Antilithiasic effect of β-cyclodextrin in LPN hamster: comparison with cholestyramine

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Abstract β-Cyclodextrin (BCD), a cyclic oligosaccharide that binds cholesterol and bile acids in vitro, has been previously shown to be an effective plasma cholesterol lowering agent in hamsters and domestic pigs. This study examined the effects of BCD as compared with cholestyramine on cholesterol and bile acid metabolism in the LPN hamster model model for cholesterol gallstones. The incidence of cholesterol gallstones was 65% in LPN hamsters fed the lithogenic diet, but decreased linearly with increasing amounts of BCD in the diet to be nil at a dose of 10% BCD. In gallbladder bile, cholesterol, phospholipid and chenodeoxycholate concentrations, hydrophobic and lithogenic indices were all significantly decreased by 10% BCD. Increases in bile acid synthesis (+110%), sterol 27-hydroxylase activity (+106%), and biliary cholate secretion (+140%) were also observed, whereas the biliary secretion of chenodeoxycholate decreased (−43%). The fecal output of chenodeoxycholate and cholate (plus derivatives) was increased by +147 and +64%, respectively, suggesting that BCD reduced the chenodeoxycholate intestinal absorption preferentially. Dietary cholestyramine decreased biliary bile acid concentration and secretion, but dramatically increased the fecal excretion of chenodeoxycholate and cholate plus their derivatives (+328 and +1940%, respectively). In contrast to BCD, the resin increased the lithogenic index in bile, induced black gallstones in 34% of hamsters, and stimulated markedly the activities of HMG-CoA reductase (+670%), sterol 27-hydroxylase (+310%), and cholesterol 7α-hydroxylase (+390%). Thus, β-cyclodextrin (BCD) prevented cholesterol gallstone formation by decreasing specifically the reabsorption of chenodeoxycholate, stimulating its biosynthesis and favoring its fecal elimination. BCD had a milder effect on lipid metabolism than cholestyramine and does not predispose animals to black gallstones as cholestyramine does in this animal model.—Boehler, N., M. Riottot, J. Férézou, M. Souidi, F. Milliat, C. Sérougne, J. L. Smith, and C. Lutton. **Antilithiasic effect of β-cyclodextrin in LPN hamster: comparison with cholestyramine.** ***J. Lipid Res.*** **1999.** 40: **726–734.**

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Beta-cyclodextrin (BCD) is a cyclic oligosaccharide containing seven glucopyranose units all linked in α (1–4) position (1), produced by the enzymatic cleavage of amylomaize starch. Its cone-shaped structure, with a hydrophilic surface and a hydrophobic cavity, confers a remarkable ability of BCD to form inclusion complexes (1). In particular, BCD is able to bind and solubilize cholesterol and bile acids in vitro (2, 3). When orally administered to rats, this carbohydrate does not exert any measurable toxic, genotoxic, or carcinogenic effects (4, 5). BCD absorption by the small intestine is minimal (6, 7) with slow degradation by the microflora in the hindgut (8).

We have previously reported that BCD, added to cholesterol-enriched diets, had a marked hypocholesterolemic effect in rats, hamsters, and pigs. This poorly digestible carbohydrate stimulated bile acid synthesis and modified the bile acid species, and the results supported decreased intestinal absorption of cholesterol (9, 10). We also reported that autoclaved amylomaize starch, another poorly digestible carbohydrate, prevented cholelithiasis in hamsters fed a fat-free lithogenic diet (11) by decreasing hepatic cholesterol synthesis and increasing the transformation of cholesterol into bile acids. While a high concentration of dietary amylomaize starch is necessary to produce beneficial effects on cholesterol and bile acid metabolism, significantly lower amounts of BCD (⩽10%) were required for comparable effects (9).

To determine the potential of BCD as an antilithiasic agent and to further explore its effects on cholesterol and bile acid metabolism, we have examined the effects of BCD in LPN hamsters fed a low-fat, sucrose-based lithogenic diet (12). In order to obtain a detailed picture of the mechanistic actions of BCD, the following key parame-

Abbreviations: BCD, β-cyclodextrin; CTR, cholestyramine; L, lithogenic diet; HMG-CoA R, HMG-CoA reductase; C7αOH, cholesterol 7α-hydroxylase; S27OH, sterol 27-hydroxylase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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ters were determined: lipid concentrations in plasma, bile, and liver; hepatic enzyme activities of HMG-CoA reductase, cholesterol 7α-hydroxylase, and sterol 27-hydroxylase; protein levels of HMG-CoA reductase, cholesterol 7α-hydroxylase and LDL receptor in liver; intestinal absorption of dietary cholesterol, and the composition of bile acids in both feces and bile. The effects of BCD were compared with those of cholestyramine (CTR), a bile acid sequestrant known to decrease plasma cholesterol in various animal species (13) and to prevent gallstone formation in the hamster (14).

