyzed as described by Mazur et al. (1990). A polyvalent control serum (Biotrol-33 plus, Biotrol) was treated in parallel to samples and served as control of accuracy of results in the analysis of triglycerides and cholesterol. When cholesterol was present in the diet without GG, there was a dramatic rise in the cecal SCFA concentration, there was a marked elevation of the cecal SCFA pool from about 200 μmol in controls to 600 μmol in those fed the GG diets. Furthermore, there were specific changes in the SCFA molar ratio due to dietary GG. The propionic acid pool was 4–5 times higher in GG-fed rats, whereas the acetate and the butyric acid pools were only 1.7–2.5 times higher (data not shown). The cecal plasma flow was 0.8–0.95 mL/min in control rats, and it was significantly greater in those fed GG (1.25–1.30 mL/min).

### RESULTS

**Effects of dietary guar gum and cholesterol on body weight and cecal fermentation**

Table 2 shows the effects of dietary guar gum and cholesterol on body weight and cecal fermentations.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight (g)</th>
<th>Cecal weight (g)</th>
<th>Cecal pH</th>
<th>Cecal plasma flow (mL/min)</th>
<th>Cecal SCFA pool (μmol)</th>
<th>Acetate/propionate/butyrate molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>304 ± 12</td>
<td>1.66 ± 0.11</td>
<td>7.41</td>
<td>0.80 ± 0.12</td>
<td>199 ± 8</td>
<td>64.3/21.6/14.1</td>
</tr>
<tr>
<td>Guar gum</td>
<td>288 ± 9</td>
<td>3.76 ± 0.25</td>
<td>5.92</td>
<td>1.25 ± 0.15</td>
<td>609 ± 35</td>
<td>56.2/35.8/8.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>306 ± 7</td>
<td>1.94 ± 0.14</td>
<td>7.42</td>
<td>0.95 ± 0.10</td>
<td>230 ± 20</td>
<td>66.5/23.5/10.0</td>
</tr>
<tr>
<td>Cholesterol/Guar gum</td>
<td>283 ± 9</td>
<td>3.88 ± 0.35</td>
<td>6.02</td>
<td>1.30 ± 0.20</td>
<td>644 ± 25</td>
<td>57.8/36.2/6.1</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10.
2 Means in a column not sharing the same superscript are significantly different (P < 0.05).
3 Data for the treatment groups that did not meet the assumption of equal variance were log-transformed before statistical analysis.
4 Abbreviations used: SCFA, short-chain fatty acids; GG, guar gum; chol, cholesterol; NS, not significant (P > 0.05).

In rats fed the cholesterol-free diet, there was a slight but significant plasma cholesterol-lowering effect of GG (Table 3). Cholesterol supplementation of the control diet led to significantly greater plasma cholesterol. In these cholesterol-fed rats, GG feeding elicited a potent cholesterol-lowering effect, because plasma cholesterol did not differ from that in cholesterol-free controls. GG was also effective in lowering plasma triglycerides in both cholesterol-free and cholesterol-fed groups. The cholesterol diet had a triglyceride-raising effect, but GG exerted a more potent effect on triglyceridemia in the cholesterol-fed rats (−0.66 mmol/L) than in those fed cholesterol-free diets (−0.26 mmol/L).

The liver weight was significantly increased by cholesterol supplementation of the diet (+29%), but the liver weight in rats adapted to the GG/cholesterol diet was not significantly different than the value found in controls. In the absence of dietary cholesterol, GG feeding did not affect liver cholesterol or triglyceride concentrations. In rats fed a cholesterol-containing diet, however, there were large increases in the cholesterol (chiefly esterified) and triglyceride concentrations of the liver. GG supplementation drastically reduced this lipid accumulation, to concentrations that were still significantly higher than in control rats (cholesterol) or not significantly different (triglycerides).

