Viscosity and Fermentability as Attributes of Dietary Fiber Responsible for the Hypocholesterolemic Effect in Hamsters[1-3]

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ABSTRACT The attribute(s) of soluble dietary fibers responsible for cholesterol lowering is currently uncertain. A series of experiments were conducted in which viscosity and fermentability was assessed independently for their effect on plasma and liver cholesterol concentration. Hamsters were divided into four dietary groups and fed diets containing 0.12% cholesterol and 5% fiber as high viscosity hydroxypropyl methylcellulose (HV-HPMC group), low viscosity hydroxypropyl methylcellulose (LV-HPMC group), high viscosity guar gum (HV-GG group) or low viscosity guar gum (LV-GG group). Hydroxypropyl methylcellulose is essentially nonfermentable, whereas guar gum is highly fermentable. Plasma cholesterol concentrations at 3, 6 and 11 wk and liver cholesterol concentrations at 6 and 11 wk were significantly lower in the HV-HPMC group relative to the LV-HPMC group ($P < 0.05$). Intestinal content viscosities of the LV-HPMC and HV-GG groups were similar; consequently, these two groups were compared to examine the independent effect of fermentation. Plasma and liver cholesterol were significantly lower in the HV-GG group compared with the LV-HPMC group at 6 wk ($P < 0.05$), but not at 3 or 11 wk. Hepatic sterol synthesis rates were not affected by any of the diets. This study shows that greater viscosity of intestinal contents is strongly associated with cholesterol reduction, but that the contribution of fiber fermentation remains uncertain. J. Nutr. 123: 244-252, 1993.

INDEXING KEY WORDS:

- *dietary fiber* • *cholesterol*
- *viscosity* • *fermentation*
- *hamsters*

Substantial effort has been devoted to identifying and establishing the hypocholesterolemic properties of dietary fibers. For example, guar gum, pectin and psyllium have repeatedly been shown to reduce total plasma cholesterol and/or LDL cholesterol concentrations, whereas cellulose and wheat bran have consistently failed to reduce plasma cholesterol (Chen and Anderson 1979, Tsai et al. 1976). The physiologic mechanism(s) by which cholesterol-lowering fibers elicit their effect remains to be established. Numerous hypotheses have been proposed to explain the hypocholesterolemic effect of fiber, including binding of bile acids by fiber (Story and Kritchevsky 1976), interference with micelle formation (Vahouny et al. 1980) and reduced hepatic cholesterol synthesis by propionate, a product of fiber fermentation (Anderson and Chen 1979). However, none of these hypotheses are entirely consistent with the body of accumulated observations. It is plausible that multiple mechanisms are involved in the hypocholesterolemic response and that the profile of operative mechanisms may vary considerably among different dietary fiber sources.

An examination of hypocholesterolemic fibers indicates that most share at least one of two common attributes: they are viscous and/or fermentable within the gastrointestinal tract. Given the possible complexity of multiple mechanisms, studying hypocholesterolemic responses in relation to these fiber attributes could provide a clearer focus for the elucidation of specific hypocholesterolemic mechanisms. It is possible that hypocholesterolemic fibers that are viscous but not fermentable in the

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intestinal tract may elicit different metabolic responses than hypocholesterolemic fibers that are both viscous and fermentable. We report here on a series of animal studies designed to study the hypocholesterolemic effect of dietary fiber by examining the effect of two attributes of fiber, viscosity and susceptibility to fermentation, on cholesterol and bile acid metabolism.

MATERIALS AND METHODS

Animals and diets. Male Golden Syrian hamsters weighing between 80 and 100 g (Charles River, Wilmington, MA) were used in all experiments. All animals were housed and used in compliance with the University of Minnesota Policy on Animal Care and Use.

The experimental diets were based on the AIN-76A diet (AIN 1980). The diets were formulated as follows (g/kg): casein, 200; cornstarch, 497.8; sucrose, 100; palm oil, 100; dietary fiber, 50 (see below); AIN-76 mineral mix, 35; AIN-76 vitamin mix, 10; DL-methionine, 3.0; choline bitartrate, 2.0; cholesterol, 1.2 (0.069 mg/kJ); butylated hydroxytoluene, 1.0. In addition, 1.33 mg/kg of menadione sodium bisulfite complex was added to each diet. The cholesterol, butylated hydroxytoluene and menadione sodium bisulfite complex were dissolved into the palm oil. This diet was found to induce a moderate hypercholesterolemia such that plasma cholesterol concentrations approximated those of moderately hypercholesterolemic humans (unpublished observations). Guar gum and hydroxypropyl methylcellulose (HPMC)[5] were used as the fiber sources. Native guar gum is both highly viscous and highly fermentable; it consistently reduces plasma cholesterol in both animals and humans. Hydroxypropyl methylcellulose is a semi-synthetic cellulosic ether that is essentially unfermentable in rats (Braun et al. 1974) and is available in a wide range of viscosities.