MATERIALS AND METHODS

Chemicals and isotopes

β-Cyclodextrin (Kleptose®, water content 12%, purity 99%) was kindly provided by Société Roquette Frères, (62 136 Lestrem, France). Cholestyramine was kindly provided by Bristol-Meyer-Squibb, (92057 Paris Cedex 24, France). Kits were purchased from Boehringer-Mannheim (Meylan, France) for the determination of cholesterol (method CHOD-PAP), and from Wako, Oxoid (Rungis, France) for the measurement of triglycerides and phospholipids. A polyclonal antibody raised in rabbits against the LDL receptor purified from bovine adrenal cortex was kindly provided by Paul Roach (Adelaide, Australia). Antibodies raised against HMG-CoA reductase and cholesterol 7α-hydroxylase were kindly provided by Gene Ness (Tampa, FL) and Roger Davis (San Diego, CA), respectively. Deriva-sil and BSTFA were purchased from Chrompack (Les Ulis, France) and Pierce (Rockford, IL, USA) respectively. Intralipid 20% was purchased from Pharmacia AB (Sweden). [1,2-^{3}H]cholesterol and enhanced chemiluminescence reagent (ECL) were purchased from Amersham, (Les Ulis, France), [^{14}C]taurocholate sodium salt, l-3-[glutaryl-3-^{14}C] hydroxymethylglutaryl coenzyme A, [5-^{3}H]mevalonolactone, and [4-^{14}C]cholesterol were obtained from DuPont-NEN Products (Les Ulis, France). LSC-cocktail for radioactivity counting, Emulsifier-Safe, was purchased from Packard Instrument Company (Meriden, CT).

Animals and diets

Four-week-old male golden Syrian hamsters (*Mesocricetus auratus*) from our breeding unit (designated LPN hamsters) were randomly assigned to one of the following six semi-synthetic dietary groups for 30 days: lithogenic diet (LBCD0), lithogenic diet containing 2% (L BCD2), 5% (L BCD5), or 10% (L BCD10) BCD, 2% CTR (L CTR), or 5% lactose (L LACTOSE), in place of sucrose. The LBCD0 is composed as follows: sucrose 67.5%, casein 20%, lard 5%, mineral mix 5% (11), vitamin mix 2.5% (11), without cholesterol added. Hamsters were individually caged and had free access to food and water. Lighting conditions were controlled according to a 12-h light and 12-h dark cycle (7 am–7 pm).

Experimental design

Three experiments were performed to analyze the effect of dietary BCD on cholesterol lithogenesis in the hamster.

Experiment 1: Cholesterol and bile acid metabolism

Fifteen–43 hamsters per group were used. At the end of the 30-day dietary period, animals were anesthetized with an intramuscular injection of a solution containing Tiletamine and Zolazepam (Zoletil 50, Virbac, 06116 Carros, France) at a dose of 100 mg/kg body weight, and were killed by collecting blood from a heart puncture using a syringe containing heparin. Plasma was separated from blood cells by centrifugation (10 min at 2600 *g*). The abdomen was opened by a midline incision and the gallbladder was examined for the presence of gallstones. The bile was directly aspirated from the gallbladder using a calibrated plastic insulin syringe (0.5 ml) fitted with a 30G needle and was diluted (1:20) with 0.9% saline solution and stored at −20°C for further analysis. The liver was excised and weighed, and aliquots were taken for cholesterol measurement, LDL receptor protein assay, and enzymatic assays. Colon, small intestine, and cecum were also excised, weighed, and stored at −20°C.

Plasma and lipoproteins. Plasma lipids were measured by enzymatic procedures using commercial kits. Lipoproteins were fractionated by ultracentrifugation of plasma samples (0.5 ml) in a density gradient (15), using a SW41 rotor in a L8-70 apparatus (Beckman Instruments, Gagny, France). On the basis of the cholesterol profile in the gradient, the level and the composition of the major lipoprotein classes, i.e., very low density lipoproteins (VLDL) and chylomicrons (VLDL + chylomicrons, $d < 1.010$ g/ml), low density lipoproteins (LDL, $1.010 \leq d < 1.063$ g/ml), and high density lipoproteins (HDL, $1.063 \leq d \leq 1.20$ g/ml), were calculated from the sum of appropriate fractions according to their density.

Liver. Frozen liver samples (0.5 g) were thawed and homogenized in 5 ml isopropanol, using an Ultra-Turrax apparatus (Janke & Kunkel Gmb & Co., Staufen, Germany). After incubation at 60°C for 1 h and centrifugation for 5 min at 3000 *g*, the supernatant was collected and the pellet was re-extracted with 5 ml isopropanol. Triglycerides and total cholesterol were measured enzymatically on the pooled isopropanolic extracts, using the appropriate assay kits. Free and esterified cholesterol were separated by thin-layer chromatography on silica gel plates eluted with diethyl ether, taken to dryness, and dissolved in isopropanol prior to enzymatic cholesterol determination.

Microsomes and mitochondria were prepared from fresh liver samples (1 g) by homogenization in 7 ml buffer (Tris/HCl 50 mm, sucrose 300 mm, DTT 10 mm, EDTA 10 mm, NaCl 50 mm, pH 7.4) at 4°C, by means of a Teflon pestle. Microsomal and mitochondrial fractions were isolated according to the procedure described by Einarsson et al. (16) and Souidi et al. (17), respectively. HMG-CoA reductase activity was determined, in the microsomes, in the presence of alkaline phosphatase using the radioisotopic technique of Philipp and Shapiro (18). Cholesterol 7α-hydroxylase was assayed, in the microsomal fractions, according to a radioisotopic method using [4-^{14}C]cholesterol, solubilized, and carried by hydroxypropyl-β-cyclodextrin (19). An NADPH regenerating system was used to inhibit the 3β-hydroxy-Δ5-C27-steroid oxydoreductase which is able to convert 7α-OH-cholesterol into 7α-OH-4-cholesten-3-one, in the presence of $NADP^{+}$ (19). Sterol 27-hydroxylase was assayed in the mitochondrial fractions by a radioisotopic method using [4-^{14}C]cholesterol solubilized in hydroxypropyl-β-cyclodextrin (17).

