Dietary fish oil potentiates bile acid-induced cholesterol secretion into bile in rats[1]

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Abstract Recently we demonstrated that dietary fish oil (FO) causes changes in intrahepatic cholesterol transport and hypersecretion of cholesterol into bile in rats (*J. Clin. Invest.* **88:** 943–951, 1991). We have now investigated in more detail the relationship between cholesterol and bile acid secretion in rats with chronic bile diversion fed purified diets supplemented (9% wt/wt) with either FO or corn oil (CO) for 2 weeks. Effects of FO on biliary cholesterol secretion (+ 400% as compared to CO after 14 days) were much more pronounced than previously observed in rats with intact enterohepatic circulation (+50%). Biliary bile acid (+30%) and phospholipid (+120%) secretion were increased to a much lesser extent than that of cholesterol resulting in the formation of bile supersaturated with cholesterol. The biliary cholesterol/bile acid molar ratio was 0.069 and 0.032 in FO- and CO-fed rats, respectively, at noon of day 14. This ratio increased to 0.108 in FO-fed rats at midnight, when bile acid output was maximal, but remained unchanged in CO-fed rats during the day–night cycle. Intravenous administration of taurochenodeoxycholic acid (15 μmol/kg) resulted in a 2-fold increase in bile acid output and a simultaneous 1.6-fold stimulation of cholesterol secretion in both groups, implying that administration of the bile acid induced the secretion of 2–3 times as much cholesterol in FO- than in CO-fed rats. Likewise, administration of bilirubin ditaurate (30 μmol/kg), an inhibitor of bile acid-induced biliary lipid secretion, reduced cholesterol output in both groups by about 50% while bile acid output remained unchanged. It is concluded that, in rats, dietary fish oil increases the disposition of cholesterol into bile by potentiating bile acid-dependent cholesterol secretion, presumably by facilitating the recruitment of bile-destined cholesterol.—**Smit, M. J., H. J. Verkade, R. Havinga, R. J. Vonk, G. L. Scherphof, G. In 't Veld, and F. Kuipers.** Dietary fish oil potentiates bile acid-induced cholesterol secretion into bile in rats. *J. Lipid Res.* 1994. **35:** 301–310.

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The hepatobiliary pathway is the main route for removal of cholesterol from the body, either after conversion to bile acids or in the form of free cholesterol. The mechanism of biliary cholesterol secretion and the regulation of this process are not fully understood. Biliary output of cholesterol and phospholipids appears to be regulated, at least in part, by bile acid secretion. Stimulation of bile acid output, within physiological limits, generally evokes an increase in lipid secretion (1). The coupling between bile acid and lipid output appears to be accomplished at the level of the bile canaliculus (2, 3). A relationship between the hydrophobicity of the different bile acid species and their capacity to induce biliary lipid secretion has been reported (4–6). However, this relationship is highly species-specific, indicating that the physicochemical characteristics of bile acids are not the only determinants of bile acid-induced lipid secretion. Recent data from our laboratory suggest that species-dependent differences in the magnitude of the bile acid-independent fraction of bile flow also play a role (7). In addition, it is well established that cholesterol output can be dissociated from bile acid output by pharmacological and dietary means. Various hydrophilic organic anions inhibit biliary cholesterol and phospholipid secretion in experimental animals without affecting bile acid secretion (2, 3, 8–10) by interacting with canalicular bile acids (2, 3). Feeding of a cholesterol-enriched diet reduces cholesterol output relative to that of bile acids (and phospholipids) in the rat (11). On the other hand, treatment with progesterone (12), acute cessation of lovastatin treatment (13), and diets containing fish oil (14, 15) or plant sterols such as diosgenin (16) lead to a marked hypersecretion of cholesterol in this species. These latter observations indicate that, in addition to the "driving force" of bile acids, factors exist that

Abbreviations: FO, fish oil; CO, corn oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TCDC, taurochenodeoxycholic acid; BDT, bilirubin ditaurate; VLDL, very low density lipoprotein; LDL, low density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase.

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coregulate the amount of cholesterol secreted into the bile.

We have recently compared the effects on hepatic cholesterol metabolism of a diet supplemented (9% wt/wt) with fish oil (FO), enriched in the highly unsaturated n-3 fatty acids eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), with those of corn oil (CO, n-6 unsaturated fatty acids) in rats (14). We found that FO feeding reduced plasma cholesterol and triglyceride levels by 38% and 69%, respectively, presumably due to the well-established inhibitory effects of FO on hepatic VLDL production (17). The plasma lipid-lowering effects of FO were associated with an increase in bile acid pool size by 28%; a 4-fold increase in the cholic acid/chenodeoxycholic acid ratio in bile; a preferential use of endocytosed cholesterol for bile acid synthesis; and an increase in biliary cholesterol secretion by 51%.

To gain further insight in the mechanism(s) involved in FO-induced hypersecretion of cholesterol, we have now studied the relationship between bile acid and cholesterol secretion in detail in FO- and CO-fed rats with chronic bile diversion (18). We have recently published a detailed account of the effects of prolonged interruption of the enterohepatic circulation on hepatic cholesterol and bile acid metabolism and biliary lipid secretion in rats (19). This experimental set-up allowed us to study cholesterol secretion in a longitudinal fashion under the conditions of low, stable bile acid output (derived from de novo synthesis only) in the absence of bile acid flux through the liver. Using this model, we investigated the effects of the experimental diets on plasma lipids and biliary lipid composition, on the day-night variation in biliary cholesterol output and on the capacity of taurochenodeoxycholic acid (TCDC) and bilirubin ditaurate (BDT) to stimulate and to inhibit, respectively, biliary cholesterol output.