Experimental design. These characteristics allowed us to construct a series of studies to examine viscosity and fermentability as main effects in a 2 × 2 factorial design. Hamsters were divided into four dietary groups and fed diets containing 5% fiber as high viscosity HPMC (HV-HPMC group), low viscosity HPMC (LV-HPMC group), high viscosity guar gum (HV-GG group) or low viscosity guar gum (LV-GG group). METHOCEL K100LV (Dow Chemical, Midland, MI) was used as the low viscosity HPMC and had an in vitro viscosity of 100 cP, based on the manufacturer's specifications; METHOCEL K100M (Dow Chemical) was used as the high viscosity HPMC and had an in vitro viscosity of 100,000 cP. High viscosity (native) guar gum was obtained from Sigma Chemical (St. Louis, MO). The low viscosity guar gum was prepared by limited acid hydrolysis of the native guar gum to a final viscosity closely matched to the low viscosity HPMC preparation. Hydrolysis was achieved by exposure of the native guar gum to hydrogen chloride gas for a length of time determined empirically.

Three trials were conducted using the experimental design indicated above. In Experiment 1, animals were fed their respective diets for 3 wk, deprived of food overnight for 16 h, and presented with a 2-g meal of their diet the following morning. Two hours after presentation of the meal, the animals were anesthetized with ethyl ether, blood collected by cardiac puncture for plasma cholesterol determination and the small intestine removed and the contents collected by gentle finger-stripping of the intestine. The intestinal contents were centrifuged at 30,000 × *g* in a J2-21 high speed centrifuge using a JA 20.1 Rotor (Beckman Instruments, Spinco Division, Palo Alto, CA) for 1 h, and the viscosity of the supernatant was measured at 37°C with a Wells-Brookfield cone/plate viscometer (model RVT, Brookfield Engineering, Stoughton, MA). For the supernatants from the HV-HPMC group, a CP-51 cone was used at a shear rate of 2.3 s^{-1}. For the supernatants from the other groups, a CP-51 cone was used at a shear rate of 23.0 s^{-1}, except for two samples from the HV-GG group, for which a CP-40 cone was used.

In Experiment 2, animals were fed their respective diets for 6 wk, deprived of food overnight for 16 h and anesthetized; blood was collected by cardiac puncture for isolation of plasma lipoproteins and the liver removed for determination of cholesterol and cholesteryl esters. The small intestine and gallbladder were excised and combined, lyophilized, weighed and stored at −20°C until analyzed for bile acids. Cecal pH was measured using a combination spear-tip pH electrode (model 81-63, Orion Research, Boston, MA). During the last week, a 3-d fecal collection was made for determination of fecal bile acid excretion. Feces were lyophilized, weighed, ground in a coffee mill and stored at −20°C until analyzed.

In Experiment 3, animals were fed their respective diets for 11 wk. After being deprived of food overnight for 16 h, each animal was given an intraperitoneal injection of 370 MBq of ^3H_2O (NEN Products, Du Pont, Boston, MA) and killed 2 h later to determine in vivo sterol synthesis rates (Lakshmanan and Veech 1977). Liver and small intestine were excised, and aliquots of plasma were taken for determination of plasma water specific radioactivity and cholesterol concentration.

[5]Abbreviations used: cP, centipoise; HPMC, hydroxypropyl methylcellulose. Dietary group abbreviations: HV-HPMC group, group fed 5% fiber as high viscosity HPMC; LV-HPMC group, group fed 5% fiber as low viscosity HPMC; HV-GG group, group fed 5% fiber as high viscosity guar gum; LV-GG group, group fed 5% fiber as low viscosity guar gum.

Lipid and lipoprotein analysis. Plasma lipoproteins were isolated by sequential centrifugation by the method of Havel et al. (1955) in a model L2-65B ultracentrifuge using a 50.2 Ti rotor (Beckman Instruments, Spinco Division) using plasma pooled from two hamsters. A preservative solution (6% of plasma volume) was added to protect lipoproteins from enzymatic degradation (Edelstein and Scanu 1986). The density (d) flotation intervals used to isolate lipoprotein fractions were as follows: VLDL, $d < 1.006$ kg/L; LDL, $1.006 < d < 1.055$ kg/L; HDL, $1.055 < d < 1.22$ kg/L. Preliminary characterization experiments indicated that these density cut-points resulted in minimal cross-contamination of apolipoproteins among isolated lipoprotein fractions. In each fraction, cholesterol and triglyceride were assayed enzymatically using commercial kits (no. 352 and no. 336, respectively, Sigma Chemical). Protein was determined by the method of Smith et al. (1985). High density lipoprotein apolipoproteins were separated by gradient SDS-PAGE (7.5–20%) (Laemmli 1970), stained with Comassie Brilliant Blue and individual apolipoprotein bands quantified by densitometric scanning using Image Version 1.17 software (National technical information service P89-212732). Corrections for differences in dye uptake between apolipoprotein bands were made. Lipids were extracted from livers by the method of Folch et al. (1957). In Experiment 2, total and unesterified cholesterol contents were determined enzymatically (Warnick et al. 1982), with cholesterol esters determined as the difference. In Experiment 3, total cholesterol was determined enzymatically using a commercial kit (no. 352, Sigma Chemical).

