Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: influence on fecal sterol and bile acid profiles

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Abstract Recent findings suggest that the effects of cholestyramine and psyllium in combination could be additive for cholesterol-lowering. We therefore examined the effect of both agents, alone and in combination, on lipoprotein cholesterol and neutral and acidic steroid excretion in the hamster. Animals (n = 8/group) were fed for 21 days, either a basal chow diet supplemented with 10% palm oil and 0.2% cholesterol, or one of four treatments consisting of the basal diet plus: 5.5% cellulose; 5% psyllium with 0.5% cellulose; 0.5% cholestyramine with 5% cellulose; or 5% psyllium with 0.5% cholestyramine. Psyllium and cholestyramine both had significant hypocholesterolemic effects, but in combination produced additive reductions in lipoprotein and hepatic cholesterol. Psyllium, cholestyramine, and the combination increased total bile acid excretion by 26%, 57%, and 79%, respectively. Psyllium affected only unconjugated bile acid excretion while cholestyramine also increased the excretion of conjugated and primary bile acids. Neither agent, nor the combination, affected fecal neutral sterol excretion. We conclude that, while both agents lower cholesterol by a mechanism of increased bile acid excretion, these studies indicate that psyllium does not bind bile acids in vivo and lend further support for the concomitant use of these agents for cholesterol-lowering.—**Daggy, B. P., N. C. O'Connell, G. R. Jerdack, B. A. Stinson, and K. D. R. Setchell.** Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: effect on fecal sterol and bile acid profiles. *J. Lipid Res.* 1997. **38:** 491–502.

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Two agents that have been clinically proven to lower serum cholesterol concentrations are the bile acid binding resin, cholestyramine (1), and the soluble fiber source psyllium hydrophilic mucilloid (psyllium) (2–5). Animal studies suggest that psyllium, in common with other agents that lower LDL cholesterol (LDL-C), can reduce the progression of atherosclerosis (6, 7). Studies in the hamster (8, 9) and in humans (10, 11) suggest that psyllium and bile acid binding resins, when used in combination, may be more effective in lowering serum cholesterol than either agent alone. The mechanism of action of cholestyramine is well known and involves selective binding of bile acids to anionic sites on the resin thereby interrupting their enterohepatic recycling and increasing fecal bile acid excretion (12–14). A consequence of this increased fecal bile acid loss is enhanced hepatic clearance of LDL-C, resulting in lower serum cholesterol concentrations (15–17).

There are some similarities in the mechanism of action of psyllium with those of cholestyramine, including increased fecal sterol excretion in animals (9, 18) and in humans (19–22), consistent with the observed increase in bile acid synthesis (16, 23, 24). Both agents have been shown to increase hepatic sterol synthesis and LDL receptor activity (16, 25).

There are, however, subtle differences between these two agents. Unlike cholestyramine, psyllium does not bind bile acids in vitro (25, 26). In the hamster the two agents have contrasting effects on biliary bile acid output, with cholestyramine decreasing and psyllium increasing biliary bile acid secretion, effects that are consistent with the intraluminal binding of bile acids by the resin (25). Intestinal sterol synthesis is elevated by cholestyramine but unchanged under the same conditions by psyllium (25). Clearly there are marked differences in the potency of psyllium and cholestyramine in altering cholesterol metabolism (9, 16).

Given the differences in the chemical structures of these two agents, including the opposite nature of the charged groups (27, 28), it is perhaps not surprising that their mechanisms of action should differ. Using the cholesterol-fed Golden Syrian hamster as an animal

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TMS, trimethylsilyl; Me-TMS, methyl ester-trimethylsilyl; GC–MS, gas chromatography–mass spectrometry.

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model, because of its similarity in sterol and bile acid metabolism to the human (29, 30), we have examined the effect of psyllium and cholestyramine, alone and in combination, on fecal bile acid and sterol excretion. Using gas chromatography–mass spectrometry to determine the complete bile acid and sterol profile, including the bile acid conjugation state, these studies extend earlier, more limited, reports of the effects of these agents on steroid output, and provide further information on the mechanism of action of psyllium.

MATERIALS AND METHODS

Animals and diets

Male Golden Syrian hamsters (n = 40), of approximately 60 days of age (90–100 g), were obtained from Charles River, Wilmington, MA. The animals were pair-housed in plastic cages with raised bottom racks to limit coprophagy. Food and water were provided ad libitum throughout the study. On arrival the animals were fed a ground commercial chow (Purina Rodent Laboratory Chow #5001) and were accustomed to a reverse 12-h light cycle (lights on at 1:00 P.M.). After 7 days of adaptation to the facility, the hamsters were randomized (n = 8/group) to test diets formulated by Research Diets, Inc. (New Brunswick, NJ). A basal diet was prepared that consisted of ground Wayne Lab Blox mixed with cholesterol (0.2% w/w; Byron Chemical Co., Long Island City, NY) and palm oil (10% w/w; Procter & Gamble Co., Cincinnati, OH). Microcrystalline cellulose (Avicel PH-101, FMC Corp., Philadelphia, PA), psyllium husk fiber (Procter & Gamble Co.), and cholestyramine resin (Sigma Chemical Company, St. Louis, MO) were added to the basal diet as described in **Table 1.** Where added to the diets, psyllium and cholestyramine were present at 5% (w/w) and 0.5%, respectively. These dosages appear to reasonably model human intake of these materials, as discussed elsewhere (9).

Sample collections and analytical methods

After 21 days on the diets, the hamsters were anesthetized singly in a carbon dioxide chamber. Blood was collected from the inferior vena cava into a syringe containing EDTA. Plasma was isolated by refrigerated centrifugation and stored at 4°C. The livers were excised, blotted, weighed, and stored at −70°C.

Lipoproteins were fractionated from plasma by sequential ultracentrifugal flotation (31). VLDL ($d < 1.020$), LDL ($1.020 \leq d \leq 1.063$), and HDL ($d > 1.063$) were collected and stored at −70°C. Lipoprotein and liver cholesterol concentrations were determined after saponification by internal standard-based capillary gas chromatography.