The protein levels of HMG-CoA reductase, cholesterol 7α-hydroxylase, and LDL receptor were determined by immunoassay (20). Nitrocellulose membranes containing microsomal proteins spotted using dot-blot apparatus were incubated with specific antibodies after incubation with anti-IgG antibody linked to horseradish peroxidase and detection was performed using enhanced chemiluminescence reagents (ECL).

Bile. Phospholipid and cholesterol concentrations in diluted bile samples were measured using commercial kits. Total bile acid concentration was estimated by an enzymatic method with 3α-hydroxysteroid dehydrogenase (21). The cholesterol saturation index (lithogenic index) of bile was calculated according to the method of Carey (22) and Thomas and Hofmann (23). Bile

acid composition was determined by gas–liquid chromatography (GLC) after deconjugation by choloylglycine hydrolase EC 3.5.1.24 (Sigma Chemical, St. Louis, MO) and extraction by diethylether, methylation with diazomethane, and silylation with deriva-sil and BSTFA. Analyses were performed on a Carlo Erba HRGC 5160 (Thermoquest, Les Ulis, France) apparatus equipped with a standard fused silica WCOT capillary column packed with OV1701 (Spiral, Dijon, France) (length: 25 m, film thickness: 0.2 μm; oven temperature: 240°C; flow rate of hydrogen carrier gas: 2 ml/min). Individual molecular species of conjugated bile acids were determined by HPLC, as previously described (24) and their molar proportions were expressed as percent of the total bile salt concentration. Hydrophobicity index of the bile acid pool was calculated for each bile sample on the basis of the hydrophobicity index previously determined for common conjugated bile salts (25).

Fecal samples. Lipids of fecal samples were extracted by boiling ethanol in a Soxhlet apparatus for 48 h. After the addition of known amounts of radioactive markers ([1,2-^{3}H]cholesterol and [^{14}C]taurocholate sodium salt), the ethanolic extract was saponified in boiling ethanolic and 2 m potassium hydroxide for 3 h. The sterol-containing unsaponifiable fraction was extracted with petroleum ether and ^{3}H radioactivity was measured in a liquid scintillation counter. In the aqueous phase, bile acids were deconjugated by the method of Grundy, Ahrens, and Miettinen (26) and extracted with diethylether. ^{14}C radioactivity was used to correct for procedural losses. Free bile acids were methylated with diazomethane and trimethylsilyl (TMS) and the derivatives were assayed by GLC, as described above. Neutral sterols were analyzed as TMS derivatives, using an HRGC 5160 apparatus equipped with a standard fused silica WCOT capillary column (length, 25 m; film thickness, 0.2 μm) cross-linked with CPSil5CB (Chrompack, Les Ulis, France). The oven temperature was 240°C and the flow rate of hydrogen carrier gas was 2 ml/min. Molar proportions and total amounts of sterols and bile acids were assessed using calibration curves obtained from the analysis of standard mixtures (26).

Experiment 2: Biliary secretion of cholesterol and bile acids.

Six–7 hamsters were used per group: L BCD0, L BCD5, L BCD10 and L CTR. Animals were anesthetized as above and gallbladder bile was aspirated. The common bile duct was ligated and the cystic duct was cannulated with polyethylene tubing (PE 10) that was introduced into the gallbladder and hepatic bile was collected for 30 min. The volume of bile secreted during collection period was determined. Phospholipid and cholesterol concentrations in hepatic bile samples were measured using commercial kits and total bile acid concentration and cholesterol saturation index of bile were estimated as described above.

Experiment 3: Intestinal absorption of cholesterol

Six hamsters per group were used: L BCD0, L BCD10, and L CTR. Each hamster received an intravenous dose of approximately 2.5 μCi of [1,2-^{3}H]cholesterol contained in Intralipid 20%, followed by an intragastric dose of about 1.0 μCi of [4-^{14}C]cholesterol contained in walnut oil, according to the dual isotope ratio method was adapted for hamsters by Turley, Herndon, and Dietschy (27). Seventy two hours after labeled cholesterol administration, animals were anesthetized and killed by collecting blood from a heart blood puncture using a syringe containing heparin. Plasma and liver samples were taken for the measurement of total cholesterol concentration. ^{3}H and ^{14}C radioactivity of two plasma samples (100 μl each) was measured and the cholesterol absorption coefficient was calculated as follows:

$$\text{Percent cholesterol absorption} = \frac{\text{Percent of intragastric } ^{14}\text{C dose per plasma sample}}{\text{Percent of intravenous } ^{3}\text{H dose per plasma sample}} \cdot 100$$

Statistical analysis

Results are given as mean values and their SEM. Statistical differences among the groups were determined by multivariant analysis and the Newman-Keuls test. A value of $P < 0.05$ was considered significant.

RESULTS

Physiological status and incidence of cholesterol gallstones

BCD, at doses of 2 or 5%, had no effect on the final body weight, daily weight gain, or food intake (**Table 1**). With a dose of 10% BCD, the cecum weight increased markedly (4.3% of body weight versus 2.8% in L group, $P = 0.05$). The feces quantity also increased in this group by a similar magnitude (0.43 versus 0.23 g/day in L group, $P = 0.05$). The addition of CTR increased food consumption and feces excretion. Sixty-five percent of the hamsters receiving the lithogenic diet had cholesterol gallstones in their gallbladder. The replacement of 5% sucrose by 5% lactose did not modify the gallstone incidence. In contrast, the incidence of cholesterol gallstones decreased with BCD supplementation to be nil at the 10% dose. This incidence was inversely proportional to the concentration of BCD in the diet when the four hamster groups were considered ($r = -0.962$, $P < 0.001$). Although CTR also prevented cholesterol gallstone formation, it induced pigmented gallstones in 34% of the hamsters, a phenomenon not observed for BCD.