In vivo sterol synthesis. Digitonin-precipitable sterols were isolated from samples of liver and small intestine after addition of a small amount of [4-^{14}C]cholesterol (NEN Products, Du Pont) to correct for losses. To eliminate contamination with ^3H_2O (Jeske and Dietschy 1980), the digitonin precipitates were dissolved in pyridine and the free sterols were quantitatively recovered with diethyl ether (Sperry 1963). The ether extracts were dried and counted for ^{3}H and ^{14}C using an external standard-channels ratio method with automatic quench compensation. Synthesis rates are expressed as nanomoles of [^{3}H]water incorporated into digitonin-precipitable sterols, calculated from the specific radioactivity of plasma water as determined by the following formula (Jeske and Dietschy 1980):

$$\frac{(\text{cpm } ^3\text{H/mL plasma})\ (1.09)}{(0.055 \times 10^9 \text{ nmol } H_2O/\text{mL})\ (0.92 \text{ mL } H_2O/\text{mL plasma})}.$$

Bile acid analysis. Bile acids were extracted from samples of lyophilized small intestine or feces by sequential extraction with ethanol and chloroform-methanol, then partially purified using C18 solid phase extraction cartridges (Bakerbond SPE, J. T. Baker, Phillipsburg, NJ), as described by Locket and Gallaher (1989). This procedure results in recovery of >90% of tissue and fecal bile acids.

Total quantities of bile acids in extracts of small intestinal/gallbladder tissue samples were determined using a modification of the spectrophotometric assay of Sheltawy and Losowsky (1975). High performance liquid chromatography was used to quantify individual bile acids present in extracts from fecal samples, as described by Gallaher et al. (1992). Briefly, separation of bile acids was achieved with a reverse-phase Novo-Pak C_{18} 4 μm cartridge (5 mm × 10 cm) housed in a radial compression module (Waters Chromatography Division, Milford, MA). A step-wise gradient elution system was used, composed of two mobile phases, ammonium dihydrogen phosphate (10 mmol/L, pH 7.8) and acetonitrile. Hyocholic acid was used as an internal standard. Bile acids were detected by use of a second column (5 × 0.5 cm i.d., Alltech, Deerfield, IL) containing 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50, 50 units, Sigma Chemical) mounted on glutaraldehyde-treated aminopropyl glass beads (Sigma Chemical) (Marshall 1973). A buffer containing NAD (0.1 mol/L Tris-HCl, pH 8.5, 2.7 mmol/L EDTA, 1.63 mmol/L dithiothreitol and 0.01 mmol/L NAD) was introduced by a tee between the first and second columns at a constant rate of 1 mL/min. NADH produced by the reaction of bile acids and NAD with the immobilized enzyme was detected fluorometrically. Peak areas were calculated using a chromatography software program (712 System Controller, Gilson Medical Electronics, Middleton, WI). Bile acids in samples were identified by comparison to retention times of authentic bile acids (Sigma Chemical) and quantified by comparison with peak areas of known amounts of standards.

Statistical analysis. Results of ex vivo viscosity measurements precluded factorial statistical analysis. Therefore, one-way ANOVA was performed using the SAS statistical software (SAS 1985). After a significant F test, Duncan's multiple range test was used to inspect differences among group means. Statistical significance was accepted at $P < 0.05$. For ex vivo viscosity values, it was found that the SD were approximately proportional to the means. Therefore, a logarithmic transformation was used to stabilize the variance. All values are reported as means ± SEM.

RESULTS

Body weight, food intake and cecal pH. **Table 1** shows body weights of the animals from the four dietary groups over the 11-wk period of Experiment 3. Although the average body weight of the HV-HPMC group was less than that of all other groups from the second week onward, statistically significant differences occurred only at wk 2 and 6. In Experiment 2,

TABLE 1

Body weight of hamsters fed diets containing guar gum or hydroxypropyl methylcellulose (HPMC) of different viscosities for 11 wk (Experiment 3)[1]

Time point	Dietary group			
	LV-GG	HV-GG	LV-HPMC	HV-HPMC
	g			
Initial	86.8 ± 1.1	87.3 ± 2.0	87.0 ± 1.5	87.8 ± 1.6
Wk 2	100.8 ± 2.8^{ab}	102.9 ± 2.4^{a}	102.0 ± 3.4^{ab}	94.1 ± 1.9^{b}
Wk 4	116.5 ± 4.2	116.4 ± 2.6	114.0 ± 3.5	108.0 ± 2.5
Wk 6	127.9 ± 5.0^{a}	124.7 ± 2.8^{ab}	128.1 ± 4.2^{a}	114.0 ± 2.9^{b}
Wk 8	132.0 ± 5.2	128.1 ± 3.9	134.6 ± 4.7	122.1 ± 2.8
Wk 10	134.7 ± 5.5	133.7 ± 4.1	138.1 ± 4.8	127.1 ± 2.8
Wk 11	132.4 ± 5.4	135.8 ± 3.9	136.9 ± 3.7	127.2 ± 3.4

[1]Values are means ± SEM, n = 10 for each group. Values in a row with different superscripts are significantly different ($P < 0.05$). Dietary group abbreviations: HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

there were no significant differences in body weight among the groups at the 6-wk time point when the animals were killed (data not shown).