METHODS

Materials

Taurochenodeoxycholic acid (TCDC) and [24-^{14}C]TCDC were purchased from Calbiochem (La Jolla, CA) and CBN (La Jolla, CA), respectively. Bilirubin ditaurate (BDT) was from Porphyrin Products (Logan, UT). Fatty acid standards were obtained from Sigma (St. Louis, MO). All reagents used were of analytical grade.

Animals

Male Wistar rats (280-320 g) bred at the Central Animal Laboratory, University of Groningen, were used throughout. The rats were housed individually in plexiglass cages in a light- (light on from 6 AM to 6 PM) and temperature (20°C)-controlled room, and maintained on commercial rat pellets (RMHB, Hope Farms, Woerden, The Netherlands). The rats were fitted with permanent silastic catheters in the bile duct and heart as described (18). Animals were allowed to recover from surgery for 7 days before experiments were started. Bile was led outside the cage by means of polyethylene tubing with a swivel joint (20) allowing free movement and undisturbed food intake. During this 7-day period, hepatic cholesterol and bile acid metabolism stabilized at a new steady state (18, 19). Experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Groningen.

Experimental procedures

Rats were switched to their experimental diets, which were provided ad libitum in powdered form, 9 days after surgery (day 0). The composition of the diets is given in **Table 1:** the type of fat (9% wt/wt), i.e., CO (Knorr Caterplan GmbH, Heilbronn, FRG) or FO (HydroMartens, Bergen, Norway), was the only variable.

From day −2 until day 14, bile was continuously collected in 24-h samples (noon-noon) and the amount of bile produced was determined by weight. Blood samples

TABLE 1. Fat composition of the experimental diets

Composition	Corn Oil	Fish Oil
Ingredients (g)		
Constant components[a]	90	90
Corn oil	10	1
Fish oil		9
Cholesterol	0.08	0.07
Chemical analysis		
Cholesterol (g/100 g)	0.08	0.08
Crude fat (g/100 g)	10.1	10.1
Fatty acids (g/100 g fatty acid)		
C14:0	nd[b]	6.2
C16:0	10.3	17.7
C16:1	nd	7.5
C18:0	1.9	3.0
C18:1	29.5	15.0
C18:2	55.8	7.6
C18:4(n-3)	nd	2.1
C20:1(n-9)	0.3	2.1
C20:3(n-3)	nd	1.0
C20:5(n-3)	nd	13.0
C22:1(n-9)	nd	2.2
C22:5(n-3)	nd	2.1
C22:6(n-3)	nd	8.4
Total		
Saturated	12.2	26.9
Monounsaturated	29.5	24.7
Polyunsaturated (n-6)	55.8	7.6
Polyunsaturated (n-3)		26.6

[a] Constant components (g) consisted of: casein (17), starch (21), dextrose (19), molasses (11), cellulose (16), dicalcium phosphate (0.6), calcium carbonate (0.7), magnesium carbonate (0.07), magnesium oxide (0.03), potassium bicarbonate (1.9), sodium chloride (0.5), vitamin premix (1.2), and mineral premix (1.0).

[b] Not detectable.

were collected via the heart catheter at indicated time intervals, transferred to heparinized test tubes, and centrifuged to obtain plasma. From noon on day 14, bile was collected at timed intervals, as indicated, for a period of 12 h to include the day–night rhythm. At noon of days 15 and 16, rats from both dietary groups received an intravenous bolus injection of either [^{14}C]TCDC (15 μmol/kg) or BDT (30 μmol/kg) in a random order. In these experiments, bile was collected in 15-min samples for a 2-h period.

Analyses

Biliary bile acid concentration was measured by an enzymatic fluorimetric assay. Bile acid composition in selected samples was determined by capillary gas chromatography as described elsewhere (21). Cholesterol and phospholipids in bile were measured after lipid extraction (22), according to the methods of Gamble et al. (23) and Böttcher et al. (24), respectively. Fatty acid composition of biliary phospholipids was determined in selected bile samples by capillary gas chromatography after transmethylation as described by Lepage and Roy (25), using C17:0 as an internal standard. Individual fatty acids were identified by comparison of their retention times with those of authentic standards. Plasma and hepatic cholesterol (ester) and plasma triglyceride concentrations were measured enzymatically using commercially available kits (Boehringer, Mannheim, FRG).

Calculations and statistics

Values are presented as means ± SD. Significance of differences between dietary groups was assessed by Student's *t*-test, at a $P < 0.05$ level of significance.

RESULTS

Lipids in plasma and liver

Plasma cholesterol concentrations decreased during the initial 8 days in rats fed FO by approximately 50% and stabilized thereafter. CO feeding did not result in lowering of plasma cholesterol levels (**Fig. 1A**). Plasma triglyceride concentrations also dropped to low levels during FO feeding and remained relatively constant during CO feeding (Fig. 1B).