**Figure 1** illustrates the effects of the diets on plasma lipoprotein-cholesterol and triglycerides. Because the rat has very low concentrations of LDL, lipoproteins with a density < 1.040 kg/L (chiefly TGRLP, together with small amounts of LDL) are contrasted with those with a density > 1.040 kg/L (HDL fractions). In rats fed cholesterol-free diets, GG lowered cholesterol only in the HDL fraction. In rats fed the cholesterol diet without GG, there was a dramatic rise in the d < 1.040 kg/L lipoprotein cholesterol (especially in TGRLP), but HDL cholesterol was unchanged. When cholesterol was present in the diet, GG had no significant effect on HDL cholesterol.
TABLE 3
Effects of dietary guar gum in rats fed cholesterol-free or 0.3% cholesterol diets on plasma and liver lipid concentrations¹,²

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma lipids (mmol/L)</th>
<th>Liver lipids (g)</th>
<th>Liver lipids (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>Liver weight</td>
</tr>
<tr>
<td>Control</td>
<td>1.62 ± 0.08b</td>
<td>0.99 ± 0.10b</td>
<td>11.73 ± 0.65A,b</td>
</tr>
<tr>
<td>Guar gum</td>
<td>1.40 ± 0.08a</td>
<td>0.73 ± 0.08A</td>
<td>10.44 ± 0.59A</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.44 ± 0.14c</td>
<td>1.59 ± 0.12c</td>
<td>15.10 ± 0.35c</td>
</tr>
<tr>
<td>Cholesterol/Guar gum</td>
<td>1.65 ± 0.09b</td>
<td>0.93 ± 0.08b</td>
<td>12.40 ± 0.54b</td>
</tr>
</tbody>
</table>

ANOVA (P value)⁴
GG × Chol <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
GG <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
Chol <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
GG × Chol NS²

¹ Values are means ± SEM, n = 10.
² Means in a column not sharing the same superscript are significantly different (P ≤ 0.05).
³ Data for the treatment groups that did not meet the assumption of equal variance were log-transformed before statistical analysis.
⁴ Abbreviations used: GG, guar gum; chol, cholesterol; NS, not significant (P ≥ 0.05).

whereas cholesterol in the lower density fraction was strongly decreased (by more than 50%). A triglyceride-lowering effect of GG was observed in the d < 1.040 kg/L fraction in rats fed the cholesterol-free diets. Cholesterol feeding led to a dramatic rise of triglyceride in this fraction, but GG had a potent effect in this case because triglycerides were reduced by more than 50%.

**Effects of dietary guar gum and cholesterol on the liver microsomal enzymes governing cholesterol metabolism.** HMG CoA reductase activity was strongly induced by GG in rats fed cholesterol-free diets (Fig. 2); the enzyme was significantly repressed by dietary cholesterol but, in this case, dietary GG significantly induced the enzyme activity (over twofold). In rats fed cholesterol-free diets, the activity of cholesterol 7α-hydroxylase was low, whereas it was markedly induced by GG feeding. In rats fed a cholesterol-containing diet, there was a significant induction of cholesterol 7α-hydroxylase; this induction was still greater when the diet also contained GG, but the activity was not higher than in rats fed a GG/cholesterol-free diet.

**Effects of dietary guar gum and cholesterol on bile acid production, intestinal reabsorption and fecal excretion.** As shown in Table 4, the bile acid flux from the liver to the intestine (µmol/h), calculated from bile flow and concentration, was strongly affected by diet conditions. It was increased both by GG (+50%) and by cholesterol feeding (+30%), and the effect of dietary cholesterol and GG were apparently additive: the highest flux was measured in rats fed the GG/
cholesterol diet (+94%). The daily fecal excretion of bile acids in rats fed the control diet was 11.2 μmol/d, corresponding to 1.7% of the biliary production (extrapolated over 24 h). This excretion was markedly enhanced by GG (+74%). In rats fed the cholesterol diet, the fecal excretion was very high as it was when GG was also present in the diet. In such conditions, the fecal excretion represented 3% of total biliary flux.