Plasma and liver lipid concentrations

Supplementation of the lithogenic diet with 10% BCD did not modify the plasma cholesterol concentration [1.33 ± 0.04 (n = 40) in the LBCD10 group, 1.40 ± 0.04 mg/mL (n = 43) in the LBCD0 group] whereas CTR decreased it by 14% (1.15 ± 0.05 mg/mL, n = 41). The basal concentrations of plasma phospholipids and triacylglycerols in LBCD0 group (2.7 ± 0.1 and 1.5 ± 0.2 mg/mL, respectively) were not modified by adding 10% BCD (2.7 ± 0.1 and 1.4 ± 0.2 mg/mL) or CTR (3.08 ± 0.1 and 1.20 ± 0.2 mg/mL) in the lithogenic diet. While BCD had no effect on cholesterol distribution among lipoprotein classes, CTR markedly decreased the cholesterol content of VLDL (−70%), LDL (−57%) and HDL (−17%) (results not shown). In the liver, the concentrations of free and esterified cholesterol and triglycerides (2.0 ± 0.2, 1.0 ± 0.1 and 6.6 ± 0.8 mg/g, respectively) in the LBCD0 group were not significantly altered by BCD or CTR addition.

Regulators of hepatic cholesterol metabolism

The activities and the protein levels of HMG-CoA reductase and of cholesterol 7α-hydroxylase and the protein level of LDL receptor were similar for the LBCD0 and LBCD10 groups (**Table 2**). However, supplementation of

TABLE 1. Final body weight, cecum weight, daily weight of excreted feces, and incidence of cholesterol and black gallstones in hamsters fed a lithogenic diet (L) supplemented with different proportions of β-cyclodextrin (0, 2, 5, or 10% BCD) or with 5% of lactose or with 2% cholestyramine (CTR)

Diets	n	Final Body Weight	Cecum Weight	Daily Weight of Excreted Feces	Number of Hamsters with Cholesterol Gallstones	Number of Hamsters with Black Gallstones
		g	*% of body weight*	*g/day*		
LBCD 0	40	69.5 ± 1.2	2.8 ± 0.1^{b}	0.23 ± 0.01^{c}	26 (65%)	3 (7.5%)
LBCD 2	15	70.7 ± 1.9	2.7 ± 0.2^{b}	0.42 ± 0.02^{a,b}	8 (53%)	0 (0%)
LBCD 5	25	70.9 ± 1.3	2.5 ± 0.2^{b}	0.42 ± 0.02^{a,b}	4 (16%)	2 (8%)
LBCD 10	43	66.5 ± 1.5	4.3 ± 0.2^{a}	0.43 ± 0.03^{a,b}	0 (0%)	1 (2%)
L Lactose	17	72.3 ± 1.5	3.1 ± 0.2^{b}	0.31 ± 0.02^{c}	13 (76%)	0 (34%)
LCTR	41	67.0 ± 1.7	2.8 ± 0.1^{b}	0.48 ± 0.02^{a}	2 (5%)	14 (34%)

Results are expressed as means ± SEM, n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$).

L diet with 10% BCD doubled sterol 27-hydroxylase activity. In contrast, the addition of CTR increased both activity (+674%) and protein level (+518%) of HMG-CoA reductase. CTR also increased the activities of cholesterol 7α-hydroxylase (+387%) and of sterol 27-hydroxylase (+176%), and raised the protein level of the LDL receptor (+114%).

Lipid concentrations of gallbladder bile

As compared to the LBCD0 diet, the LBCD5 and 10 diets markedly decreased the cholesterol and phospholipid concentrations (−32% and −42%, respectively) and their molar proportions (−28 and −22%, respectively), whereas the concentration and molar proportion of bile acids were unchanged (**Table 3**). Thus, the lithogenic indices determined by the method of Carey (22) and Thomas and Hofmann (23) were significantly decreased (−21% and −23%) by the LBCD10 diet. CTR markedly decreased the concentration of cholesterol, phospholipids, and bile acids (−60, −71.5 and −57%, respectively). The molar proportions for cholesterol and bile acids were unchanged whereas that for phospholipids were decreased (−28%). The Carey lithogenic index was increased by 24% by CTR.

Hepatic secretion of biliary lipids

The volume of bile secreted per hour was not significantly modified by the type of diet or by the addition of BCD or CTR (**Table 4**). In the LBCD0 group the three lipid concentrations in the hepatic bile were 5-fold lower than in the gallbladder bile but the lithogenic indices were similar. The addition of 10% BCD to the L diet increased the bile acid secretion (+50%), but had no effect on cholesterol or phospholipid secretions. The lithogenic index therefore decreased (−38 and −53%, according to Thomas and Hofmann (23) and Carey (22), respectively). The addition of CTR to the L diet decreased both the phospholipid and cholesterol secretion rates (−72 and −71%, respectively) and their concentrations (−63 and −64%, respectively). The lithogenic indices were similarly decreased (−40%).