Food intake did not differ among the groups at any time point in any of the three experiments (data not shown). Food intake was 8–9 g/d at wk 10 of Experiment 3.

Cecal pH, measured at the 6-wk time point (Experiment 2), did not differ among the groups. The pH values were as follows (n = 10–12): LV-GG group, 7.29 ± 0.22; HV-GG group, 7.34 ± 0.19; LV-HPMC group, 7.31 ± 0.15; HV-HPMC group, 7.37 ± 0.12.

Viscosity. There was a lack of agreement between the viscosities estimated in vitro and those estimated from the intestinal contents' supernatants as measured ex vivo (**Table 2**). In spite of similar in vitro viscosities, the high viscosity HPMC showed a much higher viscosity ex vivo than did the native guar gum. Likewise, ex vivo viscosity estimates for low viscosity HPMC were higher than those for the hydrolyzed guar gum. The ex vivo viscosities of the intestinal contents supernatants were much lower than those of the 2% solutions measured in vitro for both the high viscosity guar gum and the HPMC fibers.

These findings indicate that the 2 × 2 factorial experimental design was inappropriate. Consequently, we selected comparisons to independently examine effects of the viscosity and fermentability attributes of the fibers. To examine the role of viscosity, we compared the HV-HPMC group with the LV-HPMC group, because the two HPMC preparations are essentially unfermentable. For examining the role of fermentation, the LV-HPMC group was compared with the HV-GG group, because the low viscosity HPMC and the native guar gum have similar ex vivo viscosities.

Cholesterol and lipoproteins. **Figure 1** shows plasma cholesterol concentrations of each group at 3, 6 and 11 wk. Comparison of the HV-HPMC group with LV-HPMC group indicated that greater ex vivo viscosity was associated with a significant hypocholesterolemic effect throughout the observed time frame. Plasma cholesterol concentrations were also lower in the HV-GG group compared with the LV-HPMC group; however, this difference was statistically significant only at wk 6 (Experiment 2). These results suggest that fermentation may also influence total plasma cholesterol concentrations.

Table 3 shows plasma lipoprotein lipid and protein composition, determined at wk 6 (Experiment 2). Low

TABLE 2

Viscosity of the dietary fibers, intestinal contents supernatants and intestinal contents supernatant volumes of hamsters fed diets containing guar gum or hydroxypropyl methylcellulose (HPMC) for 3 wk (Experiment 1)[1]

Dietary group	Viscosity		Intestinal contents supernatant volume
	Dietary fiber 2% solution (in vitro)	Intestinal contents (ex vivo)	
	cP		*mL*
HV-GG	100,000	230 ± 89^{bc}	0.7 ± 0.1^{b}
LV-GG	100	56 ± 19^{c}	0.5 ± 0.0^{b}
HV-HPMC	100,000	2665 ± 648^{a}	1.0 ± 0.1^{a}
LV-HPMC	100	274 ± 63^{b}	0.5 ± 0.0^{b}

[1]In vitro viscosities were determined in distilled water at 20°C. Ex vivo viscosities were determined at 37°C and are means ± SEM of 6–9 samples. Values in a column with different superscripts are significantly different ($P < 0.05$). Dietary group abbreviations: HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