The present data indicate that the major part of the bile acid pool is present in the small intestine, but a substantial portion (23–34%) was also found in the cecum. GG led to a marked enlargement of the bile acid pool in the small intestine (+25%) and in the cecum (+98%). Cholesterol feeding was very effective in raising the small intestine (+92%) and the cecal (+144%) pools, but the maximal value was attained in rats fed the GG/cholesterol diet, which exhibited a very high bile acid pool in the cecum (a total of 33 μmol), representing a nearly fourfold increase over the control. The cecal vein-artery difference of bile acids (+0.13 ± 0.02 mmol/L in control rats) was not significantly modified by GG or cholesterol feeding alone (+0.14 ± 0.02 or +0.17 ± 0.01 mmol/L, respectively), but it was significantly increased in rats fed the GG/cholesterol diet (+0.21 ± 0.02 mmol/L). These data, combined with plasma flow measurements, provide a measure of the cecal reabsorption of bile acids; this process represents a substantial part (23% in control rats) of biliary bile acid influx. The cecal absorption was significantly enhanced by dietary GG or cholesterol (to ~10 μmol/h). Absorption of 16.4 μmol/h was observed when both GG and cholesterol were present in the diet; in such conditions, the cecal reabsorption represented 31% of biliary influx. The estimation of the small intestine reabsorption (by difference between biliary influx and cecal absorption plus fecal excretion) shows that the principal reabsorption site of bile acids is the small intestine. This reabsorption was greater in rats fed GG, in parallel to changes in the small intestine pool. In contrast, the small intestine absorption of bile acids was not significantly increased over control levels in cholesterol-fed rats, even though the small intestine pool was doubled in the latter group. Rats fed cholesterol and GG had the highest rate of small intestinal bile acid reabsorption (35.0 μmol/h), but this represented a lower percentage of total bile acid flux (66%) than that of rats fed cholesterol-free diets (>70%).

As shown in Table 5, the biliary flux of cholesterol was not significantly enhanced by cholesterol feeding; furthermore, this flux represented a negligible supply compared with the daily intake of cholesterol by cholesterol-fed rats (186 μmol/d). GG increased bile cholesterol flux, to ~1 μmol/h. Compared with controls, the cecal pool of neutral sterols was 71% 

![FIGURE 2](image)

**FIGURE 2** Effects of dietary guar gum in rats fed cholesterol-free or 0.3% cholesterol diets on hepatic activity of hydroxymethylglutarylcoenzyme A reductase and cholesterol 7α-hydroxylase. Each value is a mean ± SEM, n = 8. Data were log-transformed before statistical analysis. P values from ANOVA in guar gum (GG), cholesterol (Chol) and GG × Chol were < 0.001 in all cases for HMG CoA reductase and cholesterol 7α-hydroxylase activities. Values not sharing a common letter are significantly different (P < 0.05).

<table>
<thead>
<tr>
<th>Bile</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.91 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Guar gum</td>
<td>1.15 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.91 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GG × Chol</td>
<td>1.16 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10 except for fecal excretion values obtained on groups of two rats over three consecutive days.
2 Means in a column not sharing the same superscript are significantly different (P < 0.05).
3 Bile acid flux = biliary bile acid concentration × bile flow.
4 Data for the treatment groups that did not meet the assumption of equal variance were log-transformed before statistical analysis.
5 Small intestinal absorption was calculated as bile acid flux – (ecal absorption + fecal excretion), with all variables expressed as μmol/24 h.
6 Values (in %) between parentheses represent absorption, as a the percentage of bile acid flux, for the small intestine and the cecum.
7 Abbreviations used: GG, guar gum; chol, cholesterol; NS, not significant (P ≠ 0.05).
TABLE 5
Effects of dietary guar gum in rats fed cholesterol-free or 0.3% cholesterol diets on the biliary cholesterol flux and fecal excretion of neutral sterols

<table>
<thead>
<tr>
<th>Diet</th>
<th>Bile cholesterol flux</th>
<th>Cecal sterol pool</th>
<th>Daily fecal sterol excretion</th>
<th>Fecal sterols Coprostanol/Cholesterol/Cholestanol molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/h</td>
<td>µmol</td>
<td>µmol/24h</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.59 ± 0.07a</td>
<td>8.5 ± 1.0a</td>
<td>20.2 ± 1.7a</td>
<td>73/20/7</td>
</tr>
<tr>
<td>Guar gum</td>
<td>1.04 ± 0.10b</td>
<td>14.6 ± 1.6b</td>
<td>40.5 ± 3.0b</td>
<td>79/14/7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.77 ± 0.09a</td>
<td>26.2 ± 2.1c</td>
<td>112.1 ± 9.5c</td>
<td>60/32/8</td>
</tr>
<tr>
<td>Cholesterol/Guar gum</td>
<td>1.05 ± 0.12b</td>
<td>63.8 ± 5.3d</td>
<td>162.4 ± 14.6d</td>
<td>49/40/11</td>
</tr>
</tbody>
</table>

ANOVA (P value)5
GG <0.001 <0.001 <0.001
Chol NS <0.001 <0.001
GG × Chol NS 0.01 <0.001

1 Values are means ± SEM, n = 10 except for fecal excretion values obtained on groups of two rats over three consecutive days.
2 Means in a column not sharing the same superscripts are significantly different (P < 0.05).
3 Data for the treatment groups that did not meet the assumption of equal variance were log-transformed before statistical analysis.
4 Data on fecal excretion were determined for two rats in a cage, over three consecutive days.
5 Abbreviations used: GG, guar gum; chol, cholesterol; NS, not significant (P > 0.05).