Composition of biliary bile acids

In the L BCD0 group, the concentrations of cholic acid plus its derivatives and that of chenodeoxycholic acid plus its derivatives were similar (**Table 5**). This similarity was also observed with the glyco/tauroconjugated ratios of cholic and chenodeoxycholic acids. The addition of 10% BCD increased the concentration of cholic acid (+35%) and decreased that of chenodeoxycholic acid (−68%), leading to a markedly lower hydrophobic index (−49%) when compared with the LBCD0 diet. Moreover, the glyco/tauroconjugated ratio increased for both the cholic and the chenodeoxycholic acids (+77% and +103%, respectively) in the LBCD10 group. The addition of CTR tended to decrease the concentration of cholic acid and markedly reduced that of chenodeoxycholic acid (−94%), thus decreasing the hydrophobic index (−65%). In contrast to the effect of BCD, the glyco/tauroconjugated ratios

TABLE 2. HMG-CoA R and cholesterol 7α-hydroxylase activities and protein mass, sterol 27 hydroxylase activity, and LDL receptor mass in liver from hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD (0, 5, or 10%) or with 2% cholestyramine

Diets	n	HMG-CoA R Activity	HMG-CoA R Mass	C7αOH Activity	C7αOH Mass	S27 OH Activity	LDL Receptor
		pmol/min/mg protein	*AU*	*pmol/min/mg protein*	*AU*	*pmol/min/mg protein*	*AU*
LBCD 0	6	47.8 ± 12.6^{b}	100.0 ± 26.0^{b}	46.9 ± 6.8^{b}	100.0 ± 7.3	27.3 ± 2.9^{c}	100.0 ± 13.2^{b}
LBCD 5	6	73.5 ± 9.3^{b}	66.6 ± 3.7^{b}	49.4 ± 6.9^{b}	79.0 ± 3.0	ND	117.1 ± 8.7^{b}
LBCD 10	6	51.3 ± 13.7^{b}	94.3 ± 14.9^{b}	49.4 ± 6.9^{b}	81.1 ± 6.3	56.3 ± 5.9^{b}	108.7 ± 8.9^{b}
LCTR	6	370.0 ± 44.3^{a}	617.4 ± 34.9^{a}	228.4 ± 12.2^{a}	80.9 ± 8.9	111.6 ± 11.8^{a}	213.5 ± 18.6^{a}

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$). The masses are given in arbitrary units with 100 attributed to L group (AU); ND, not determined.

TABLE 3. Lipid concentrations in the gallbladder and lithogenic indices of bile in non-fasting hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD or with 2% cholestyramine

Diets	n	Bile Acids		Phospholipids		Cholesterol		Lithogenic Index	
		μmol/ml	mol %	μmol/ml	mol %	μmol/ml	mol %	Thomas and Hofmann (23)	Carey (22)
LBCD 0	25	151.9 ± 11.7^a	90.7 ± 0.6^b	11.2 ± 1.0^a	6.8 ± 0.5^a	4.0 ± 0.3^a	2.5 ± 0.1^a	0.34 ± 0.02^a	0.66 ± 0.04^b
LBCD 5	12	129.5 ± 10.1^a	93.2 ± 0.4^a	6.5 ± 0.4^b	4.8 ± 0.3^b	2.7 ± 0.2^b	2.0 ± 0.2^{ab}	0.30 ± 0.02^{ab}	0.56 ± 0.05^{bc}
LBCD 10	29	133.9 ± 9.1^a	92.8 ± 0.3^a	7.6 ± 0.6^b	5.3 ± 0.3^b	2.7 ± 0.3^b	1.8 ± 0.1^b	0.27 ± 0.01^b	0.51 ± 0.03^c
LCTR	24	65.4 ± 5.5^b	92.7 ± 0.5^a	3.2 ± 0.3^c	4.9 ± 0.4^b	1.6 ± 0.2^b	2.4 ± 0.2^a	0.36 ± 0.03^a	0.82 ± 0.07^a

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$).

for both cholic (C) and chenodeoxycholic (CDC) acids for the CTR group were decreased (−79%).

Composition of fecal bile acids

In the LBCD0 group, primary bile acids (C + CDC) accounted for only 5% of the total fecal elimination of bile acids, the major part (95%) corresponding to their microbial derivatives (**Table 6**). The fecal elimination of CDC plus its derivatives (70.9 ± 2.9% of the total) was higher than that of C plus its derivatives. Addition of 10% BCD increased the fecal elimination of total bile acids (+111%). The effect was relatively more marked for CDC + derivatives (+133%), as particularly highlighted by the increases of lithocholic acid (+173%) and isolithocholic (+243%), than for C + derivatives (+72%). The addition of CTR into the L diet also increased the fecal elimination of lithocholic (+300%), isolithocholic (+114%), ursodeoxycholic acids (+88%), and deoxycholic acid (+3177%). Thus, the fecal elimination of CDC + derivatives was 3.7-fold greater in LCTR than in LBCD0 groups. This phenomenon was markedly magnified for C plus its derivatives, being 31.5-fold higher than in LBCD0 group. Finally, the fecal elimination of total bile acids by CTR was increased 11.1-fold.

Composition of fecal neutral sterols

In the LBCD0 group, cholesterol accounted for 35% of the fecal output of neutral sterols, the remaining part corresponded to its microbial derivatives (**Table 7**). Though the addition of BCD did not statistically modify the fecal elimination of cholesterol, that of its main microbial derivative, the coprosterol, was reduced (−40%). Therefore, the proportion of cholesterol microbial metabolites (coprosterol, epicoprosterol, cholestanol) was 65% in the LBCD0 group and 44% in the LBCD10 animals. These differences indicate that BCD reduces the microbial degradation of cholesterol. Similar to BCD, CTR markedly decreased the proportion of coprosterol in the total sterols (−38%) and increased the cholesterol content elimination (+675%) indicating a reduced microbial degradation of cholesterol as reflected by the proportion of cholesterol metabolites in the total sterols (dropping by 14%). Moreover, in contrast to BCD, the addition of CTR markedly increased the fecal elimination of neutral sterols (+215%).