Feces were collected for 7 consecutive days immediately prior to necropsy. The entire pooled 7-day fecal sample from each pair of hamsters was lyophilized and weighed, and individual sterols and bile acids were measured by gas chromatography–mass spectrometry (32). The general method of analysis is shown schematically in **Fig. 1** and utilized established and well-validated techniques (33, 34). Bile acids and sterols were exhaustively extracted from the pooled feces by reflux in 90% ethanol (300 mL) followed by chloroform–methanol (1:1 by vol, 250 mL) and, after removal by filtration of the organic phases, the residual fecal pellet was extracted with 0.2 M ammonium carbonate in 80% ethanol (250 mL). The combined organic extracts were taken to dryness by evaporation. The internal standards, nordeoxycholic acid (10 μg) and cholestane-3β,5α-diol (10 μg) were added to 1/50th of the extract. In addition, [^{14}C]taurocholate, [^{14}C]cholate, and [^{14}C]cholesterol were added to determine the relative recoveries of bile acids and sterols throughout the method, which were quantitative and in accord with previously published data (32). The extract was purified by combined liquid-gel and liquid-solid chromatography and separated on a lipophilic anion exchange gel into specific fractions containing neutral sterols, unconjugated bile acids, and conjugated bile acids (32). Trimethylsilyl (TMS) ether and methyl ester-trimethylsilyl ether (Me-TMS) derivatives (35) were prepared for the neutral sterols and unconjugated bile acids, respectively. Nordeoxycholic acid (1 μg) was added to the conjugated bile acid fraction, which was then solvolyzed (36) and hydrolyzed (37) and the resulting unconjugated bile acids were isolated by liquid-solid extraction (38) and lipophilic anion ex-

TABLE 1. Composition of test diets (g/kg)

Ingredient	Basal	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine
Wayne Lab Blox	898	848.6	848.6	848.6	848.6
Cholesterol	2	1.9	1.9	1.9	1.9
Palm oil	100	94.5	94.5	94.5	94.5
Cellulose	0	55.0	5.0	50.0	0
Psyllium	0	0	50.0	0	50.0
Cholestyramine	0	0	0	5.0	5.0

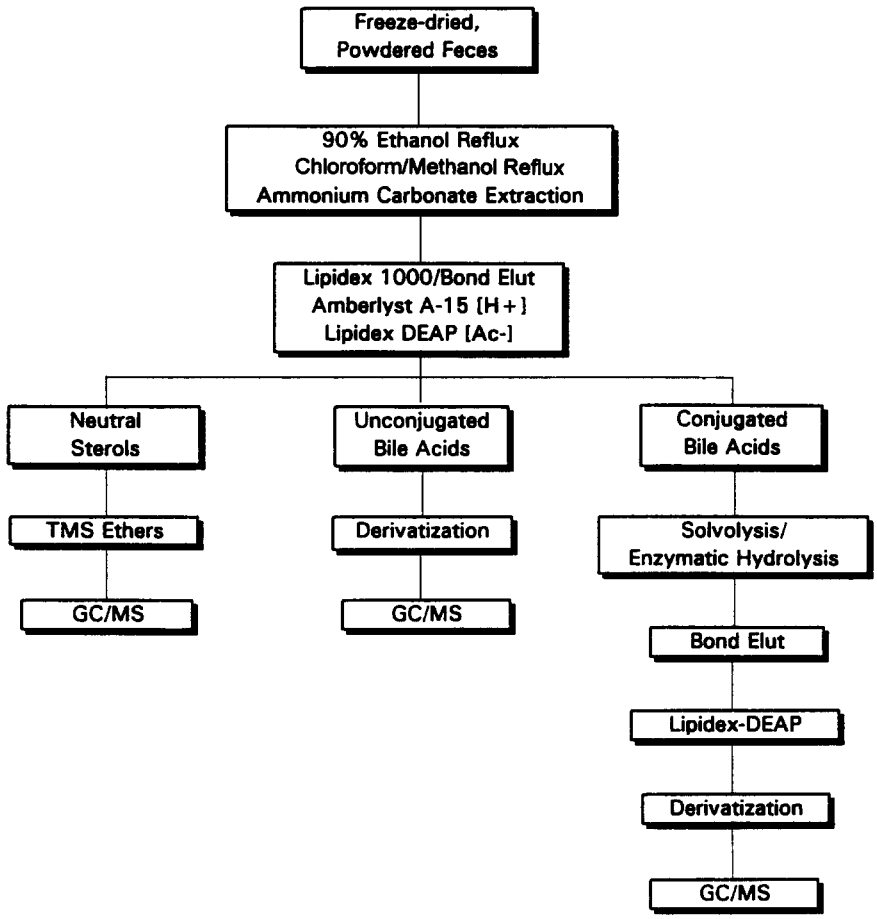

Fig. 1. General scheme for the analysis of fecal bile acids and neutral sterols.

change chromatography (39), and then converted to Me-TMS ether derivatives. A second internal standard, coprostanol (equal to the amount of the added nordeoxycholic acid internal standard), was added to each of the bile acid fractions immediately before preparation of the volatile derivatives. All derivatives were finally purified by passage through a small column of Lipidex-5000 (40). Bile acids and sterols were identified and quantified by gas chromatography and mass spectrometry (41).

Statistical analyses

Unless otherwise stated, test results are presented as mean ± SEM. Comparisons between the two negative control treatments (basal and cellulose groups) were made by two-sample *t*-tests. Tests for interaction effect were done by two-way analysis of variance. When the interaction was not significant, a test of each treatment effect was done after eliminating the interaction term. When the interaction was significant, each treatment effect was tested within each dose level of the other treatment. The above analyses were repeated on the log values.

RESULTS

Evaluation of cellulose as a negative control fiber

Table 2 summarizes some of the main observations comparing the basal and 5.5% cellulose diets. The cellulose-fed animals consumed more food and had a lower feed efficiency (g body weight gain per g of food consumed) than those fed the basal diet, consistent with a caloric dilution effect. Cellulose caused a significant increase in fecal dry weight. There were no significant differences observed for lipid variables between these two diet regimens. Therefore, the 5.5% cellulose diet was selected as the negative control for the analysis of interactions between psyllium and cholestyramine.