Livers of the animals were harvested at day 17 of the experimental dietary regimes. No significant differences between FO- and CO-fed rats in liver weight (10.8 ± 1.3 vs. 10.7 ± 0.4 g) or between the hepatic concentrations of cholesterol (30.4 ± 0.9 vs. 31.3 ± 2.9 μmol/g protein) and cholesteryl ester (0.9 ± 0.5 vs. 1.1 ± 0.7 μmol/g protein) were found.

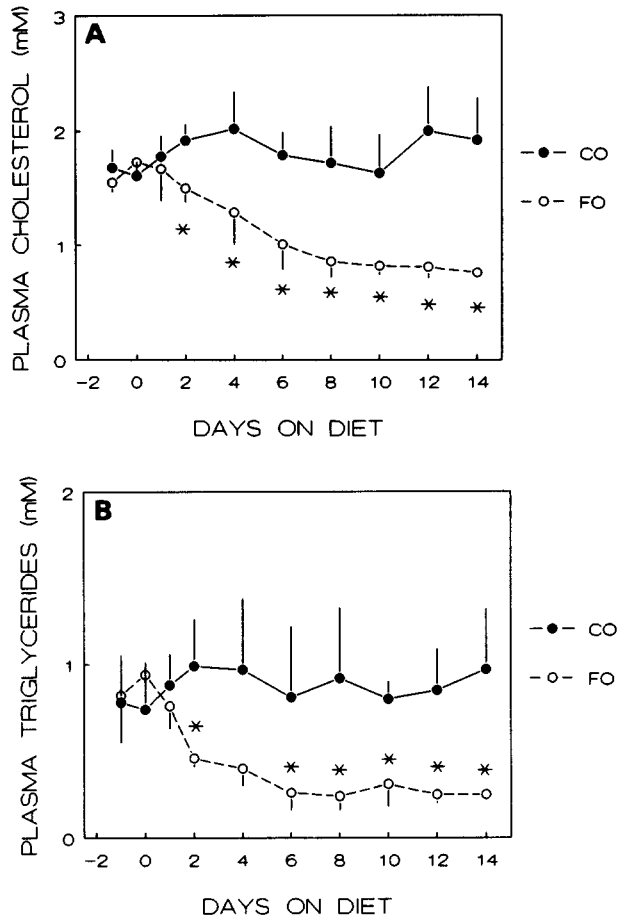

Fig. 1. Plasma concentrations of cholesterol (A) and triglycerides (B) in rats with chronic bile diversion fed purified diets enriched (9% wt/wt) with fish oil (FO) (open symbols) or corn oil (CO) (closed symbols) during a 14-day period. Blood samples were collected from the unanesthetized rats via a permanent heart catheter (18). Mean values ± SD (n = 5 in both groups) are shown; * indicates significant difference between groups.

Bile formation and bile acid synthesis

FO feeding resulted in a stimulation of bile flow by 20% when compared to the control period; no effect of CO on the amount of bile produced was observed (not shown). The stimulation of bile flow induced by FO was mainly due to an increase in the so-called bile acid-independent fraction of bile flow.

FO feeding caused a gradual increase in mean daily bile acid production, i.e., from 44.9 ± 4.1 μmol/kg per h during the control period to 51.2 ± 4.2 μmol/kg per h at day 14 of the experimental diet. Corresponding values for the CO group were 44.8 ± 4.1 and 39.5 ± 1.0 μmol/kg per h ($P < 0.05$ when compared to FO), respectively. FO resulted in similar qualitative changes in hepatic bile acid synthesis as reported previously in rats with intact enterohepatic circulation (14); chenodeoxycholic acid synthesis

was decreased relative to that of cholic acid, resulting in an increase in the ratio between cholic acid and chenodeoxycholic acid from 2.1 ± 0.4 (control period) to 4.3 ± 0.7 at day 14 ($P < 0.05$), as determined in 24-h samples. This ratio was not affected by CO feeding; 2.4 ± 0.4 (control) and 2.4 ± 0.5 (day 14). A clear day–night variation in bile acid synthesis existed in both groups. After 14 days on the experimental diets, bile acid synthesis increased from 35.0 ± 4.7 and 27.9 ± 6.7 μmol/kg per h at noon in FO- and CO-fed rats, respectively, to 75.3 ± 4.8 and 60.2 ± 5.1, respectively, at midnight.

Biliary lipid secretion

FO feeding resulted in a continuous increase in biliary cholesterol secretion (**Fig. 2A**). Cholesterol output increased from a mean daily value of 0.9 μmol/kg per h in the control period to 6.5 μmol/kg per h after 14 days on the experimental diet. CO feeding resulted in a small increase in cholesterol disposition into bile, i.e., from 0.8 to 1.3 μmol/kg per h; thus, cholesterol output was increased by 400% in rats fed FO compared to those fed CO after 14 days on the experimental diets. Bile acid and phospholipid output were stimulated to a much smaller extent than that of cholesterol by the FO diet, i.e., by 30% and 118%, respectively, at day 14 when compared to CO. As a consequence, the molar ratios between cholesterol and bile acids (Fig. 2B) and cholesterol and phospholipids (not shown) in bile became significantly elevated in FO-fed rats. The disproportional increase in biliary cholesterol secretion relative to that of bile acids and phospholipids resulted in the formation of supersaturated bile in FO rats. After 14 days on the diet, the mean cholesterol saturation index, calculated according to Carey (26), was 1.91 in FO-fed rats as compared to 0.87 in the CO-fed animals. During the control period in which normal rat chow was given, mean saturation indices of 0.58 and 0.54 were found for the FO and the CO group, respectively.