Intestinal absorption of dietary cholesterol

The addition of 10% BCD or 2% CTR to the lithogenic diet significantly decreased the intestinal absorption coefficient by −33% and −63%, respectively (percent of cholesterol absorption for LBCD0: 86.2 ± 8.4%, LBCD10: 57.7 ± 3.7%, LCTR: 32.0 ± 4.0% of the dietary cholesterol ingested, n = 6 per group).

DISCUSSION

Hamsters from the LPN strain are predisposed to develop cholesterol gallstones when fed a sucrose-enriched diet (11, 12, 17, 28, 29). In the lithogenic diet used in the present study, the lipid amount (5%) and the distribution of fatty acids are similar to that reported by Trautwein, Siddiqui, and Hayes (30), but the cholesterol content provided by lard and casein is negligible (10 mg per 100 g) compared with that of most lithogenic diets (300–400 mg per 100 g) (30, 31). Under this dietary condition, LPN hamsters differ from control animals (fed a starch-based diet) by an enrichment of gallbladder bile in cholesterol

TABLE 4. Hepatic biliary lipid secretions and concentrations and lithogenic index of bile in hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD (0 or 10%) or with 2% cholestyramine

Diets	n	Volume of Bile Secreted	Bile Acid		Phospholipid		Cholesterol		Lithogenic Index	
			Secretion	Concentration	Secretion	Concentration	Secretion	Concentration	Thomas and Hofmann (23)	Carey (22)
		μl/h	μmol/h	μmol/ml	μmol/h	μmol/ml	μmol/h	μmol/ml		
LBCD 0	6	270.7 ± 20.2	7.16 ± 0.80^b	27.34 ± 3.63^{ab}	0.65 ± 0.18^a	2.34 ± 0.57^a	0.24 ± 0.03^a	0.88 ± 0.10^a	0.40 ± 0.03^a	1.12 ± 0.08^a
LBCD 10	7	298.0 ± 42.8	10.77 ± 1.43^a	37.95 ± 4.87^a	0.60 ± 0.10^a	2.02 ± 0.16^a	0.22 ± 0.04^a	0.76 ± 0.11^a	0.25 ± 0.03^b	0.53 ± 0.05^b
LCTR	7	227.7 ± 29.7	4.0 ± 0.49^c	18.20 ± 1.76^b	0.18 ± 0.06^b	0.84 ± 0.25^b	0.07 ± 0.01^b	0.33 ± 0.03^b	0.24 ± 0.03^b	0.71 ± 0.06^b

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$).

TABLE 5. Gallbladder bile acid composition, glyco/tauroconjugated bile acid ratio, and hydrophobic index of bile in hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD (0, 5, or 10%) or with 2% cholestyramine

Diets	n	C	DC	CDC	LC	OXO Bile Acids	Cholate G/T Ratio	Chenodeoxycholate G/T Ratio	Hydrophobic Index
				μmol/l					
LBCD 0	11	66.2 ± 11.9^{b}	5.6 ± 1.7^{ab}	64.3 ± 11.8^{a}	4.8 ± 1.8^{ab}	14.1 ± 3.1^{a}	3.9 ± 1.0^{b}	3.8 ± 1.0^{b}	0.37 ± 0.01^{a}
LBCD 5	8	92.5 ± 7.5^{a}	8.4 ± 1.0^{a}	16.2 ± 7.1^{b}	9.6 ± 0.6^{a}	5.1 ± 2.0^{b}	3.5 ± 0.7^{b}	2.9 ± 0.6^{bc}	0.21 ± 0.01^{b}
LBCD 10	10	89.0 ± 2.2^{a}	8.7 ± 1.1^{a}	20.4 ± 1.3^{b}	4.9 ± 0.9^{ab}	6.9 ± 0.5^{b}	6.9 ± 0.4^{a}	7.7 ± 0.9^{a}	0.19 ± 0.01^{b}
LCTR	12	45.7 ± 2.1^{b}	4.2 ± 0.5^{b}	3.7 ± 0.4^{b}	3.1 ± 1.1^{b}	5.1 ± 0.6^{b}	0.7 ± 0.2^{c}	0.8 ± 0.2^{c}	0.13 ± 0.01^{c}

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$). LC, lithocholic acid; DC, deoxycholic acid; C, cholic acid; CDC, chenodeoxycholic acid; G, glyco amidate moiety; T, tauro amidate moiety; G/T, ratio glyco/tauro amidate.

and chenodeoxycholic acid, which increases bile acid hydrophobicity and facilitates the formation of cholesterol gallstones (32 and unpublished data). In the present study, we have used this LPN strain model of cholelithiasis to examine the effects of BCD on the incidence of gallstones and on lipid metabolism and compared these effects with those of cholestyramine.