TABLE 2. Comparison of effects (mean ± SEM) of basal and cellulose diets in hamsters fed for 21 days

Variable	Basal	Cellulose	*P*
Initial body weight (g)	103.9 ± 1.0	104.2 ± 1.4	0.856
Weight gain (g)	35.6 ± 2.0	30.8 ± 3.2	0.134
Food consumption (g/animal pair)	430 ± 13	464 ± 16	0.020
Water consumption (g/animal pair)	484 ± 44	512 ± 79	0.815
Feed efficiency (wt gain/g food)	0.165 ± 0.003	0.132 ± 0.012	0.015
Plasma cholesterol (mg/dL)	342 ± 10	342 ± 19	0.983
VLDL cholesterol (mg/dL)	107 ± 7	107 ± 12	0.991
LDL cholesterol (mg/dL)	106 ± 8	100 ± 9	0.551
HDL cholesterol (mg/dL)	129 ± 4	135 ± 10	0.518
Liver weight/100 g body weight	5.54 ± 0.09	5.64 ± 0.16	0.492
Liver cholesterol (mg/g)	16.5 ± 1.2	16.6 ± 0.7	0.904
Fecal dry weight (g/7 d/animal pair)	15.9 ± 0.7	19.6 ± 1.1	0.016
Unconjugated bile acids (μg/day)	1379 ± 43	1298 ± 86	0.558
Conjugated bile acids (μg/day)	203 ± 10	178 ± 13	0.494
Total bile acids (μg/day)	1582 ± 53	1476 ± 96	0.485
Total cholesterol-derived neutral sterols (μg/day)	1998 ± 55	1860 ± 68	0.642

Effects of psyllium and cholestyramine on antemortem measures, blood lipids, and liver tissue

All of the hamsters gained weight and appeared healthy throughout the feeding study. Antemortem measurements are summarized in **Table 3.** There were no significant differences in food or water consumption. Weight gain was significantly lower for animals fed psyllium. Feed efficiency tended to be lower with psyllium and higher with cholestyramine, although the changes were not statistically significant.

Upon necropsy, it was noted that the livers of the basal diet- and 5.5% cellulose-fed hamsters were relatively large, and had a fatty, mottled appearance in comparison to the other treatments. No other abnormalities of organs or tissues were noted.

When cellulose was partially replaced with 5% psyllium or 0.5% cholestyramine, a moderate cholesterol-lowering effect was observed (**Table 4**). Psyllium tended to be more effective than cholestyramine at these doses. The combination of psyllium and cholestyramine produced lower mean plasma and lipoprotein (VLDL, LDL, and HDL) cholesterol than either agent alone. For all of the plasma lipid variables, the test for interaction revealed that the reductions were statistically additive, in the direction of synergy. The synergistic trend for LDL-C is illustrated in **Fig. 2.**

The most striking improvement brought about by the single treatments was the reduction in liver cholesterol (Table 4). Relative liver weights and liver cholesterol were also reduced by the combination. Normalized liver weight exhibited a synergistic decrease. Seven out of eight hamsters in the combination group had liver cholesterol values of 3 mg/g or less, approaching the values reported in chow-fed male hamsters (9, 42).

Qualitative and quantitative bile acid and sterol excretion

Chromatographic separation of bile acids according to their mode of conjugation revealed that fecal bile acids were mainly excreted as unconjugated bile acids. More than 20 unconjugated bile acids were identified by mass spectrometry (**Fig. 3; Table 5**) and these were mostly secondary bile acids. Total fecal bile acid excretion for the control group of animals was 1298 ± 86 μg/day and comprised mainly deoxycholic and lithocholic acids and their respective stereoisomers. The primary

TABLE 3. Antemortem measurements (mean ± SEM) in hamsters fed cellulose, psyllium, cholestyramine, or psyllium plus cholestyramine for 21 days

					P Values		
	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine	Psyllium	Cholestyramine	Interaction
Initial body weight (g)	104.2 ± 1.4	103.5 ± 0.9	104.1 ± 1.5	104.1 ± 0.9	0.792	0.825	0.780
Weight gain (g)	30.8 ± 3.2	25.8 ± 2.1	35.5 ± 1.5	27.4 ± 2.0	0.006	0.162	0.494
Food consumption (g/animal pair)	464 ± 16	458 ± 26	459 ± 20	412 ± 5	0.173	0.196	0.275
Water consumption (g/animal pair)	512 ± 79	581 ± 116	579 ± 86	562 ± 75	0.767	0.782	0.643
Feed efficiency (wt gain/g food)	0.13 ± 0.01	0.12 ± 0.02	0.16 ± 0.01	0.13 ± 0.01	0.097	0.086	0.809

TABLE 4. Blood and liver cholesterol levels (mean ± SEM) in hamsters fed cellulose, psyllium, cholestyramine, or psyllium plus cholestyramine for 21 days

	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine	*P* Values		
					Psyllium	Cholestyramine	Interaction
Plasma cholesterol (mg/dL)	342 ± 19	252 ± 15	286 ± 8	175 ± 16	0.0001	0.0002	0.113
VLDL cholesterol (mg/dL)	107 ± 12	56 ± 6	76 ± 7	29 ± 5	0.0001	0.0003	0.137
LDL cholesterol (mg/dL)	00 ± 9	84 ± 6	94 ± 5	67 ± 9	0.007	0.099	0.215
HDL cholesterol (mg/dL)	135 ± 10	112 ± 9	116 ± 3	79 ± 3	0.0001	0.0005	0.113
Liver wt/100 g body wt	5.64 ± 0.16	4.74 ± 0.09	5.54 ± 0.08	4.18 ± 0.10	—	—	0.018[b]
Liver cholesterol (mg/g)	16.6 ± 0.7	6.1 ± 0.8	8.6 ± 0.7	3.1 ± 0.6	0.0001	0.0001	0.900

[a]For these variables, *P* values are reported for the log of the actual data. This transformation of the data was performed to generate a more realistic assessment of additivity for shrinking biological variables. For example, had actual values been used, liver cholesterol in the combination group would have needed to drop to negative values just to be perfectly additive. The log transformation retains the values within the bounds of reality. However, the use of log values versus actual values did not alter the statistical significance of any of these observations at $\alpha = 0.05$.

[b]Due to the significant interaction, *P* values were not determined for the main effects.