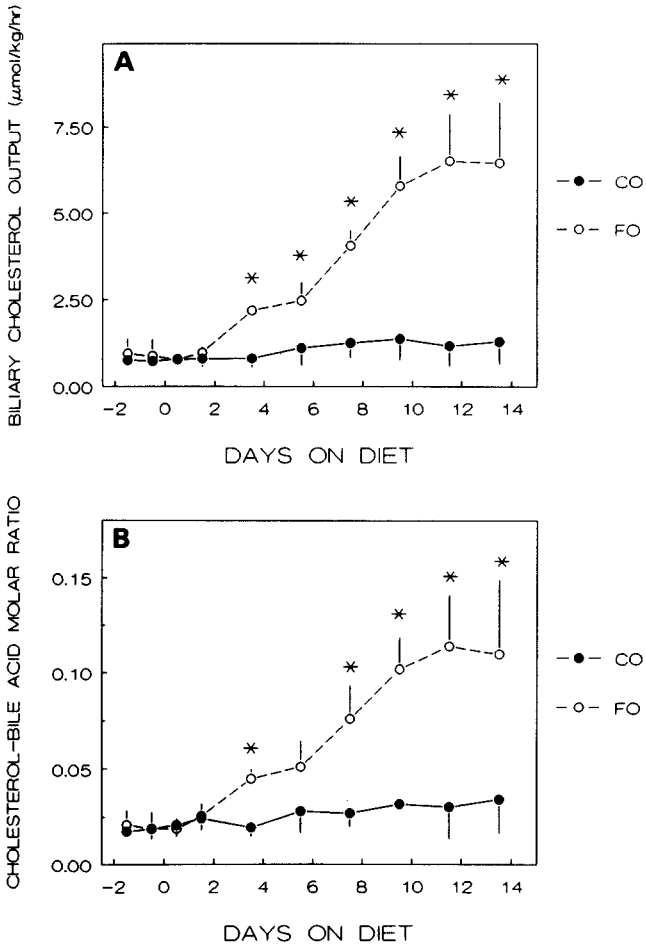

Fig. 2. Average daily cholesterol output in bile (A) and the cholesterol/bile acid molar ratio in bile (B) in rats with chronic bile diversion fed purified diets enriched with fish oil (FO) (open circles) or corn oil (CO) (closed circles) during a 14-day period. Mean values ± SD (n = 5 in both groups) are shown; * indicates significant difference between groups.

Cholesterol output increased during the night in both groups (**Fig. 3A**). In CO-fed rats the molar ratio between cholesterol and bile acids remained virtually constant from noon to midnight (Fig. 3B). In contrast, in FO-fed rats cholesterol output increased to a substantially larger extent than bile acid output, leading to an 1.57-fold increase in cholesterol/bile acid molar ratio at midnight as compared to noon.

The fatty acid composition of biliary phospholipids, recently implicated as an important determinant of the "cholesterol-holding" capacity of bile (e.g., ref. 27), was differentially affected by the dietary regimes (**Table 2**). The most prominent changes observed were the relative enrichment of biliary phospholipids with the FO-specific fatty acids (C20:5, C22:6) and the relative reduction in C20:4-containing species in the FO group.

Coupling between cholesterol and bile acid output

To study the coupling between cholesterol and bile acid output, rats of both groups were injected intravenously with taurochenodeoxycholic acid (TCDC, 15 μmol/kg) or bilirubin ditaurate (BDT, 30 μmol/kg), an inhibitor of bile acid-induced lipid secretion (3). **Fig. 4** shows that TCDC induced similar relative changes in bile acid (upper panels) and cholesterol (lower panels) output in both groups, in spite of the fact that basal cholesterol output was 2.6 times higher in FO- than in CO-fed rats. Thus, a similar increase in bile acid output resulted in secretion of about 2.6 times as much cholesterol in the FO group. Consequently, no differences in the relative values of the cholesterol/bile acid molar ratio were observed between the groups (not shown). Recovery of radioactivity in bile was essentially complete within 1 h after injection; no attempts were made to analyze the bile for radiolabeled metabolites of TCDC. BDT inhibited cholesterol output by about 50% in both groups without altering bile acid