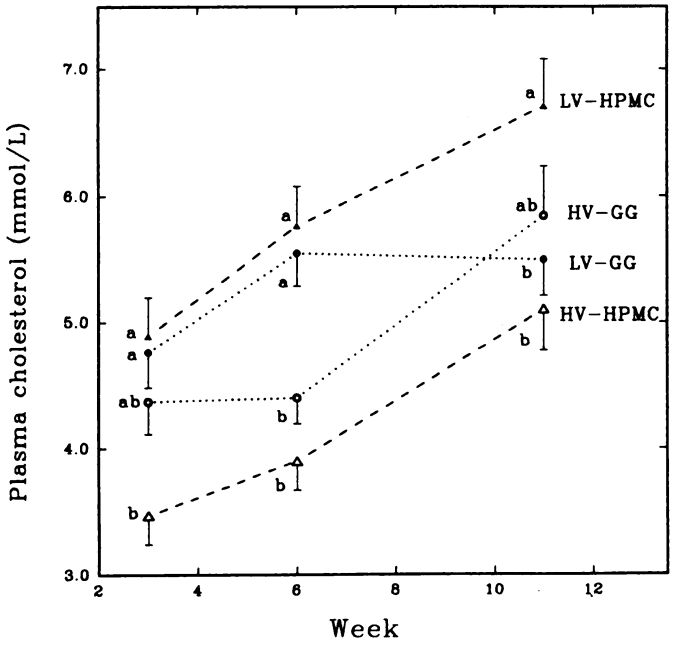

FIGURE 1 Plasma cholesterol concentrations in hamsters fed 5% guar gum (GG) or hydroxypropyl methylcellulose (HPMC) of high and low viscosities for 3 wk (Experiment 1), 6 wk (Experiment 2), or 11 wk (Experiment 3). Values represent means ± SEM; n = 9–10 animals per group in Experiment 1, n = 16 in Experiment 2, and n = 10 in Experiment 3. Means at the same time point with different superscripts are significantly different ($P < 0.05$). Dietary group abbreviations: LV-GG, low viscosity GG; HV-GG, high viscosity GG; LV-HPMC, low viscosity HPMC; HV-HPMC, high viscosity HPMC.

density lipoprotein cholesterol concentrations were unaffected by the dietary treatments. VLDL cholesterol concentrations were approximately the same as those observed within the LDL fraction. However, the HV-HPMC group had a significantly lower VLDL cholesterol concentration relative to the LV-HPMC group. The greatest dietary effects were found within the HDL fraction. Again, the HV-HPMC group had a significantly lower HDL cholesterol concentration than did the LV-HPMC group. In addition, the group fed native guar gum (HV-GG group) had a significantly lower HDL cholesterol concentration compared with the LV-HPMC group. Overall, the differences in protein concentrations mirrored the differences in cholesterol concentrations, suggesting that there were not major compositional changes observed within the isolated lipoprotein fractions.

The HDL apolipoprotein profile, determined by gradient gel electrophoresis, was determined at wk 6 (Experiment 2). Approximately 70% (range 68–74%) of the apolipoprotein content was composed of apo A-1. Various apo C composed most of the remainder (15–22%), with only traces of apo E present (1–2%). No statistically significant changes in the apolipoprotein profile were observed among the dietary treatments. The results of the lipoprotein ana-

TABLE 3

***Effect of the diets on plasma lipoprotein composition in hamsters after 6 wk (Experiment 2)*[1]**

Dietary group	Cholesterol				Triglyceride			Protein		
	Total	VLDL	LDL	HDL	Total	VLDL	LDL	HDL	VLDL	LDL	HDL
	mmol/L								*mg/L*		
HV-GG	4.40^{b} ± 0.21	0.60^{ab} ± 0.05	0.69 ± 0.05	2.54^{b} ± 0.16	1.36 ± 0.08	0.95 ± 0.06	0.22 ± 0.01	0.19 ± 0.03	164 ± 20	192 ± 8	2406^{ab} ± 157
LV-GG	5.55^{a} ± 0.26	0.72^{a} ± 0.09	0.68 ± 0.04	3.20^{a} ± 0.22	1.68 ± 0.19	1.11 ± 0.14	0.21 ± 0.02	0.36 ± 0.11	198 ± 20	205 ± 11	2629^{ab} ± 183
HV-HPMC	3.90^{b} ± 0.23	0.43^{b} ± 0.05	0.68 ± 0.03	2.08^{b} ± 0.14	1.20 ± 0.11	0.79 ± 0.09	0.21 ± 0.01	0.21 ± 0.04	143 ± 20	196 ± 6	2198^{b} ± 179
LV-HPMC	5.77^{a} ± 0.31	0.64^{a} ± 0.06	0.74 ± 0.06	3.34^{a} ± 0.24	1.41 ± 0.08	1.00 ± 0.13	0.21 ± 0.02	0.21 ± 0.04	198 ± 37	225 ± 16	2740^{a} ± 152

[1] Values are means ± SEM of pools of plasma from two animals; n = 8. Values within the same column with different superscripts are significantly different ($P < 0.05$). Dietary group abbreviations:HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

TABLE 4

Effect of the diets on liver weight and cholesterol concentration in hamsters after 6 wk (Experiment 2) or 11 wk (Experiment 3)[1]

	Dietary group	Liver weight	Total cholesterol	
			Cholesteryl esters	Free cholesterol
		g	*μmol/g liver*	
Expt. 2 (6 wk)	HV-GG	4.9 ± 0.2[a]	19.8 ± 2.1[b]	6.2 ± 0.3[b]
	LV-GG	4.2 ± 0.1[bc]	30.3 ± 3.3[a]	7.3 ± 0.5[ab]
	HV-HPMC	4.3 ± 0.1[b]	7.2 ± 0.9[c]	6.2 ± 0.3[b]
	LV-HPMC	3.8 ± 0.1[c]	31.2 ± 2.7[a]	8.3 ± 0.8[a]
Expt. 3 (11 wk)	HV-GG	6.9 ± 0.3[ab]	24.6 ± 2.6[ab]	
	LV-GG	7.0 ± 0.4[a]	32.1 ± 5.2[a]	
	HV-HPMC	5.9 ± 0.3[b]	14.2 ± 3.4[b]	
	LV-HPMC	6.4 ± 0.2[ab]	31.3 ± 3.4[a]	