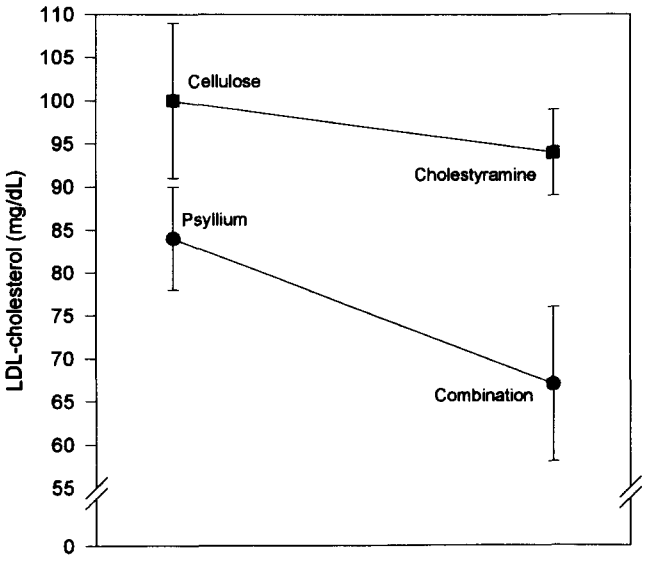

Fig. 2. LDL cholesterol concentrations (mean ± SEM) in hamsters fed diets containing either: 5.5% (w/w) cellulose; 0.5% cholestyramine resin (plus 5% cellulose); 5% psyllium (plus 0.5% cellulose); or 0.5% cholestyramine with 5% psyllium. The diet to which these materials were added was made hypercholesterolemic by addition of 0.2% cholesterol and 10% palm oil. After 21 days on the diets, anesthetized hamsters (n = 8/group) were exsanguinated for lipoprotein cholesterol determination. LDL (d 1.20–1.63) was isolated by ultracentrifugation. The statistical analysis tested whether the two lines were not parallel, i.e., whether there was a significant interaction indicative of interference or synergy. For LDL-C, simple additivity was shown.

bile acids, cholic and chenodeoxycholic acids, were not detected in the unconjugated bile acid fraction from control or psyllium-alone fed animals, but cholic acid did appear in the unconjugated bile acid fraction of those animals fed diets containing cholestyramine. Total unconjugated bile acid excretion was increased significantly by both psyllium (+28%, psyllium vs. cellulose; $P = 0.009$ for overall psyllium effect) and cholestyramine (+49%, $P = 0.0001$). The interaction was not statistically significant ($P = 0.6584$), indicating additivity.

In the conjugated bile acid fraction, cholic and chenodeoxycholic acids were identified in all groups of animals (Fig. 3; **Table 6**), accounting for 25–30% of the total conjugated bile acids excreted for the control and psyllium-fed animals and slightly higher proportions (>30%) for those animals fed the diets containing cholestyramine. Cholestyramine caused a greater than 2-fold increase in the fecal excretion of total conjugated bile acids ($P = 0.0001$), including the primary bile acids. Psyllium had no apparent effect on conjugated bile acid excretion ($P = 0.1263$), and there was no significant interaction between psyllium and cholestyramine for this variable ($P = 0.4463$).

The principal neutral sterols excreted in hamster feces comprised the endogenous sterols, coprostanol, epicoprostanol, cholesterol, cholestanol, and cholestanone, and a number of plant sterols, the predominant one being 24α-ethyl-coprostanol (Fig. 3; **Table 7**). The patterns of neutral sterol excretion were similar across treatment groups, although epicoprostanol was essentially absent from the fecal extracts of hamsters fed psyllium, with or without cholestyramine. Quantitatively, neither agent, alone or in combination, was found to cause a significant increase in the excretion of cholesterol-derived neutral sterols, based on either the raw data, or by using plant sterols as a marker.

Calculated bile acid synthesis rates

Primary bile acid synthesis rates were determined from the sum of the respective cholic and chenodeoxy-

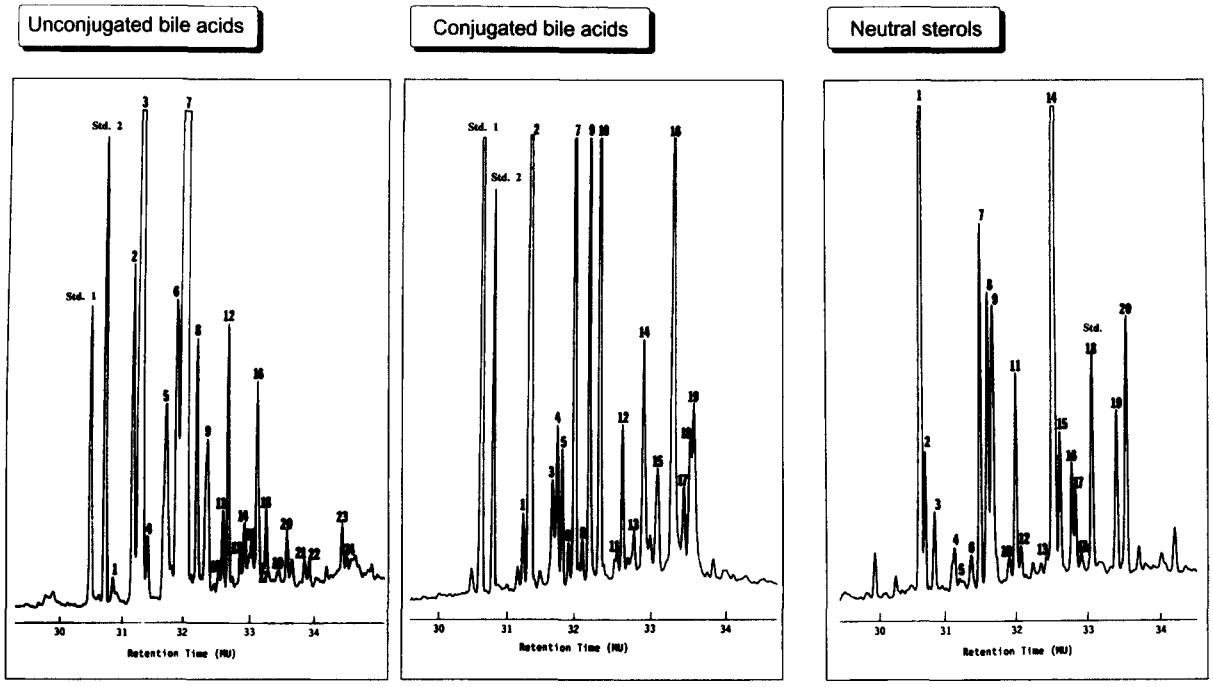

Fig. 3. Representative gas chromatographic profiles for the methyl ester-trimethylsilyl ethers of bile acids isolated as unconjugated bile acids (left panel) and conjugated bile acids (middle panel), and trimethylsilyl ethers of neutral steroids (right panel) from hamster feces. Confirmation of peak identities was made by mass spectrometry and individual compounds are numbered in accordance with the peak numbers in the respective tables.