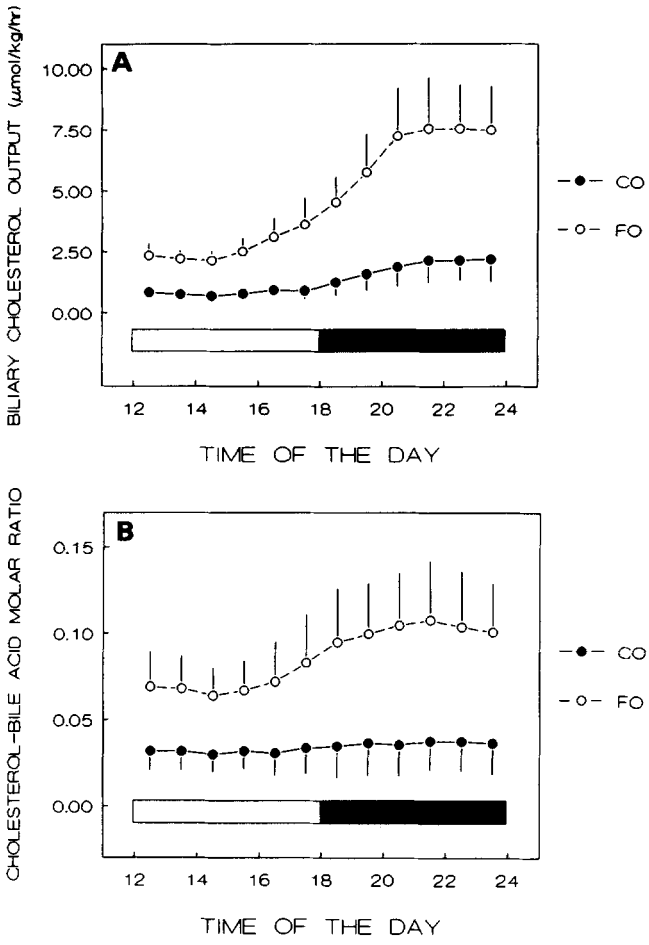

Fig. 3. Cholesterol output into bile (A) and the cholesterol/bile acid molar ratio in bile (B) from noon to midnight in rats with chronic bile diversion at the 14th day of feeding purified diets enriched with fish oil (FO) (open symbols) or corn oil (CO) (closed symbols). Mean values ± SD (n = 4 in both groups) are shown.

output, i.e., it resulted in a much stronger reduction of cholesterol output in absolute terms in FO-fed rats. The relative changes in cholesterol output appeared slightly more pronounced in CO- than in FO-fed rats (**Fig. 5**, lower panels), but the differences did not reach statistical difference. The biliary secretion profile of BDT was similar in both groups, with peak secretion (about 3 μmol/15 min) occurring in the second 15-min interval after injection.

DISCUSSION

In the present study we used rats with chronic bile diversion to study the effects of FO feeding on the hepatic processing of cholesterol and the relationship between biliary cholesterol and bile acid secretion. Long-term bile diversion in rats leads to a 6- to 8-fold increase in the hepatic activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and cholesterol 7α-hydroxylase (19), the rate-limiting enzymes in biosynthesis of cholesterol and bile acids, respectively. Unlike the situation in most other species, however, this situation does not lead to a persistent reduction of plasma cholesterol levels and an altered hepatic LDL-receptor activity in rats (18, 19). Yet, the cholesterol-lowering effect of FO under these experimental conditions was more pronounced than previously observed in rats with an intact enterohepatic circulation (14). It may be that bile diversion potentiates the reported stimulatory effects of FO on hepatic LDL receptor activity (28). It is also possible that the influence of FO on VLDL assembly and/or secretion (see ref. 17) and/or the inhibitory effect of FO on incorporation of cholesteryl esters into VLDL particles (29) is more effective in hepatocytes of bile-depleted rats. Thus, although the mechanism remains unclear, it is evident that the FO-induced changes in plasma cholesterol are influenced by the status of the enterohepatic circulation.

The relationship between biliary cholesterol and bile acid output in rats can, in general, be described by a hyperbolic function (1), i.e., cholesterol output is relatively high at low bile acid output. The biliary cholesterol/bile acid molar ratio is 2 times higher in bile-depleted rats than in animals with intact enterohepatic circulation (19), which may, in part, be related to the marked difference in bile acid output rate. Feeding FO to bile-depleted rats resulted in an almost continuous increase of this ratio during the experimental period whereas CO had only a relatively small effect. The extremely high cholesterol saturation index at day 14 in FO rats indicated the formation of bile supersaturated with cholesterol. The occurrence of supersaturated bile is quite uncommon in rats under any experimental condition and was not observed in our previous study in which rats with intact entero-

TABLE 2. Phospholipid secretion and fatty acid composition of biliary phospholipids in rats with chronic bile diversion fed purified diets enriched with fish oil or corn oil for 14 days (24-h bile collections)

	Corn Oil	Fish Oil
	μmol/kg/h	
Phospholipid secretion	6.8 ± 1.14	14.8 ± 0.80[a]
Fatty acid	%	
Palmitic, 16:0	36 ± 2.3	34 ± 1.5
Palmitoleic, 16:1	1 ± 0.4	4 ± 0.1[a]
Stearic, 18:0	7 ± 0.5	7 ± 0.7
Oleic, 18:1	4 ± 1.0	7 ± 0.5[a]
Linoleic, 18:2	28 ± 1.5	23 ± 1.3[a]
Arachidonic, 20:4	17 ± 1.2	7 ± 0.5[a]
Eicosapentaenoic, 20:5	nd[b]	9 ± 1.1[a]
Docosahexaenoic, 22:6	1 ± 0.1	3 ± 0.4[a]
Unidentified	5 ± 3.8	6 ± 2.2

Number of animals = 5 in both groups.
[a]Indicates significant difference between groups.
[b]Not detectable.