Effects of BCD

BCD supplementation resulted in a dose-dependent reduction in cholesterol gallstone formation with a 10% BCD diet totally abolishing cholesterol gallstones. This marked decrease is not related to the slight reduction in dietary sucrose (necessary to include BCD) as the gallstone incidence is unaffected by replacing 5% sucrose with 5% lactose, a disaccharide that is resistant to digestion similar to BCD. In fact, the addition of BCD to the lithogenic diet produced a fall in the molar proportion of cholesterol and phospholipids in gallbladder bile, which was reflected in a lower lithogenic index. Moreover, BCD induced a spectacular decrease in the hydrophobic index of the gallbladder bile, associated with two major changes in the bile acid composition, i.e., an increased proportion of cholic acid at the expense of chenodeoxycholic acid and an increase in the glyco/tauroconjugation ratio of these two bile acids. In the BCD groups compared to the lithiasic hamsters (LBCD0), the modification of the bile acid composition was associated with a stimulation of the biliary secretion of bile acids, together with an increase in both bile acid synthesis and bile acid intestinal absorption. The fractional bile acid flow due to synthesis was doubled in BCD-fed as compared to lithiasic hamsters, supporting the results of previous experiments in hamsters and pigs by our group (9, 10). The enhanced bile acid synthesis is associated with a 2-fold stimulation in hepatic sterol 27-hydroxylase activity and no change in cholesterol 7α-hydroxylase expression (activity and protein level), suggesting a channelling of cholesterol through the acidic bile acid pathway in BCD-fed hamsters. This hypothesis is supported by the fecal elimination of chenodeoxycholic and cholic acids (plus their microbial derivatives) in BCD-fed compared with lithiasic hamsters, being 2.5- and 1.6-fold higher, respectively. These results are consistent with previous observations in hamsters and mice fed diets containing either starch or BCD in place of sucrose (9, 33).

Taken together, these results suggest that BCD binds a greater proportion of chenodeoxycholic than cholic acid in the small intestine, decreasing the reabsorption of chenodeoxycholic acid. In vitro data also support the observation that BCD binds hydrophobic bile acids (lithocholic and chenodeoxycholic acids) more strongly than the hydrophilic cholic acid (34, 35) and that this binding is higher for tauro- versus glyco-conjugated bile acids (3). This could partly explain the bile enrichment in glycoconjugated bile acids and the increased fecal output of lithocholic acid in BCD-fed hamsters. This latter effect may be responsible for the minimal colonic damage by this cytotoxic bile acid (9, 36). The hepatic synthesis of bile acids, as determined by the fecal bile acid elimination, and the biliary bile acid secretion were 2.1- and 1.5-fold higher in 10% BCD-fed compared with lithiasic hamsters, respectively. The secretion of bile acids by hepatocytes is the sum

TABLE 6. Composition and daily fecal elimination of bile acids in hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD (0, 5, or 10%) or with 2% cholestyramine

Diets	n	CDC	ILC	LC	UDC	C	DC	OXO Bile Acids	Total Fecal Elimination
					μmol/day·10^{-1}				*μmol/day*
LBCD 0	6	0.49 ± 0.07^{b}	1.28 ± 0.23^{bc}	9.26 ± 0.38^{c}	1.81 ± 0.52^{b}	0.38 ± 0.03^{c}	4.65 ± 0.62^{b}	0.87 ± 0.10^{b}	1.87 ± 0.08$^{b,\heartsuit}$
LBCD 5	6	0.35 ± 0.03^{b}	3.21 ± 0.41^{ac}	14.70 ± 1.47^{c}	0.39 ± 0.14^{b}	0.33 ± 0.04^{c}	6.21 ± 0.71^{b}	1.38 ± 0.37^{b}	2.66 ± 0.10$^{b,\clubsuit}$
LBCD 10	6	0.38 ± 0.04^{b}	4.39 ± 1.07^{a}	25.28 ± 2.67^{b}	0.31 ± 0.09^{b}	0.46 ± 0.04^{b}	7.35 ± 0.89^{b}	1.59 ± 0.18^{b}	3.97 ± 0.37$^{b,\spadesuit}$
LCTR	6	2.34 ± 0.70^{a}	2.74 ± 0.52^{ac}	36.83 ± 2.90^{a}	3.40 ± 0.65^{a}	0.84 ± 0.03^{a}	152.39 ± 13.96^{a}	8.83 ± 1.53^{a}	20.74 ± 1.82^{a}

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscript letters are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$). In the last column, two analyses were performed, an ANOVA in four groups (superscript letters) and an ANOVA in three groups (superscript symbols). In this latter analysis, LCTR results were eliminated because the mean and SEM were too high in comparison with other groups, which prevented an accurate statistical analysis. CDC, chenodeoxycholic acid; ILC, isolithocholic acid; LC, lithocholic acid; UDC, ursodeoxycholic acid; C, cholic acid; DC, deoxycholic acid.

TABLE 7. Composition, daily fecal elimination of neutral sterols in hamsters fed a lithogenic diet (L) supplemented with different proportions of BCD (0, 5, or 10%) or with 2% cholestyramine

Diets	n	CH	COP	EPICOP	Cholestanol	Fecal Elimination
				μmol/day		
LBCD 0	6	1.82 ± 0.47^b	3.24 ± 0.38^a	0.05 ± 0.01	0.05 ± 0.01	5.17 ± 0.65^b
LBCD 5	6	1.76 ± 0.18^b	1.76 ± 0.18^b	0.10 ± 0.03	0.03 ± 0.01	3.59 ± 0.33^b
LBCD 10	6	2.49 ± 0.23^b	1.92 ± 0.34^b	0.05 ± 0.01	0.05 ± 0.01	4.51 ± 0.48^b
LCTR	6	14.12 ± 0.56^a	2.03 ± 0.42^b	0.06 ± 0.01	0.60 ± 0.01	16.35 ± 0.34^a

Results are expressed as means ± SEM; n, number of hamsters per dietary group. Values with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls post hoc test ($P < 0.05$). CH, cholesterol; COP, coprosterol; EPICOP, epicoprosterol.