TABLE 5. Unconjugated bile acid excretion by the male Golden Syrian hamster (mean ± SEM, μg/day)

Peak #	Bile Acid	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine
1.	Dihydroxy bile acid	8.4 ± 0.7	9.3 ± 0.8	9.3 ± 0.9	10.0 ± 0.8
2.	3β-Hydroxy-5β-cholanoic	98.0 ± 7.9	126.1 ± 9.5	118.6 ± 2.9	119.6 ± 5.1
3.	3α-Hydroxy-5β-cholanoic (lithocholic)	332.5 ± 21.1	427.4 ± 24.9	447.1 ± 13.2	465.6 ± 28.7
4.	3-Oxo-5β-cholanoic	21.6 ± 0.7	22.9 ± 3.3	32.3 ± 1.9	30.2 ± 2.5
5a.	3α,12α-Dihydroxy-5α-cholanoic	23.1 ± 2.5	26.3 ± 2.6	30.0 ± 1.9	42.7 ± 6.0
5b.	3α,12β-Dihydroxy-5β-cholanoic	31.3 ± 3.7	43.6 ± 3.6	48.8 ± 2.4	66.3 ± 6.0
6.	3β,12α-Dihydroxy-5β-cholanoic	51.1 ± 3.9	69.0 ± 5.9	79.0 ± 2.1	98.8 ± 6.3
7.	3α,12α-Dihydroxy-5β-cholanoic (deoxycholic)	340.7 ± 33.0	506.3 ± 47.3	580.6 ± 43.1	851.5 ± 66.2
8.	3β-Hydroxy-5α-cholanoic	57.7 ± 3.4	70.4 ± 4.9	126.1 ± 5.1	87.7 ± 10.0
9a.	3α,7α,12α-Trihydroxy-5β-cholanoic (cholic)	N.D.	N.D.	75.7 ± 8.6	54.5 ± 3.1
9b.	3-Oxo-12α-hydroxy-5β-cholanoic	54.3 ± 1.5	50.9 ± 4.2	N.D.	N.D.
10.	3β,6α-Dihydroxy-5β-cholanoic	8.4 ± 0.3	10.2 ± 1.7	11.7 ± 2.4	9.3 ± 0.5
11.	Dihydroxy bile acid + trace oxo-dihydroxy bile acid	12.2 ± 1.6	14.5 ± 2.1	18.6 ± 1.5	29.0 ± 4.1
12.	3β,12α-Dihydroxy-5α-cholanoic	41.5 ± 2.6	61.1 ± 10.3	67.0 ± 3.1	100.2 ± 2.5
13.	12-Oxo-3β-hydroxy-5β-cholanoic	10.4 ± 0.7	14.8 ± 1.5	10.9 ± 0.8	12.5 ± 1.3
14a.	3β,4β,12α-Trihydroxy-5β-cholanoic	23.8 ± 0.7	N.D.	31.1 ± 3.2	20.9 ± 1.5
14b.	3α,6α,7α,12α-Tetrahydroxy-5α-cholanoic	N.D.	17.3 ± 1.3	N.D.	N.D.
15.	3β,12β-Dihydroxy-5α-cholanoic	10.5 ± 0.9	10.7 ± 0.8	19.7 ± 3.2	17.2 ± 1.3
16.	12-Oxo-3α-hydroxy-5β-cholanoic	66.5 ± 3.5	41.1 ± 3.1	91.3 ± 7.1	66.6 ± 4.1
17.	3x,4x,7x-Trihydroxy bile acid	8.4 ± 1.6	10.5 ± 0.6	10.7 ± 0.3	9.5 ± 1.0
18.	3α,6α,7α,12α-Tetrahydroxy-5β-cholanoic	22.5 ± 1.5	31.1 ± 2.5	25.4 ± 3.2	25.7 ± 0.9
19.	3α,7β,22-Trihydroxy-5β-cholanoic + tetrahydroxy bile acid	8.6 ± 0.9	9.6 ± 1.1	9.3 ± 0.3	9.5 ± 0.7
20.	3x,4x,12x-Trihydroxy bile acid	21.4 ± 1.0	20.5 ± 1.6	25.9 ± 2.4	19.6 ± 2.3
21a.	Trihydroxy + tetrahydroxy bile acids	10.5 ± 0.9	12.7 ± 1.2	N.D.	10.7 ± 0.3
21b.	7-Oxo-3α,12α-dihydroxy-5β-cholanoic	N.D.	N.D.	22.5 ± 2.4	N.D.
22.	Oxo-hydroxy + oxo-trihydroxy bile acids	13.4 ± 1.4	14.8 ± 1.3	11.3 ± 0.8	9.7 ± 0.6
23.	3x,4x,12x-Trihydroxy bile acid	14.3 ± 1.7	30.0 ± 5.0	16.3 ± 0.7	18.9 ± 0.9
24.	2β,3α,7α,12α-Tetrahydroxy-5β-cholanoic	6.8 ± 1.0	8.4 ± 0.3	10.2 ± 0.8	9.1 ± 1.2
Totals		1298 ± 86	1659 ± 122	1929 ± 83	2195 ± 125

N.D., none detected.

TABLE 6. Conjugated bile acid excretion by the male Golden Syrian hamster (mean ± SEM, μg/day)