[1]Values are means ± SEM of 16 and 10 animals, respectively. Values within the same column with different superscripts are significantly different ($P < 0.05$). For Experiment 3, total hepatic cholesterol only was determined. Dietary group abbreviations: HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

lyses suggest that the changes in plasma cholesterol concentration were primarily due to changes in the number of HDL particles and not to compositional changes within HDL particles.

Hepatic cholesterol concentrations were determined in Experiments 2 and 3 (**Table 4**). In Experiment 2, cholesterol ester concentrations were significantly lower in the HV-HPMC group compared with the LV-HPMC group. Also, the cholesterol ester concentration of the HV-GG group was significantly lower than that of the LV- HPMC group. Unesterified (free) cholesterol concentration was also influenced by the dietary treatments; a significantly lower concentration was found in the HV-HPMC group compared with the LV-HPMC group and in the HV-GG group compared with the LV-HPMC group. In Experiment 3, only total hepatic cholesterol concentration was determined. Again, the lowest concentration was found in the HV-HPMC group, and this concentration was significantly lower than that found in the LV-HPMC group. The concentration in the HV-GG group was not significantly different from the concentration in the LV-HPMC group.

Rates of sterol biosynthesis in liver and small intestine were examined. **Table 5** shows hepatic and small intestinal rates of cholesterol synthesis at wk 11, as measured in vivo using incorporation of tritiated water into digitonin-precipitable sterols. Synthesis rates are expressed on a per organ basis. There were no differences in hepatic sterol synthesis rates among the dietary treatments. In the small intestine, synthesis rates tended to be lowest in the LV-HPMC group; this group had the highest plasma cholesterol and HDL cholesterol concentrations.

Bile acids. Bile acid pool size was estimated as total small intestinal and gallbladder bile acids. The pool size of animals at wk 6 (Experiment 2) did not differ significantly among the four dietary groups. The values were as follows (μmol/animal, *n* in parentheses): HV-GG group, 20.3 ± 1.1 (16); LV-GG group, 21.7 ± 1.5 (15); HV-HPMC group, 23.2 ± 1.0 (15); LV-HPMC group, 20.7 ± 1.1 (16).

Fecal bile acid excretion rates were estimated to determine whether the observed reductions in plasma cholesterol could be associated with an increase in bile acid excretion. Daily fecal bile acid excretion at wk 6 (Experiment 2) is shown in **Table 6**. Total daily bile acid excretion was highest in the HV-GG group and lowest in the LV-HPMC group; this difference was statistically significant. Thus, the greater total bile acid excretion in the HV-GG group was associated with the lower plasma cholesterol seen at the 6-wk time point (Figure 1) and with the highly fermentable nature of guar gum.

TABLE 5

Sterol biosynthesis rates in liver and small intestine of hamsters at 11 wk (Experiment 3)[1]

Dietary group	Liver	Small intestine
	nmol ^3H_2O incorporated into DPS/(h·organ)	
HV-GG	653 ± 152	1067 ± 134[a]
LV-GG	519 ± 59	792 ± 59[ab]
HV-HPMC	480 ± 84	920 ± 75[ab]
LV-HPMC	468 ± 40	724 ± 73[b]

[1]Values are means ± SEM; *n* = 9–10 animals per group. Values within the same column with different superscripts are significantly different ($P < 0.05$). DPS = digitonin-precipitable sterols. Dietary group abbreviations: HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

TABLE 6

Daily fecal bile acid excretion and fecal weight in cholesterol-fed hamsters fed guar gum or hydroxypropyl methylcellulose (HPMC) of different viscosities for 6 wk (Experiment 2)[1]