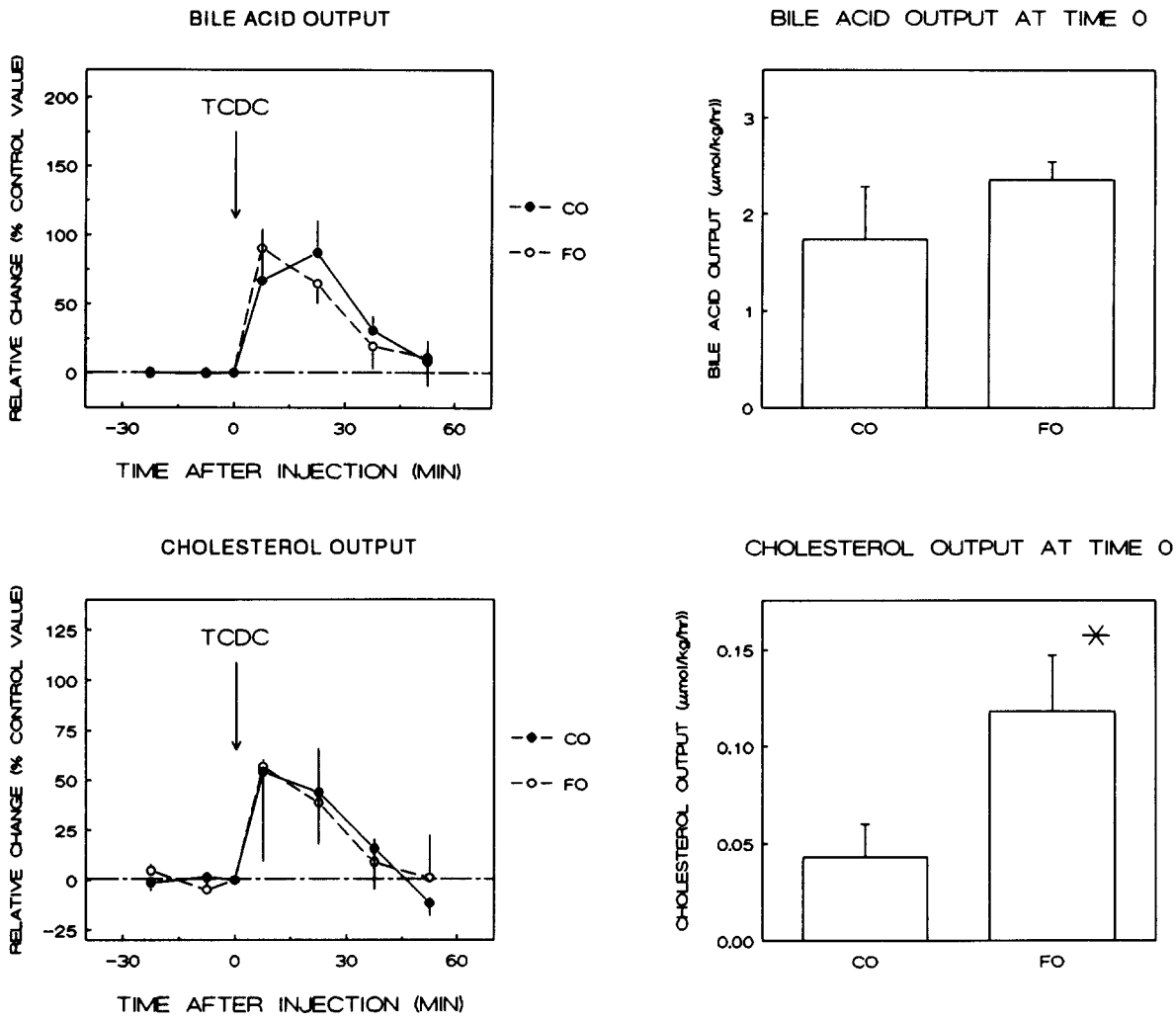

Fig. 4. Relative changes (circles) and basal values (bars) of bile acid output (upper panels) and cholesterol output (lower panels) in bile, after administration of radiolabeled taurochenodeoxycholic acid (15 μmol/kg) to rats with chronic bile diversion after more than 2 weeks of feeding semisynthetic diets enriched with fish oil (FO) (open symbols) or corn oil (CO) (closed symbols). Mean values ± SD (n = 4 in both groups) are shown; * indicates significant difference between the groups.

hepatic circulation received exactly the same diet (14). Apparently, the combination of bile diversion and FO feeding leads to a situation in which the hypersecretion-inducing effect of FO is enhanced (see below). The experiments with TCDC and BDT surprisingly demonstrated that under the condition of FO-induced hypersecretion, biliary cholesterol secretion is still regulated by bile acid secretion. Thus, both stimulation of biliary cholesterol secretion by TCDC and its inhibition by BDT occurred in a proportional fashion in both dietary groups. Consequently, the absolute changes in cholesterol output were much larger in the FO group than in the CO group. Apparently, FO feeding potentiates the efficacy of bile acids to induce secretion of cholesterol.

