Effect of Cholestyramine on Fecal Bile Salt Excretion in Rats Fed Diets Containing Medium-Chain Triglycerides or Corn Oil (37260)

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In vitro studies have shown that binding of pure bilt salts dissolved in isotonic saline as well as of mixed bile salts in animal or human bile approaches the theoretical anion-exchange capacity of cholestyramine (1). Adding fatty acids of chain length greater than 10 carbons markedly depresses binding of bile salts to the resin (2). In contrast, fatty acids of less than 10 carbons have little effect on bile salt binding (2). Johns and Bates (3) suggested that fatty acids generated during pancreatic lipolysis of long-chain dietary fat may compete with bile salt for binding sites on cholestyramine and contribute to its lower *in vivo* bile salt binding ability. Since medium-chain fatty acids compete very little with bile salt for resin binding sites (2), feeding diets containing triglycerides composed of 80% $C_{8/0}$ and 20% $C_{10/0}$ fatty acids (MCT) should result in minimal fatty acid competition and bile salt binding by the resin might be increased. This concept was tested by measuring fecal bile salt excretion in rats fed diets containing two levels of MCT or corn oil and graded levels of cholestyramine.

Materials and Methods. Two-hundred-twenty male Wistar weanling rats[1] were allotted to 4 groups of 55 animals each on the basis of body weight. The animals were housed individually in screen-bottom cages in an air-conditioned animal room. Two groups were fed diets containing 20% casein and 10% corn oil (4) or MCT. In two other groups fed 20% corn oil or MCT the additional fat was added at the expense of carbohydrate (4). After 11 days, 50 animals from each pretest group were selected on a body weight basis, subdivided into 5 test groups of 10 animals each, and fed their respective basal diets to which 0, 0.25, 0.5, 1, or 2% cholestyramine was added. Feces from each animal were collected during the first and third weeks of the 3-week experimental period and individually analyzed for fecal bile salts. Dry, pulverized fecal samples weighing 500 mg were placed into 16 × 150-mm screw-cap test tubes. The feces were extracted with 10 ml of 0.1 *N* HCl in 80% ethanol by intermittent agitation for 1 hr at ambient temperature on a Vortex mixer. The tubes were centrifuged at 2500*g* for 10 min and a 2-ml aliquot of supernatant fluid transferred to a screw-cap tube. Nonpolar lipids were removed from the alcoholic extract by liquid–liquid partitioning into two volumes of pet ether. Two-tenths-milliliter aliquots of the "lipid-free" alcoholic extract were assayed for bile salts using hydroxysteroid dehydrogenase (5). Fecal nonesterified fatty acids were determined by Method B of Van de Kamer (6).

In order to collect a bile sample at the conclusion of the experiment, the rats were anesthesized with diethyl ether, a laparotomy performed, and the duodenum isolated by ligatures placed 1 cm proximal and 1 cm distal to the ampulla of Vater. The abdominal incision was closed with wound clips and the animal was returned to the cage. Forty-five minutes later the animal was sacrificed by cervical dislocation and the bile-distended duodenum was removed. The bile was collected in 50 μl capillary tubes and stored at −18°. The relative amounts of taurine and glycine conjugates of tri- and dihydroxy bile salts were determined by reflectance densitometry using a chromatogram spectro-

[1] Obtained from Harlan Industries, Cumberland, IN.

TABLE I. Effect of Cholestyramine on Fecal Fatty Acid and Bile Salt Excretion in Rats Fed Diets Containing Corn Oil or Medium Chain Triglyceride (MCT).[a]

Fat	Cholestyramine	Fecal bile salt excretion		Nonesterified fatty acid excretion		Anion-exchange capacity of ingested resin[b]	
		Corn oil	MCT	Corn oil	MCT	Corn oil	MCT
% of diet				μmole/rat/day			
10	0	15 ± 4[c]	18 ± 5	299 ± 156	44 ± 23	—	—
10	0.25	33 ± 10	44 ± 9[d]	293 ± 141	59 ± 15	186 ± 33	202 ± 27
10	0.50	52 ± 11	63 ± 13	404 ± 195	82 ± 14	382 ± 80	366 ± 77
10	1.00	95 ± 18	79 ± 15[d]	786 ± 221	130 ± 32	797 ± 90	757 ± 133
10	2.00	109 ± 29	86 ± 11[d]	1,349 ± 412	265 ± 68	1,597 ± 316	1,719 ± 239
20	0	17 ± 7	19 ± 4	534 ± 234	49 ± 20	—	—
20	0.25	34 ± 8	43 ± 8[d]	560 ± 313	52 ± 16	182 ± 25	190 ± 28
20	0.50	48 ± 11	57 ± 16	506 ± 302	89 ± 36	335 ± 34	362 ± 45
20	1.00	70 ± 14	80 ± 17	807 ± 503	124 ± 34	645 ± 153	685 ± 125
20	2.00	120 ± 20	91 ± 14[e]	1,555 ± 436	234 ± 81	1,465 ± 302	1,454 ± 228