	Dietary group			
	HV-GG	LV-GG	HV-HPMC	LV-HPMC
	μmol/d			
3α,7α-Diol-12-one-5β-cholanic acid	1.42 ± 0.47[a]	0.68 ± 0.17[b]	0.37 ± 0.10[b]	0.29 ± 0.10[b]
Hyodeoxycholic acid	0.03 ± 0.00[b]	0.05 ± 0.01[a]	0.03 ± 0.01[b]	0.04 ± 0.01[ab]
Cholic acid	0.29 ± 0.12[a]	0.06 ± 0.02[b]	0.03 ± 0.01[b]	0.03 ± 0.01[b]
3α-Ol-12-one-5β-cholanic acid	0.14 ± 0.02[a]	0.15 ± 0.02[a]	0.13 ± 0.02[a]	0.06 ± 0.01[b]
Chenodeoxycholic acid	0.18 ± 0.04[ab]	0.26 ± 0.05[a]	0.16 ± 0.03[ab]	0.12 ± 0.02[b]
Deoxycholic acid (DCA)	0.55 ± 0.10	0.39 ± 0.07	0.45 ± 0.08	0.37 ± 0.06
Lithocholic acid (LCA)	0.35 ± 0.07[c]	0.63 ± 0.11[b]	1.17 ± 0.10[a]	0.71 ± 0.12[b]
Total bile acids[2]	2.97 ± 0.48[a]	2.24 ± 0.36[ab]	2.35 ± 0.13[ab]	1.63 ± 0.19[b]
LCA:DCA ratio	0.85 ± 0.20[c]	1.84 ± 0.23[b]	3.30 ± 0.50[a]	2.23 ± 0.39[b]
Daily fecal weight, *g*	0.72 ± 0.05[c]	0.84 ± 0.07[c]	1.26 ± 0.06[a]	1.05 ± 0.08[b]

[1]Values are means ± SEM, n = 11–12 per group. Differences among means were inspected using Duncan's multiple range test. Values in a row with different superscripts are significantly different ($P < 0.05$). Dietary group abbreviations: HV-GG, high viscosity (native) guar gum; LV-GG, low viscosity (hydrolyzed) guar gum; HV-HPMC, high viscosity HPMC; LV-HPMC, low viscosity HPMC.

[2]Includes bile acids listed and ursodexoycholic acid, which was present only in small amounts (<0.02 μmol/d).

Large changes in the excretion of individual bile acids (excretion profile) (Table 6) were apparent. There was greater excretion of 3α,7α-diol-12-keto-5β-cholanic acid in the guar gum–fed groups, particularly the HV-GG group. Based on these and other experiments from our laboratory (unpublished observations), an increase in this particular bile acid seems to be associated with the feeding of fermentable compounds in hamsters. Other distinctive differences in the bile acid profile were a much greater cholic acid excretion in the HV-GG group and much greater lithocholic acid excretion in the HV-HPMC group, relative to the other groups. Overall, individual and total fecal bile acid excretion was substantially similar to that reported for hamsters by Imray et al. (1992) as determined by gas chromatography.

DISCUSSION

The unexpectedly low viscosities of the intestinal contents of the guar gum–fed animals may be due to fermentation in the hamster stomach. In hamsters, unlike rats, the stomach is separated into a pregastric pouch and a gastric pouch. The pregastric pouch has a higher pH than the gastric pouch. In addition, the presence of short-chain fatty acids in the pregastric pouch contents and the keratinized epithelial lining (similar to a rumen) of the pouch all suggest the pregastric pouch as a site of active fermentation (Hoover et al. 1969). Thus, guar gum was likely fermented sufficiently in the pregastric pouch to reduce its viscosity to the unexpectedly low levels observed ex vivo.

The extent to which HPMC could have been hydrolyzed in the hamster pregastric pouch is not known. HV-HPMC group showed reduced ex vivo viscosity estimates relative to the in vitro estimate. However, direct comparisons between the in vitro and ex vivo viscosity measurements are probably misleading, because any such relationship would certainly depend upon gastric emptying rates. These rates will influence the concentration of HPMC present in the intestinal contents. Further, the rate of movement of chyme through the tract and the rate of hydration of the HPMC are other factors that will affect the contents' viscosity. Given the ubiquitous nature of gastrointestinal microflora among species, one could also argue that if HPMC is shown to be essentially unfermentable in rats, it is likely to be unfermentable in hamsters. Nonetheless, we cannot completely exclude the possibility that some hydrolysis of HPMC occurred within the stomach.

Our results indicate that high viscosity HPMC elicits a hypocholesterolemic effect in cholesterol-fed hamsters that is largely, if not exclusively, associated with viscosity attributes. This hypocholesterolemic effect cannot be attributed to the slightly lower body weight of the animals in the HV-HPMC group. In recent experiments we have found significant plasma cholesterol reductions in high viscosity HPMC–fed animals compared with low viscosity HPMC–fed animals with equivalent body weights (unpublished observations).

Several other investigators have attempted to examine fiber viscosity independent of fermentation. Topping et al. (1988) examined the effect of feeding diets containing different viscosities of methylcel-

lulose to rats. Methylcellulose, like HPMC, is nonfermentable. Similar to our results, they found no significant effect of viscosity on hepatic cholesterol synthesis or fecal bile acid excretion. However, in contrast to our results, they found no changes in plasma cholesterol concentration. Their diets did not contain cholesterol, which may explain the lack of change, because plasma cholesterol in rats fed cholesterol-free diets is quite resistant to change.