Peak #	Bile Acid	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine
1.	3β-Hydroxy-5β-cholanoic	5.1 ± 1.0	3.6 ± 0.4	9.7 ± 3.1	7.5 ± 2.1
2.	3α-Hydroxy-5β-cholanoic (lithocholic)	13.5 ± 1.9	13.7 ± 2.3	27.8 ± 1.6	36.2 ± 4.8
3.	3α,12α-Dihydroxy-5α-cholanoic	11.7 ± 3.5	15.2 ± 4.1	8.0 ± 2.3	7.3 ± 2.4
4.	3α,12β-Dihydroxy-5β-cholanoic	2.6 ± 0.4	3.5 ± 0.6	8.0 ± 0.4	14.1 ± 2.2
5.	3β,7α-Dihydroxy-5β-cholanoic	4.4 ± 0.5	5.1 ± 0.6	15.8 ± 1.4	15.4 ± 0.8
6.	3β,12α-Dihydroxy-5β-cholanoic	1.8 ± 0.3	2.1 ± 0.5	3.4 ± 0.4	3.6 ± 0.5
7.	3α,12α-Dihydroxy-5β-cholanoic (deoxycholic)	27.6 ± 1.6	32.9 ± 2.2	48.4 ± 2.8	84.2 ± 10.2
8.	3α,7α,12α-Trihydroxy-5α-cholanoic	1.1 ± 0.0	1.4 ± 0.4	4.2 ± 0.2	4.4 ± 0.3
9.	3α,7α-Dihydroxy-5β-cholanoic (chenodeoxycholic)	19.4 ± 1.6	18.5 ± 0.9	68.1 ± 5.2	63.9 ± 5.9
10.	3α,7α,12α-Trihydroxy–5β-cholanoic (cholic)	33.8 ± 3.4	35.1 ± 2.6	67.7 ± 4.8	82.7 ± 8.9
11.	3α,7β-Dihydroxy-5β-cholanoic (ursodeoxycholic)	3.1 ± 0.3	2.8 ± 0.7	5.0 ± 1.8	2.8 ± 0.3
12.	3β,12α-Dihydroxy-5α-cholanoic	2.4 ± 0.2	2.5 ± 0.3	22.3 ± 7.3	19.7 ± 8.4
13.	3-Oxo-7α-hydroxy-5β-cholanoic	2.5 ± 0.3	3.2 ± 0.4	3.3 ± 0.2	4.8 ± 0.9
14.	3x,12x-Dihydroxy-cholanoic	8.0 ± 2.0	12.2 ± 3.6	10.1 ± 2.2	17.0 ± 5.3
15.	3β,12β-Dihydroxy-5β-cholanoic	5.3 ± 0.5	5.6 ± 0.8	8.0 ± 0.8	8.3 ± 0.9
16.	12-Oxo-3α-hydroxy-5β-cholanoic + 7-oxo-3α-hydroxy-5β-cholanoic	14.5 ± 2.0	18.2 ± 3.0	43.6 ± 5.2	40.9 ± 7.6
17.	Unknown bile acid	4.7 ± 1.3	3.8 ± 1.0	6.4 ± 0.6	7.2 ± 1.2
18.	Dihydroxy-cholanoic	8.1 ± 1.7	10.4 ± 1.4	11.7 ± 1.6	14.1 ± 3.1
19.	3x,7x,12x-Trihydroxy-cholanoic	8.7 ± 1.9	10.8 ± 1.5	12.5 ± 1.9	14.7 ± 2.9
Totals		178 ± 13	200 ± 20	384 ± 16	448 ± 46

TABLE 7. Neutral sterol excretion by the male Golden Syrian hamster (mean ± SEM, μg/day)

Peak #	Neutral Sterol	Cellulose	Psyllium	Cholestyramine	Psyllium + Cholestyramine
1.	Coprostanol	741.3 ± 14.2	911.0 ± 154.5	820.5 ± 124.9	1006.0 ± 148.8
2.	Epicoprostanol	375.3 ± 39.3	29.5 ± 29.5[a]	399.5 ± 102.9	N.D.
3.	Coprostanone	60.5 ± 6.4	75.3 ± 14.1	65.8 ± 14.7	92.0 ± 30.1
4.	Methyl-diene sterol	29.3 ± 2.6	28.3 ± 7.2	30.3 ± 4.0	32.3 ± 6.9
5.	Unknown sterol	26.5 ± 3.6	10.3 ± 1.4	27.5 ± 7.1	11.3 ± 2.1
6.	Lithocholic acid + hydroxy cholesterol	34.8 ± 4.5	40.0 ± 9.1	41.3 ± 9.0	50.3 ± 10.6
7.	Cholesterol	261.0 ± 30.2	297.8 ± 68.0	341.0 ± 21.1	340.3 ± 51.6
8.	Cholestanol	216.5 ± 3.5	186.0 ± 36.6	235.3 ± 22.3	185.8 ± 30.5
9.	Cholestanone	206.0 ± 6.9	186.3 ± 37.2	233.8 ± 35.3	197.5 ± 33.8
10.	Dioxo-C_{27} sterol	21.5 ± 1.3	22.3 ± 2.1	20.5 ± 1.2	22.8 ± 3.3
11.	Ethyl-coprostenol	139.8 ± 6.2	152.5 ± 29.5	167.3 ± 21.9	186.8 ± 31.8
12.	Ethyl-monohydroxy-Δ-C_{27} sterol	57.5 ± 8.3	15.3 ± 3.5	65.0 ± 16.1	58.8 ± 43.1
13.	Unknown sterol	30.5 ± 2.3	23.3 ± 4.3	28.8 ± 3.1	19.8 ± 4.0
14.	24α-Ethyl-coprostanol	788.3 ± 13.9	690.3 ± 126.4	878.5 ± 97.7	693.3 ± 97.9
15.	24α-Ethyl-epicoprostanol + campestanol	105.0 ± 2.1	94.3 ± 19.5	125.3 ± 11.4	99.0 ± 14.6
16.	24α-Ethyl-coprostanone + stigmasterol	75.0 ± 5.4	77.8 ± 14.3	87.5 ± 13.6	78.5 ± 14.4
17.	Ring-hydroxy cholesterol	51.5 ± 1.8	29.8 ± 6.6	36.5 ± 3.3	21.8 ± 3.8
17a.	Ethyl-cholesterol isomer	17.8 ± 2.3	15.0 ± 3.2	18.3 ± 1.3	16.3 ± 2.3
18.	Int. std. (cholestane-3β,5α-diol)	—	—	—	—
19.	β-Sitosterol	120.8 ± 13.1	120.8 ± 29.9	158.5 ± 8.2	124.5 ± 18.6
20.	β-Sitostanol	200.5 ± 4.7	177.5 ± 36.1	234.8 ± 13.7	183.0 ± 25.6
Cholesterol-derived		1860 ± 68	1686 ± 305	2096 ± 162	1822 ± 284
Plant sterol-derived		1698 ± 21	1497 ± 277	1920 ± 39	1598 ± 232
Cholesterol-derived: Plant Sterol Ratio		1.10 ± 0.03	1.13 ± 0.02	1.09 ± 0.07	1.14 ± 0.07

[a]Detected in one of four samples; N.D., none detected.

cholic acid-derived fecal metabolites (**Fig. 4**). The cholic acid synthesis rate increased significantly with both psyllium ($P = 0.0047$) and cholestyramine ($P = 0.0001$) treatments. The combination of psyllium plus cholestyramine led to a 2-fold increase in cholic acid synthesis rate, consistent with a statistical finding of additivity ($P = 0.5429$ for the interaction). The change in chenodeoxycholic acid synthesis rate was less dramatic, with the combination causing no further increase above the resin-only treatment. With the combination, there was a synergistic interaction involving a shift toward cholic acid production. The ratios of cholic:chenodeoxycholic acid synthesis rate were 1.46 ± 0.03 for cellulose, 1.50 ± 0.09 for psyllium, 1.55 ± 0.09 for cholestyramine, and 1.99 ± 0.11 for the combination ($P = 0.0469$ for the interaction).