The addition of GG to the cholesterol-free diet approximately doubled the net output of steroids. Rats fed the fiber-free cholesterol diet were the only group with a positive steroid balance: 22% of the daily cholesterol intake was apparently retained. In cholesterol-fed rats, there was a slightly negative balance when GG was present in the diet, suggesting that a substantial endogenous production of cholesterol was still occurring even when the diet contained 0.3% cholesterol.

DISCUSSION

Guar gum effectively decreases serum cholesterol concentrations in humans (Gatenby 1990, Todd et al. 1990) as well as in rodents (Anderson et al. 1994, Fernandez et al. 1995, Ide et al. 1991, Moundras et al. 1994). In the present study, GG was moderately hypocholesterolemic (−14%) in rats fed a greater in rats fed GG, 208% greater in those fed cholesterol and 65% greater (64µmol) in rats fed the cholesterol/GG diet. The daily fecal excretion of sterols in control rats was about 20 µmol/24 h, consisting primarily in coprostanol (73%) and cholesterol (20%); this excretion was doubled by GG. Neutral sterol excretion reached very high values in rats fed a cholesterol diet, and GG further increased this excretion by 50 µmol/24 h (eightfold over the control level). With the addition of cholesterol to the diets, the proportion of cholesterol in fecal sterols increased, whereas coprostanol decreased.

Figure 3 shows the respective contribution of bile acids and sterols to the overall elimination of steroids. Sterols made up a major portion of the disposal of cholesterol from the body pool even when cholesterol-free diets were fed. Figure 4 indicates the apparent digestive balance of steroids, namely, the difference between intake (186 µmol/24 h in rats fed the cholesterol diets) and excretion. In rats fed cholesterol-free diets, there was a net output of sterols from the body pool.

FIGURE 3 Respective contribution of bile acids and sterols in the overall elimination of steroids in rats fed cholesterol-free or 0.3% cholesterol diets, either fiber-free or containing 7.5% guar gum.

FIGURE 4 Apparent digestive balance of steroids in rats fed cholesterol-free or 0.3% cholesterol diets, either fiber-free or containing 7.5% guar gum. The average dietary cholesterol intake was 186 µmol/24 h in cholesterol-fed rats.
cholesterol-free diet, whereas it exerted a stronger cholesterol-lowering effect (±32%) in rats fed a cholesterol-containing diet, as previously observed (Fernandez 1995, Ney et al. 1988). GG has also been identified as one of the most potent lipid-lowering polysaccharides in rats fed diets containing a higher percentage of cholesterol (1%) and supplemented with cholic acid (Anderson et al. 1994).

The addition of GG to the diet elicits a marked rise in the viscosity of the digestive contents, which may alter emulsification and hydrolysis of lipids in duodenal medium in vitro (Pasquier et al. 1996). Nevertheless, with a moderate lipid content of the diet, the digestibility of glycerides is likely to remain very high. However, GG may delay lipid digestion, such that absorption occurs in a more distal part of the small intestine (Meyer and Dory 1988), which may affect the size and apolipoprotein composition of TGRLP in rats (Mazur et al. 1990, Redard et al. 1992).