How does dietary FO exert this potentiating effect on bile acid-induced cholesterol secretion? We recently provided evidence that the quantitative regulation of bile acid-induced lipid secretion takes place at the level of the bile canaliculus (2, 3). Factors involved in this regulation are *1)* biliary bile acid concentration; *2)* biliary bile acid composition; *3)* biliary concentration of hydrophilic organic anions; and *4)* the magnitude of the bile acid-independent fraction of bile flow. In the present study, bile acid concentration was similar in both groups, and bile acid composition in FO rats had changed into a more hydrophilic direction, i.e., a composition expected to induce less lipid secretion (4–6, 30). The absence of potential endogenous "uncouplers", i.e., organic anions able to inhibit biliary lipid secretion (factor 3) in FO bile was not evaluated; yet, all uncouplers studied thus far affect phospholipid secretion more strongly than that of cholesterol [see ref. 2 and references therein]. The magnitude of the so-called bile acid-independent fraction of bile flow, which is inversely related to the capacity of bile acids to induce

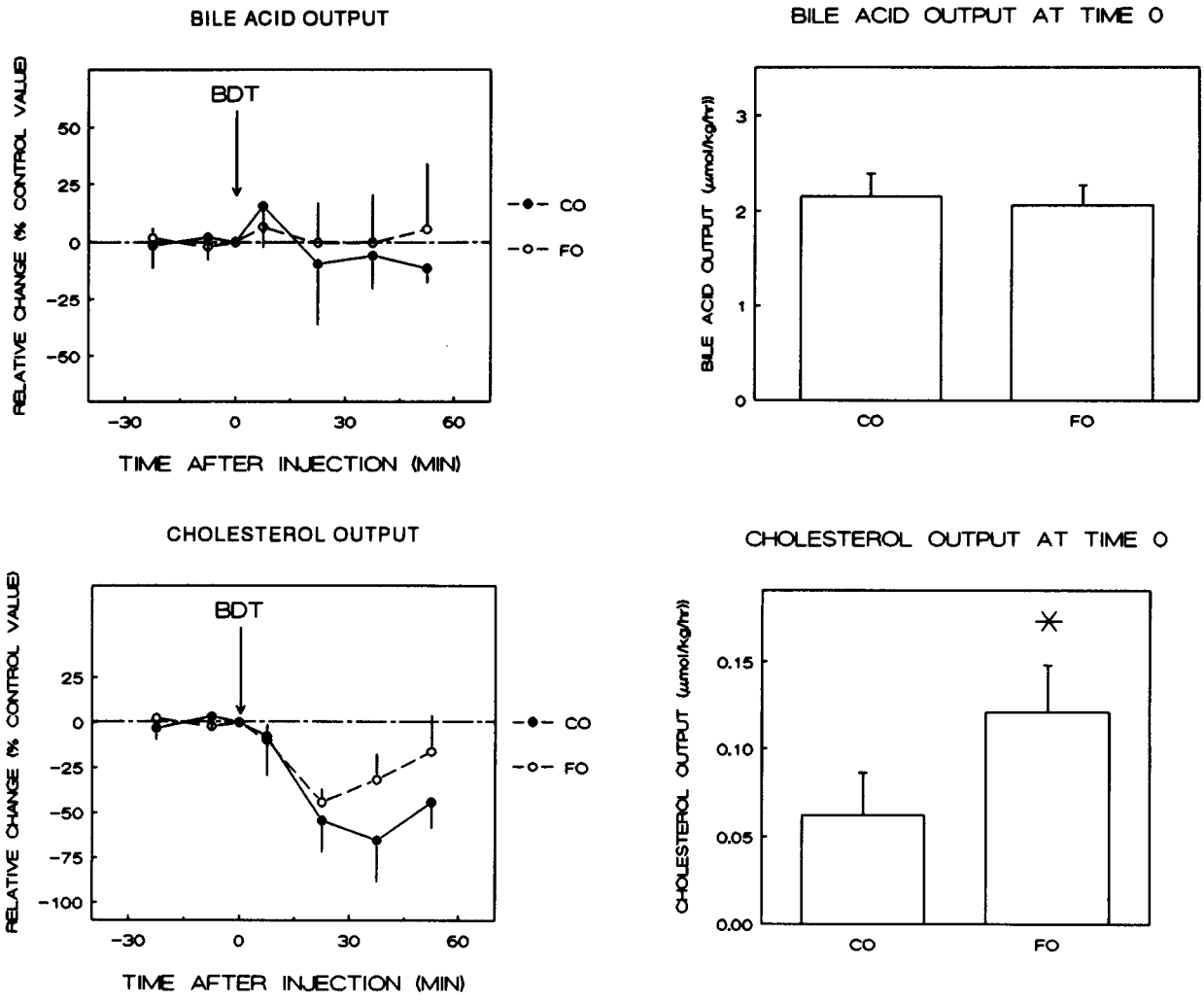

Fig. 5. Relative changes (circles) and basal values (bars) of bile acid output (upper panels) and cholesterol output (lower panels) in bile, after administration of bilirubin ditaurate (30 μmol/kg) to rats with chronic bile diversion after more than 2 weeks of feeding diets enriched with fish oil (FO) (open symbols) or corn oil (CO) (closed symbols). Mean values ± SD (n = 4 in both groups) are shown; * indicates significant difference between the groups.