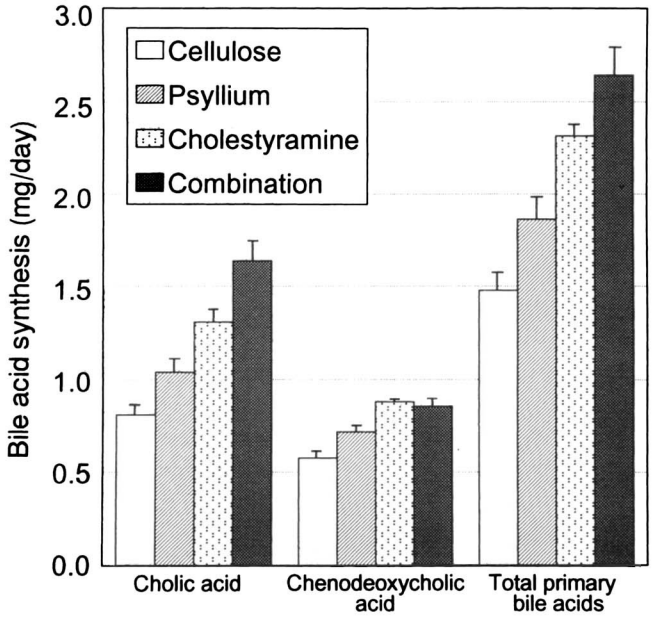

Fig. 4. Calculated primary bile acid synthesis rates (mean ± SEM) in hamsters fed diets containing either: 5.5% (w/w) cellulose; 5% psyllium (plus 0.5% cellulose); 0.5% cholestyramine resin (plus 5% cellulose); or 0.5% cholestyramine with 5% psyllium. The rates were computed from the fecal excretion of bile acids which could be positively identified by GC–MS as being derived from cholic or chenodeoxycholic acid. Cholic acid synthesis rates increased with both psyllium ($P = 0.0047$) and cholestyramine ($P = 0.0001$), with an additive increase for the combination ($P = 0.5429$ for an interaction). A significant interaction ($P = 0.0418$) was observed for chenodeoxycholic acid. Total bile acid synthesis rates increased with both psyllium ($P = 0.0063$) and cholestyramine ($P = 0.0001$), with an additive increase for the combination ($P = 0.8196$ for an interaction).

DISCUSSION

The hamster has been widely used as a suitable animal model for examining cholesterol homeostasis and the factors that influence the dynamics of cholesterol synthesis and metabolism in humans. It is therefore somewhat surprising that there have been few and only limited reported studies of bile acid metabolism and fecal excretion in this species, especially given that the pathway for bile acid synthesis represents a major pathway for cholesterol degradation, and that the traditional therapeutic approach to lowering serum cholesterol has been to enhance the elimination of bile acids by fecal excretion.

There is a wealth of literature on biliary bile acid composition of the hamster (43–48) and this has shown that cholic and chenodeoxycholic acids comprise the major products of hepatic synthesis, and in common with humans (49), these primary bile acids are predominantly conjugated to glycine. Bile acid synthesis by the hamster is significantly different from that in the rat, an animal that has also been used in studies of cholesterol metabolism, where this latter rodent synthesizes mainly taurine conjugates of cholic acid and several muricholate isomers including Δ^{22}-muricholic acid (50, 51).

To our knowledge there have been few studies of fecal bile acid excretion by the hamster, and these have mostly been limited to measurements of total bile acid excretion based on nonspecific enzymatic methods (9, 13, 52). Several studies have used gas chromatography for analysis, and these more specific methods have provided limited information on the excretion of the principal individual bile acids (30, 53–56). However, many unidentified bile acids have been found in hamster feces, and the chemical form in which these are excreted has never to our knowledge previously been examined. In the study of Malavolti et al. (54), lithocholic and deoxycholic acids were reported to be the major fecal bile acids, in accord with the findings of others (30, 53), but many unidentified metabolites quantitatively accounted for a large proportion of the total bile acids. In more recent studies, the hamster was shown to excrete many positional and stereoisomers, and oxido-reduced metabolites of the primary bile acids (30), as well as bile acid polymers (57).

We describe for the first time a detailed characterization of fecal excretion by the hamster where we have not only examined the pattern of individual bile acid metabolites, which reflect intestinal bacterial metabolism, but also the pattern of bile acid conjugation. These studies have been performed using GC–MS after selective isolation of specific bile acid fractions. The methodology has been extensively validated previously for human and rat feces (32), and utilizes a combination of liquid-solid and liquid-gel extraction and chromatographic steps to selectively isolate bile acids from feces under mild and nondestructive conditions that retain bile acids in the chemical form in which they are excreted. Unlike the routine methods for fecal bile acid analysis (58–61), the methods used in this study yield quantitative recoveries and provide information on the state of bile acid conjugation. This type of information is of considerable value, especially when examining underlying mechanisms of action of agents that influence cholesterol homeostasis by affecting fecal bile acid excretion. Minor modification of the original method (32) included an initial organic extraction step in which a counter-ion is added to ensure displacement of bile acids from any ion-exchanging groups. This is an essential step that is often omitted from techniques used in the extraction of feces containing anion exchangers such as cholestyramine (33, 62). Because in steady-state, fecal bile acid excretion is equivalent to hepatic bile acid synthesis, the specific determination of individual bile acids in feces permits calculations of cholic acid and chenodeoxycholic acid synthesis rates (63). Our

studies of fecal bile acid excretion using the approach described here extends more limited studies of fecal bile acid excretion where the effects of psyllium and cholestyramine have been examined in the hamster (9).

Total fecal bile acid excretion by control hamsters fed a cellulose diet was 1298 ± 86 μg/day. This value is similar to values reported for the hamster using essentially the same methodology (30), but lower than values reported in many other studies. These differences are best attributed to the methodological differences in measurement and physiological variations due to differences in the experimental designs of the studies. It is well established that less specific methods for measurement of bile acids tend to yield higher values for normal ranges (34). This is evident from the fact that colorimetry, enzymatic assay, packed column gas chromatography, immunoassay, and GC–MS, in this order, give decreasing values for serum bile acids. In the methodology used in this study, only those peaks in the capillary GC profiles that were positively identified as bile acid metabolites were measured. Many other extraneous peaks of unknown origin were apparent and these were excluded from quantification, which would account for lower values for fecal bile acid excretion. Furthermore, a recent study of biliary bile acid and lipid profiles has revealed striking quantitative differences among different strains of hamsters, and responses to dietary manipulations differ markedly among animals (48).