The primary hypothesis concerning the mechanism of the cholesterol-lowering effect of fibers is increased excretion of cholesterol and bile acids. Consistent with this view, GG was found to be effective in enhancing steroid excretion in the present study. Neutral sterols accounted for a major part of steroid excretion, especially in rats fed a cholesterol-containing diet, and it is noteworthy that the excretion of neutral sterols was quite responsive to dietary GG. In rats fed cholesterol-free diets, the biliary secretion of cholesterol extrapolated over 24 h represented a substantial percentage (about 70%) of the daily excretion of sterols. With the addition of GG to the diets, the biliary output of sterols was enhanced, corresponding still to about 60% of sterol excretion. Mucosal cell sloughing also represents a source of endogenous cholesterol in the intestine, and this supply is likely to be increased by dietary GG, which elicits a hypertrophy and an accelerated turnover of the rat intestinal mucosa (Ikegami et al. 1990, Pell et al. 1992). Nevertheless, data obtained in rats fed cholesterol support the view that GG is effective in depressing the absorption of exogenous cholesterol, as previously shown in guinea pigs and rats (Fernandez 1995, Gee et al. 1983), probably by slowing absorption of cholesterol from micelles by mechanisms involving increased resistance to diffusion in the aqueous luminal medium (Gee et al. 1983, Vahouny et al. 1980). This could also be due to the binding of bile acid to fibers or to inhibition of formation of bile acid micelles in the small intestine (Phillips 1986, Vahouny et al. 1980). It must be noted that the fecal loss of bile acids was relatively low because, extrapolating the biliary bile acid fluxes over 24 h, it represented no more than 2% of the biliary bile acid flux in rats fed cholesterol-free diets. In rats fed cholesterol-containing diets, this loss was higher (3.8% in controls and 3.1% in the GG diet group). A stimulatory effect of GG on the secretion of bile acids by the liver has been observed, in keeping with previous data obtained on rat models (Ide et al. 1991, Ikegami et al. 1990). This was the result of a greater bile flow combined with a higher concentration of bile acids in bile. Supplementation of the diet with cholesterol also raised the bile acid flux, with the maximal flux observed when both GG and cholesterol were present in the diet. The digestive tract comprises the largest bile acid pool in rats, and Ide and Horii (1987) have reported that the small intestine and the cecum both contain more than 95% of the pool, located chiefly in the ileum. In the present experiment, GG led to a striking enlargement of the small intestinal bile acid pool, as shown previously (Ebihara and Schneeman 1989), and of the cecal bile acid pool. In parallel, bile acid reabsorption from the small intestine (essentially in the ileum) and the flux of bile acids to the large intestine were enhanced by GG. The small intestinal pool of bile acids was diluted in a larger volume in rats fed GG than in controls, because of the presence of GG itself and the likely presence of greater amounts of endogenous materials (Gee et al. 1996, Johnson et al. 1988). These features, potentially unfavorable to bile acid absorption, could be outweighed by an up-regulation of ileal transport, which occurs in rodents when there is a spill-over of the bile acid pool (Lilienau et al. 1993). It has been hypothesized that a dietary load of cholesterol causes an inhibition of bile acid absorption in the ileum (Bjorkhem et al. 1991), which could explain the concomitant enlargement of the cecal pool in the present study. Accordingly, in rats fed the 0.3% cholesterol diet, the estimated absorption of bile acids in the small intestine was only 18% higher than in rats fed a cholesterol-free diet, even though the corresponding pool was twice as large.

Bile acid transfer into the large intestine plays a major role in the control of bile acid balance. The size of the cecal pool is a reflection of this transfer: it made up 22% of the total intestinal pool in control rats fed the cholesterol-free diet and 32–34% in those fed GG. The effectiveness of bile acid reabsorption from the large intestine is dependent on the solubility of bile acids in the cecum, which is reduced by fibers such as GG (Moundras et al. 1994). GG is readily broken down by the microflora in the cecum and is thus unlikely to play a direct role in bile acid insolubilization; rather, it acts by promoting acidification of the cecal content by the microflora, which tends to insolubilize bile acids. In addition, bacteria may be effective binding sites for bile acids (Gelissen and Eastwood 1995), and they may also synthesize insoluble forms of bile acids (Benson et al. 1993). Nevertheless, bile acid reabsorption from the large intestine corresponded to 23% of the biliary influx in control rats, and this percentage was even higher in rats fed diets containing GG or cholesterol. Thus, the physiological changes elicited by GG in the cecum, such as a greater surface area of exchange or an accelerated blood flow, might outweigh the potential inhibitory effects of GG in the cecal lumen.