In another study, Sugano et al. (1988) fed rats diets containing 0.5% cholesterol and 5% chitosans of different in vitro viscosities (range of 17–1620 cP as a 0.5% solution). They reported that all chitosans greatly reduced both plasma and liver cholesterol, but to an equivalent degree, relative to a cellulose-based control. Previous studies have shown that chitosans do not significantly increase fecal bile acid excretion (Sugano et al. 1980). The explanation for the divergent results of Sugano et al. (1988) and ours may indicate differences in the mechanism of action between the two fibers. Chitosans are polyglucosamines that display a wide range of viscosities, dependent on their molecular weight. However, they are insoluble at pH > 6.0 (Furda 1983). Because the pH of small intestinal contents of rats is ~6.8 (Gallaher and Schneeman 1986), it is questionable whether chitosans cause any viscosity increase within the small intestine and therefore doubtful that the reduction in cholesterol observed should be attributed to a viscosity effect.

The results of the present study are therefore not necessarily in disagreement with the previous studies examining the relationship of fiber viscosity to cholesterol lowering. In the case of the study of Topping et al. (1988), the discrepancy is likely due to the animal models used, i.e., rats fed a cholesterol-free diet vs. cholesterol-fed hamsters. In the study of Sugano et al. (1988), it would seem that no changes in intestinal contents viscosity were achieved with the different chitosan preparations, and therefore viscosity was not the fiber attribute responsible for cholesterol lowering.

The hypocholesterolemic effect of native guar gum in our study seems to be fermentation-related. However, this effect was clearly apparent only at the 6-wk time point (Experiment 2). Because most plasma cholesterol–lowering fibers are both fermentable and viscous, the effects of these attributes cannot usually be separated. Nondigestible but highly fermentable short-chain oligosaccharides have been used to examine fermentation alone. Levrat et al. (1991) found that dietary inulin reduced plasma cholesterol concentration in rats in a dose-related manner in a 3-wk feeding study. The cecal bile acid pool was expanded by inulin feeding, which presumably would lead to increased bile acid excretion, as was found in our study with guar gum feeding. Rotstein et al. (1981) reported a significant reduction in plasma cholesterol in hamsters fed an essential fatty acid–deficient diet containing lactulose, relative to the same diet without lactulose. These investigators found an almost threefold increase in fecal neutral steroid excretion in the animals fed lactulose, but no significant increase in fecal bile acid excretion. In contrast, Jenkins et al. (1991) reported that consumption of 18–25 g/d of lactulose by normolipidemic subjects for 2 wk increased plasma cholesterol slightly compared with the control diet. Thus, evidence for a role of fermentation as a phenomenon responsible for plasma cholesterol reduction is contradictory.

The specific mechanism(s) by which increased intestinal viscosity and fermentation produce their hypocholesterolemic effects has yet to be determined. Fermentable fibers have been hypothesized to reduce plasma cholesterol by a reduction in hepatic cholesterol synthesis, mediated by propionate produced in the large intestine as a result of increased fermentation (Anderson and Chen 1979). However, our finding of no significant differences in hepatic sterol synthesis among the diet groups (Table 4) does not support this hypothesis. Additionally, in hamsters fed psyllium mucilloid, a fermentable fiber source (Costa et al. 1989), hepatic cholesterol synthesis was significantly higher than in a cellulose control group (Turley et al. 1991). Thus, in hamsters, it seems unlikely that propionate, in the quantities produced by fiber fermentation, inhibits hepatic cholesterol synthesis.

Small differences among groups in cholesterol absorption could potentially account for the observed differences in plasma cholesterol or hepatic cholesteryl esters. A relatively modest difference in cholesterol absorption efficiency of 10% between the LV-HPMC group and the HV-HPMC group, for example, would represent a difference over 6 wk of ~70 μmol. This reduction of ~70 μmol cholesterol, combined with the increase in fecal bile acid excretion of ~30 μmol (an increase that was not statistically significant), could account for the observed difference between the LV-HPMC and HV-HPMC groups of ~99 μmol in the combined pools of plasma cholesterol and hepatic cholesteryl esters. This analysis of our results is consistent with the suggested mechanism of cholesterol lowering by psyllium in hamsters, i.e., the hypocholesterolemic effect of psyllium was due to a decrease in cholesterol absorption, an increase in bile acid excretion, or a combination of these two effects (Turley et al. 1991).

To the extent that fiber lowers plasma cholesterol by multiple mechanisms, determination of the mechanism(s) of cholesterol lowering by dietary fiber will be more difficult to establish with certainty. For example, differences in bile acid excretion determined at a single time point may be too small to detect reliably. However, over a long period of time, these differences could contribute significantly to the hypocholesterolemic effect.

Viscous materials could inhibit intestinal absorption of cholesterol by slowing diffusion of the cholesterol-containing micelles to the intestinal mucosal cells (the site of cholesterol absorption) and/or by interfering with the formation of mixed micelles. In either case, the result would be decreased intestinal absorption and increased fecal excretion of cholesterol. Studies of the effect of changes in small intestinal viscosity on cholesterol absorption are necessary to examine this possibility.

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