[a] Data obtained during the third week of cholestyramine administration.
[b] Resin intake (mg) × resin capacity (4.1 μmole/mg).
[c] Mean ± 1 SD for 10 animals.
[d] MCT vs corn oil ($p < 0.05$) by Student's t test.
[e] MCT vs corn oil ($p < 0.01$) by Student's t test.

photometer for thin-layer chromatography (TLC).[2] Five microliters of bile or standard solutions of taurocholate, glycocholate, taurodeoxycholate and glycodeoxycholate[3] were applied to 20 × 20 cm TLC plates coated with 0.25 mm silica gel G.[4] The plates were predeveloped in methanol:acetic acid (9:1) to 18.5 cm and heat-activated for 1 hr at 110° prior to application of the bile samples. The bile salt conjugates were separated by development of the plates to 15 cm in chloroform:methanol:acetic acid:water (30:10:3:2). Plates were dried at room temperature, sprayed with 50% sulfuric acid and charred for 30 min at 110°. The areas representing the four bile salt types were scanned and quantitated (7).

Results. The effects of dietary MCT or corn oil on fecal bile salt and fatty acid excretion in rats fed graded levels of cholestyramine are shown in Table I. Since results obtained during the first and third weeks were similar, only the third week data are presented. With 0.25% cholestyramine, fecal bile salt excretion in animals fed 10 or 20% dietary MCT was 30% higher than in those fed corn oil ($p < 0.05$). With 2% resin, the animals fed corn oil excreted about 30% more bile salts that did those fed MCT ($p < 0.05$). Food and resin intake were similar with either type of dietary fat. Increasing the dietary level of either fat from 10 to 20% did not markedly alter fecal bile salt excretion rates.

Fatty acid excretion in animals fed 20% corn oil with no resin added to the diet was about double that of the animals fed 10% corn oil. However, with 1 or 2% dietary resin, fatty acid excretion was more closely related to the level of dietary resin than to the level of dietary fat (Table I). Thus, fecal fatty acid excretion in animals fed dietary corn oil was increased by cholestyramine only when the anion-exchange capacity of the ingested resin exceeded the basal fatty acid excretion rates. Animals fed dietary MCT excreted markedly lower amounts of fecal fatty acid than did those fed the corn oil; this was increased only moderately by adding cholestyramine to the diet. In animals fed MCT with 2% dietary cholestyramine, fecal fatty acid excretion was lower than the fatty acid ex-

[2] Carl Zeiss, Oberkochen/Wuertt., West Germany.
[3] A Grade, Calbiochem., Los Angeles, CA.
[4] Brinkmann Instruments, Des Plaines, IL.

cretion in animals fed the basal corn oil diet.

Analysis of duodenal bile indicated that animals fed MCT secreted relatively greater amounts of taurine-conjugated bile salts than did the animals fed corn oil (Table II). This effect appeared to be related to the level of dietary corn oil since animals receiving 20% corn oil secreted lower amounts of taurine-conjugated bile salts in the bile than did those fed 10% corn oil diets. Cholestyramine increased the relative amounts of glycine-trihydroxy and decreased the amounts of taurine-dihydroxy bile salts in the bile.

Discussion. This study was designed to test whether long-chain fatty acids generated during pancreatic digestion of conventional dietary fat compete with bile salt for binding sites on cholestyramine and contribute to its lower *in vivo* bile salt binding ability. Since medium-chain fatty acid anions have a low affinity for cholestyramine *in vitro* and do not displace bile salts from the resin (2), feeding diets containing medium-chain triglycerides should result in minimal fatty acid competition. This concept is further supported by the fact that medium-chain triglycerides are well absorbed in animals (8) and humans (9) fed cholestyramine.