of the flow due to bile acid synthesis (from cholesterol) and that due to intestinal reabsorption (enterohepatic circulation). As the fractional bile acid flow from synthesis is low compared with that of secretion (37, 38), it follows that BCD increases the bile acid reabsorption. Although it appears that BCD prevents the reabsorption of chenodeoxycholic acid, the increased concentration of cholic acid in gallbladder bile and the increased secretion of total bile acids suggest that cholic acid reabsorption is increased. In fact, the hydroxyl group in position 12 prevents the inclusion of this bile acid in BCD, dramatically reducing BCD affinity for cholic and deoxycholic acids (34). Moreover, we have previously demonstrated that poorly digestible carbohydrates, such as lactose or amylomaize starch (the BCD precursor), increased the size of ileal villi and stimulated the ileal absorption of taurocholate in rats (39–42). Thus, our data suggest that BCD reduces the reabsorption of chenodeoxycholic acid and enhances that of cholic acid. In a similar manner, BCD decreases the dietary cholesterol absorption, either by encapsulation of the molecule (2), or by reducing its micellar solubilization.

The potent antilithiasic property of BCD demonstrated in LPN hamsters in the present study is apparently discordant with the previous results obtained in pigs in which BCD was shown to favor the nucleation of cholesterol in bile (10, 42). Pigs basically differ from hamsters in the transformation of cholesterol to bile acids leading mainly to chenodeoxycholic and hyocholic acids as primary bile acids (43, 44). To explain the pro-nucleation property of BCD in pigs, we think that BCD binds hyocholic acid preferentially over chenodeoxycholic acid in the small intestine, thus preventing the reabsorption of hyocholic acid and its microbial degradation. In support of this notion, gallbladder bile from BCD-fed pigs had twice the proportion of chenodeoxycholic acid and half the proportion of hyocholic plus hyodeoxycholic acids of that in control pigs. Furthermore, the daily fecal elimination values of chenodeoxycholic + lithocholic acids and that of hyocholic + hyodeoxycholic acids in BCD-fed pigs were 3- and 29-fold higher, respectively, than in control pigs (42). Thus, in pigs as in hamsters, BCD diminishes specifically the enterohepatic circulation of several bile acids, such as hyocholic and chenodeoxycholic acids which are more easily bound by BCD in the intestine. Consequently, BCD supplementation enriches pig bile with chenodeoxycholic acid and hamster bile with cholic acid. These observations emphasize the importance of the biliary bile acid composition and suggest it is one of the primary factors involved in the physiological process of cholelithiasis.

BCD versus cholestyramine

CTR treatment of LPN hamsters induced black pigmented stones as was previously described (14). CTR has also been reported to decrease lipoprotein levels (45) and increase HMG-CoA reductase and cholesterol 7α-hydroxylase activities (46) in hamsters and these effects have been confirmed in the present studies with LPN hamsters. In agreement with the increased LDL receptor-dependent LDL transport reported in hamsters treated with CTR (45), an increase in liver LDL receptor mass is also shown in the current study. However, none of these effects were found after BCD ingestion, indicating that the mode of action of BCD is somewhat different from that of CTR. Moreover, CTR markedly decreased bile acid flow compared with that of the control (−45%) and 10% BCD-fed (−63%) groups. Consequently, the bile acid concentration in gallbladder bile fell relative to that of cholesterol and phospholipids. These changes increased the lithogenic index and may partly explain the presence of black gallstones which are likely to contain some cholesterol (30). Furthermore, bile acid synthesis was markedly stimulated by CTR (+1000%), consistent with the elevation of both hepatic sterol 27-hydroxylase and cholesterol 7α-hydroxylase activities. This indicates that the bile acid reabsorption is markedly impaired by CTR, but in contrast to BCD, CTR increased the fecal output of cholic acid plus derivatives to a greater extent than that of chenodeoxycholic acid plus derivatives. In vitro, the affinity of CTR for dihydroxylated bile acids (deoxycholic and chenodeoxycholic acids) is higher than for trihydroxylated bile acids (47). Thus, CTR and BCD are both able to bind bile acids, but the ability of CTR to bind dihydroxylated bile acid is greater than that of BCD, while BCD has higher specificity for chenodeoxycholic than for deoxycholic acid (34). We speculate that the bile acid specificity of BCD also occurs in vivo (lumen of small intestine) and is likely to be a factor determining the compensatory modifications culminating in reduced cholesterol gallstone formation.

In summary, the addition of CTR or BCD to the lithogenic diet modifies cholesterol and bile acid metabolism in similar ways, but by markedly different degrees. Although the effect of BCD on lipid metabolism is less dra-

matic than that of CTR, the antilithiasic effect of this carbohydrate is more desirable than that of the resin because BCD does not induce black gallstones as CTR does. Many of the differences between BCD and CTR can be attributed to the high specificity of BCD for chenodeoxycholic in the small intestine. BCD reduces the hydrophobicity of bile acids within the enterohepatic circulation, and thus reduces the tendency for cholesterol nucleation in the gallbladder bile of hamster. BCD's low toxicity and its beneficial effects (4, 5), even at low levels (1–2%), on cholesterol and bile acid metabolism suggest it may be considered as a good alternative to the bile acid-binding resins which are poorly tolerated by humans. Another advantage of BCD supplementation is that it has both anti-lithiasic and hypocholesterolemic properties, both of which have positive beneficial outcomes for human conditions. Further studies aimed at evaluating the effects and properties of BCD in well-balanced diets in both animals and humans are presently being undertaken in our laboratory.

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