In rats fed the cholesterol diet, hypercholesterolemia was caused by a dramatic rise in TGRPLP cholesterol, whereas HDL cholesterol was unchanged. This change also elicited a substantial increase in plasma triglycerides, together with an accumulation of triglycerides and cholesterol esters in the liver. Cholesterol ester accumulation is connected to the induction of acylCoA:cholesterol acyltransferase (ACAT), as previously shown (Fernandez 1993, Suckling and Strange 1985). It has been established that cholesterol-enriched diets stimulate hepatic biosynthesis of triglyceride and depress oxidation of fatty acids in rats (Liu et al. 1995). Feeding GG resulted in an almost complete recovery from all these disturbances of lipid metabolism, because hypercholesterolemia and hypertriglyceridemia were practically abolished, and the liver lipid accumulation was drastically reduced. Numerous aspects of the lipido-lowering effects of soluble fibers such as GG occur simultaneously, including reduced availability of dietary cholesterol, changes in plasma apolipoprotein concentrations (especially apo E and apo A-I) (Moundras et al. 1994, Schneeman et al. 1984), accelerated cycling of apo-apolipoproteins (Fernandez et al. 1995, Mazur et al. 1990) and attenuation of the postprandial rise of glucose and insulin (Morand et al. 1994).

In previous investigations, GG elicited a strong induction of HMG CoA reductase activity in rat liver, in parallel to a cholesterol-lowering effect (Ide et al. 1991, Moundras et al. 1994), but this induction was not observed by Overton et al. (1994). With a cholesterol-free diet, this induction is the result of the GG-mediated diversion of the cholesterol body pool towards fecal steroid excretion. In rats fed diets containing
cholesterol and no GG, HMG CoA reductase activity was strongly repressed. When GG was added to the 0.3% cholesterol diet, hepatic cholesterylgenesis was probably reactivated, as reported in rats fed pectin or psyllium by Arjmandi et al. (1992). The mechanism of induction of the rate-limiting enzyme of cholesterylgenesis by GG is probably connected to a depletion of cholesterol from the liver. In rats fed a cholesterol-free GG diet, there was also an increase in cholesterol 7α-hydroxylase activity, suggesting a coordinated up-regulation of HMG CoA reductase and cholesterol 7α-hydroxylase (Pandak et al. 1990). However, the situation in cholesterol-fed rats, in which the two enzyme activities changed in the opposite direction, relative to the control rats, suggests a more complex regulation.

The up-regulation of bile acid synthesis by soluble fibers such as GG has been frequently observed in rats (Favier et al. 1995, Ide et al. 1991, Matheson et al. 1995, Overton et al. 1994) but not always in other species such as guinea pig (Fernandez 1995). Because bile acids, especially the nonpolar species, are able to down-regulate cholesterol 7α-hydroxylase in rats (Stange et al. 1989), an impaired reabsorption of bile acids should accelerate cholesterol oxidation. In the present experiment, because bile acid absorption remained very effective in rats fed GG, it seems unlikely that the portal concentration of bile acids would be depressed, compared with controls. Nevertheless, cholesterol 7α-hydroxylase was strongly induced. The correlation between portal bile acids and the activity of cholesterol 7α-hydroxylase has been questioned (Fukushima et al. 1995), and Pandak et al. (1995) have suggested that the down-regulation of cholesterol 7α-hydroxylase by bile acids is not an effect exerted by plasma bile acids, but rather by factor(s) released by the intestine when bile acids are present in the lumen.

In conclusion, it appears that a large part of the cholesterylreducing effect of GG is dependent on its capacity to accelerate neutral and acidic steroid excretion. When cholesterol is present in the diet, GG seems particularly effective in depressing the intestinal absorption of exogenous cholesterol. Basically, GG enhances the intestinal pool of steroids, especially bile acids. Because reabsorption of bile acids is proportional to the size of the intestinal bile acid pool, this process may be substantially enhanced in rats fed GG. Thus, in these rats, there is apparently an enhanced liver secretion and intestinal reabsorption of bile acids, and not merely a diversion of intestinal steroids towards fecal elimination. It is noteworthy that the percentage of the biliary bile acids lost in the feces remained fairly constant with the addition of GG in the diet. Hence, the higher the biliary flux, the greater the fecal elimination of bile acids. In contrast, when cholesterol was added to the diets, the enhanced fecal loss of bile acids corresponded to a less effective reabsorption. In rats fed GG diets, the losses of steroids were compensated for to a certain extent by the induction of liver HMG CoA reductase. The induction of this enzyme took place in spite of an accelerated return of bile acids to the liver; this process could limit the adaptation of choles terol synthesis and thus contribute to the cholesterylreducing effect of GG in this animal model.

LITERATURE CITED