It was found that with low levels of dietary cholestyramine, fecal bile salt excretion was higher with MCT diets than with corn oil diets. This may have been related to less fatty acid competition by medium-chain fatty acids, since fecal fatty acid excretion in the animals fed MCT and cholestyramine was always substantially less than the total anion-exchange capacity of the ingested resin. However, since animals fed MCT secreted relatively higher amounts of taurine-conjugated bile salts which are preferentially bound by cholestyramine (1), it is possible that this effect of MCT on bile composition contributed in part to increased resin binding ability. Although animals fed the 20% corn oil diet and low levels of resin excreted considerably greater amounts of fecal fatty acids than comparable groups fed the 10% corn oil diet, bile salt binding was not affected by level of corn oil in the diet. These findings suggest that under the conditions of this study, fatty acid competition does not of itself account for the relatively low *in vivo* bile salt binding ability of cholestyramine.

Fecal bile salt excretion in animals fed cholestyramine is determined by bile salt synthesis and secretion in the liver, intraluminal binding of bile salt to the resin and subsequent reabsorption of bile salt by the ileum. Since binding of bile salt to cholestyramine is a reversible process, the relatively low *in vivo* binding when low levels of resin are fed may be a consequence of active transport by the ileum, and elution

TABLE II. Bile Salt Composition of Rat Duodenal Bile.

		Taurine conjugates				Glycine conjugates			
		Trihydroxy		Dihydroxy		Trihydroxy		Dihydroxy	
Fat	Cholestyramine	Corn oil	MCT	Corn oil	MCT	Corn oil	MCT	Corn oil	MCT
% of diet		% of total bile salts							
10	0	59 ± 16[a]	61 ± 20	23 ± 12	36 ± 18	13 ± 11	2 ± 2	6 ± 6	1 ± 2
10	0.25	65 ± 13	67 ± 14	11 ± 4	20 ± 5	19 ± 9	8 ± 8	4 ± 5	5 ± 6
10	0.50	57 ± 21	70 ± 17	8 ± 2	14 ± 6	29 ± 17	12 ± 10	6 ± 4	3 ± 4
10	1.00	59 ± 13	73 ± 19	14 ± 7	16 ± 12	22 ± 8	9 ± 12	6 ± 5	2 ± 3
10	2.00	55 ± 20	77 ± 10	10 ± 6	16 ± 7	28 ± 16	5 ± 5	6 ± 6	2 ± 2
20	0	49 ± 24	66 ± 18	11 ± 7	29 ± 19	31 ± 19	3 ± 3	8 ± 7	2 ± 4
20	0.25	37 ± 21	79 ± 10	8 ± 5	13 ± 5	46 ± 19	5 ± 5	9 ± 5	2 ± 3
20	0.50	34 ± 17	63 ± 17	8 ± 5	14 ± 5	48 ± 17	20 ± 15	9 ± 5	3 ± 2
20	1.00	41 ± 12	60 ± 22	7 ± 5	19 ± 12	45 ± 13	18 ± 18	7 ± 3	4 ± 5
20	2.00	36 ± 15	58 ± 17	8 ± 8	14 ± 8	47 ± 16	21 ± 16	9 ± 6	6 ± 7

[a] Mean ± 1 SD for 10 animals.

of previously bound bile salt from the resin (10). With higher levels of resin, it appears that fecal bile salt excretion may be limited by the capacity of the liver to synthesize bile salts. In rats fed 2% resin, corn oil promoted higher bile salt excretion rates than did MCT. Under these conditions of minimal feedback inhibition, long-chain unsaturated fatty acids may promote more rapid bile salt synthesis than medium-chain or saturated fatty acids. Hofmann (11) found that in patients with ileal resection, dietary MCT was associated with lower rates of hepatic bile salt synthesis and fecal bile salt excretion than that observed with conventional dietary fat. When cholestyramine was administered to these patients, fecal bile salt excretion was about 35% lower when MCT was substituted for conventional dietary fat.

Summary. Rats were fed diets containing medium-chain triglycerides or corn oil and graded levels of cholestyramine to determine if fatty acid competition influences resin bile salt binding ability. When low levels of the resin were fed, MCT promoted a higher rate of bile salt excretion than did corn oil. With higher levels of resin, bile salt excretion apparently was limited by hepatic capacity for bile salt synthesis; animals fed corn oil had higher rates of bile salt excretion than those fed MCT. Rats fed dietary MCT had relatively greater amounts of taurine-conjugated bile salts in the duodenal bile than did rats fed dietary corn oil. Cholestyramine affected the bile salt composition of bile by increasing the relative amounts of glycine-trihydroxy bile salt, and decreasing the relative amounts of taurine-dihydroxy bile salt in the bile.

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