lipid secretion (7), was increased in rats fed FO, i.e., a situation in which less lipid secretion should occur. It thus seems that the four factors mentioned do not play a decisive role. A factor also implied in the regulation of biliary cholesterol secretion is the composition of biliary phospholipid acyl chains, which, in turn, can be influenced by the secretion of different bile acid species (31, 32). The changes in fatty acid composition of FO bile relative to that in CO bile, i.e, enrichment with eicosapentaenoic (20:5) and docosahexaenoic (22:6) acid-containing phospholipids and depletion in linoleic (18:2) and arachidonic (20:4) acid-containing species (probably reflecting changes at the *sn*-2 position), were similar to those recently described by Booker, Scott, and LaMorte (33) in prairie dogs, by Scobey et al. (34) in African green monkeys, and by Berr et al. (35) in humans. Interestingly, both in monkeys (34) and in humans (35) dietary FO reduced the cholesterol saturation index in bile, when compared to lard and the habitual diet, respectively. By linear path analysis, Berr, Schreiber, and Frick (36) found a positive correlation between the relative amount of cholesterol in human gallbladder bile and the contribution of oleic and arachidonic acids to biliary phospholipids, whereas a negative relationship with linoleic and palmitoleic acids was found. However, eicosapentaenoic and docosahexaneoic acids were not included in this analysis. Furthermore, in view of the differential effects of FO on cholesterol saturation index mentioned previously, it is not clear whether these relationships also apply to the situation in rats. Finally, from a mechanistic point of view it seems unlikely that the relatively small changes in acyl chain composition would be responsible for the observed large effect on biliary cholesterol secretion.

The answer may be related to alterations in intra-

hepatic transport of cholesterol induced by FO, leading to a facilitated recruitment of bile-destined cholesterol by bile acids. It is known that experimental procedures associated with impaired secretion of VLDL-cholesterol (ester) in rats, such as dietary FO and, as shown very recently, a bean diet (37) and orotic acid treatment (38), result in increased disposition of cholesterol into bile. The opposite, i.e., increased cholesterol secretion into plasma and reduced secretion into the bile, has, for instance, been reported to result from administration of 25-hydroxycholesterol to rats (38). These findings suggest a functional coupling between the pools of cholesterol destined for secretion into bile or blood or, alternatively, the presence of a single pool for cholesterol transport out of the cell (37–39). The observation that the changes induced by FO in plasma cholesterol and bile cholesterol (compare Figs. 1A and 2A) showed a similar time-dependency is suggestive in this respect. It has been speculated that the combined activities of HMG-CoA reductase (input) and ACAT (output) may regulate the size of this pool (38). As FO and CO do not differentially affect the activities of these enzymes in our hands (14), although inhibition of ACAT activity by FO has also been described (29), it appears that the FO-induced impairment of VLDL-cholesterol secretion per se increases the availability of bile-destined cholesterol, which is subsequently secreted in a bile acid-dependent fashion via a process that is regulated at the level of the canaliculus (2, 3). In our previous study (14), we found small, non-significant increases in the amount of cholesterol (and phospholipids) relative to protein in total hepatic plasma membranes. As the canalicular membrane, i.e., the site where lipid secretion occurs, comprises only a small fraction of the total membrane, it may be that significant changes in this domain were present; studies on this subject are in progress in our laboratory.

Why then is the FO-induced hypersecretion of cholesterol so much more pronounced in rats with an interrupted enterohepatic circulation than in intact rats? This may be related to the changes in cholesterol synthesis and esterification that are induced by long-term bile diversion. It is well established that HMG-CoA reductase activity increases after interruption of the enterohepatic circulation; in our experimental set-up we found a 6-fold increase after 8 days of bile diversion under normal feeding conditions (19). ACAT activity, on the other hand, decreased by 30% during bile diversion (19) and this effect is probably responsible for the extremely low amounts of hepatic cholesteryl ester in both groups found in the present study. A role of ACAT activity in the regulation of biliary cholesterol secretion in the rat has previously been suggested by Nervi and coworkers (39, 40). The role of cholesterol synthesis in this process is controversial. Turley and Dietschy (41) found no relation between cholesterol synthesis and biliary cholesterol secretion in rats when synthesis was varied over a wide range by manipulations such as administration of bile acid-binding resins and infusion of lipoprotein-cholesterol. In contrast, in a very recent study Robins et al. (42) found, by comparison of cholesterol synthesis and output in rats of different ages, a close relationship between synthesis and output at middark, i.e., when synthesis is high. The observation that cholesterol secretion relative to that of bile acids in FO-fed rats was even more increased during the night, confirming the observations of Robins et al. (42), appears to be in line with this possibility, but may, on the other hand, also be related to the ingestion of (FO-enriched) food during this period. Circumstantial support for a role of HMG-CoA reductase (and ACAT) in the regulation of biliary cholesterol secretion in the rat can be derived from a recent study from our laboratory, showing reduced bile acid-dependent cholesterol secretion in rats fed a cholesterol-enriched diet for 2 weeks (11). Under these dietary conditions, cholesterol synthesis is strongly decreased while esterification is increased. Furthermore, reduced biliary cholesterol secretion in humans treated with HMG-CoA reductase inhibitors (43, 44) may be explained by the putative role of synthesis in regulation of the secretion process. Thus, increased cholesterol secretion under the conditions used in the present study, i.e., FO feeding combined with bile diversion, may be due to an increased availability of bile-destined cholesterol by the combination of decreased VLDL-cholesterol secretion, decreased ACAT activity, and increased cholesterol synthesis. The important point is, however, that under these conditions cholesterol secretion still is governed by bile acid secretion or, in other words, that dietary FO apparently potentiates the efficacy of bile acids to induce secretion of cholesterol. In view of the reported differential effects of EPA and DHA on hepatic lipid metabolism (45) it remains to be established whether EPA or DHA alone or the combination of both are responsible for the effects of FO on biliary cholesterol secretion.

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