Qualitatively, fecal bile acid excretion was similar in hamsters fed the cellulose and the psyllium diets. More than 20 different bile acid metabolites were identified by mass spectrometry (Tables 5 and 6), and these were excreted predominantly in the unconjugated form (87% and 89%, respectively, for cellulose and psyllium). The secondary bile acids, lithocholic and deoxycholic acids, accounted for only 50% of the total unconjugated bile acids excreted, while the primary bile acids were not detected as unconjugated species. Cholic and chenodeoxycholic acids were, however, excreted as conjugated bile acids and accounted for 25–30% of the bile acids in this fraction (Table 6); however, these primary bile acids represented only 3.6% and 2.9%, respectively, of the total bile acids excreted when the animals were maintained on the cellulose and psyllium diets.

With the addition of cholestyramine to the diet, either alone or in combination with psyllium, the fecal bile acid profiles were qualitatively similar to those of cellulose and psyllium-alone fed animals, except for the appearance of cholic acid in the unconjugated bile acid fraction when cholestyramine was ingested (Table 5). The major effect of cholestyramine was to cause a shift in the qualitative distribution of bile acids in feces and the quantitative excretion. Specifically, the proportion of conjugated bile acids relative to the total represented 17% of the total bile acids, which is considerably higher than values for the cellulose and psyllium diets, and the relative proportion of the primary bile acids, cholic and chenodeoxycholic acids, was also higher at >32%. Quantitatively, total fecal bile acid excretion was increased by addition of psyllium or cholestyramine to the diet, either alone or in combination. Cholestyramine caused a greater than 2-fold increase in the fecal excretion of total conjugated bile acids, including the primary bile acids, and this effect was enhanced by the addition of psyllium. It should be noted that the differential effects of psyllium and cholestyramine on cholic acid excretion in the unconjugated bile acid fraction were only detectable after specific isolation of these fractions using lipophilic anion exchange chromatography coupled with detection by mass spectrometry.

The mechanism by which psyllium increases fecal bile acid excretion is unclear, and based on our detailed studies of bile acid excretion, it differs from the mechanism of action of cholestyramine. Only in the presence of cholestyramine were conjugated bile acid and cholic acid concentrations increased, and this resin increased primary bile acid excretion 2- to 3-fold. This effect is consistent with cholestyramine's capacity to bind bile acids and presumably limit the extent of intestinal bacterial biotransformation. Psyllium has not been found to bind bile acids in vitro (25, 26); indeed, the negative charge provided by the galacturonic acid residues would preclude any direct ionic binding of acidic steroids, although indirect binding through a calcium bridge has been proposed for another soluble fiber source (64). Our findings suggest that if binding does occur, it must be weak and minimal. Other less specific suggested mechanisms for the action of psyllium include decreased intraluminal mixing (65), or increased thickness of the unstirred water layer (66–68). Such changes might be expected to affect neutral sterol excretion. Neutral sterol excretion in feces was, however, unchanged by psyllium or low-dose cholestyramine, and this is in accord with previous observations by others (8, 22, 24). It is possible that the increased hepatic sterol synthesis in psyllium-fed hamsters (25), in response to increased fecal bile acid output and accompanied by increased biliary bile acid output (25, 69), maintains intraluminal cholesterol levels and cholesterol absorption. The only qualitative difference in neutral sterol excretion was the relative lack of epicoprostanol, a bacterially derived epimer of coprostanol, in the fecal extracts of psyllium-fed hamsters, suggesting that the fiber may have altered the gut microflora or the activity of the enzyme(s) responsible for the epimerization reaction. Perhaps psyllium, which has been shown to be partially fermentable by fecal bacteria in vitro (70) and in vivo (71), lowers intraluminal pH sufficiently to inactivate

the bacterial enzymes necessary for these types of reactions.

The intestine and the liver are major sites of regulation of cholesterol homeostasis in the hamster and in humans. As nonabsorbable agents, psyllium and cholestyramine both act directly at the level of the intestine, where both cause increased fecal bile acid excretion. However, both have a profound effect on the liver, and we observed a heightened response when the two agents were used in combination. In response to increased fecal bile acid excretion, there occurs a compensatory increase in bile acid synthesis. Thus psyllium and cholestyramine provide the liver with an enhanced means to eliminate excess incoming sterol. Consistent with this conclusion, both agents stimulate hepatic sterol synthesis in the hamster (18, 25, 72) and decrease hepatic storage of cholesterol, as shown here and elsewhere (25, 72). The effect on bile acid metabolism, seen here and by others using this animal model (9, 16), would support the conclusion that psyllium lowers blood cholesterol primarily through increased bile acid excretion. Clinical results with psyllium (22, 24) and another soluble fiber source, oat bran (73), support this conclusion also in humans. This does not, however, preclude additional mechanisms that may come into play with psyllium. For example, psyllium may also act via reduction of the absorption of dietary saturated fat (74) or reduction in the rate of glucose absorption (75).

In this study, the effects of psyllium and cholestyramine on plasma cholesterol reduction were found to be additive. The changes observed in plasma and LDL cholesterol were in the direction of synergy; however, the group sizes did not provide sufficient power to conclusively demonstrate this effect. Weight gain with the combination of agents tended to be higher than with psyllium alone, so that superior performance of the combination versus psyllium alone cannot be attributed to growth depression.

The test diets contained 0.2% added cholesterol, over and above the trace levels (~0.02%) present in the Lab Blox. The animals fed the combination treatment tended to have lower food consumption relative to the other treatment groups. Lower food consumption could contribute to the cholesterol-lowering effect of the combination, in that less dietary cholesterol would be consumed; however, less drug would also be consumed. Reduced food consumption in animals fed the combination would, if anything, strengthen the mechanistic finding of increased bile acid excretion. Cholesterol intake tends to stimulate bile acid synthesis in rodents, possibly via interference with bile acid uptake from the gut lumen (76), or by providing additional substrate (77), so the slightly reduced food intake with the combination would not be expected to result in the observed increase in bile acid excretion.

The significance of these findings to clinical practice is yet to be determined. Psyllium is currently recommended for use with bile acid binding resins for the prevention or relief of constipation (78). Our results suggest that there may be a second clinically important benefit of the combination, namely, improved cholesterol-lowering. This benefit may in turn allow the physician to prescribe a lower dose of resin, further reducing side effects, improving compliance, and reducing the cost of therapy.

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