Increased Biliary Calcium in Cholesterol and Pigment Gallstone Disease: The Role of Altered Bile Acid Composition

Mohammad Z. Abedin[a], Seth D. Strichartz[a,b], Sarkis Festekdjian[a] and Joel J. Roslyn[a,b,*]
[a]Research and Surgical Services, Sepulveda Veteran Administration Medical Center, Sepulveda CA and the [b]Department of Surgery, UCLA School of Medicine, Los Angeles, CA

The present study was undertaken to define the relationship between calcium metabolism and bile acid composition in animal models of diet induced cholesterol and pigment gallstones. Groups of prairie dogs were fed either a control non-lithogenic chow (N = 12), a 1.2% cholesterol enriched chow (N = 6, XOL) for two weeks, or a high carbohydrate diet deficient in iron (N = 6, CHO-FeD), or a high carbohydrate diet with normal iron levels (N = 6, CHO) for eight weeks. Hepatic (HB) and gallbladder (GB) bile samples were analyzed for total calcium, cholesterol, phospholipids, total bile acids (TBA), and individual bile acid composition.

In each of the four groups, TBA concentrations were essentially similar and taurine conjugates accounted for approximately 90% of TBA in HB bile and about 98% in GB bile. In the control group, cholic acid (CA) was the predominant bile acid and comprised 76% of TBA and chenodeoxycholic (CDCA) accounted for about 13% of the total. Feeding a diet rich in cholesterol caused a significant change in the relative concentrations of individual bile acids of hepatic bile—such that CA decreased significantly ($p < 0.001$) while CDCA increased by 300% ($p < 0.001$). The changes in secondary bile acids were insignificant. An identical shift in individual bile acid composition was noted in animals maintained on high carbohydrate diet, irrespective of iron content. Similar changes were observed in the GB in the experimental groups.

Calcium concentrations of GB bile with or without gallstone formation showed a positive linear relationship with TBA ($y = 4.35 + 0.14X$, $p < 0.001$) and taurochenodoxycholic acid (TCDCA) ($y = 15.04 + 0.46X$, $p < 0.001$), but an inverse relationship with taurocholic acid (TCA) ($Y = 55.16 - 0.41X$, $p < 0.008$). However, such relationships were absent in hepatic bile. These data indicate that diet-induced alterations in bile acid composition may modify calcium solubility or GB function, thereby contributing to the increased GB calcium observed during cholesterol and pigment gallstone formation.
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It has long been recognized that biliary calcium is an important factor in the pathogenesis of pigment gallstones (1,2). Calcium is also present as a salt in the central matrix of some, if not all, cholesterol gallstones (3,4). Recent studies suggest that both pigment (5) and cholesterol gallstone disease (6,7) are characterized by increased biliary levels of calcium. Nonetheless, the factors which are responsible for these changes in calcium metabolism remain obscure. *In vitro* studies by Moore *et al.* (8) indicate that bile acids act as important buffers for free calcium in bile and may be important determinants of calcium solubility. In addition, bile acid composition influences biliary secretion of calcium (9). In an effort to further define the relationship between bile acids and calcium metabolism during gallstone formation, the present study was undertaken to determine the bile acid composition in animal models of diet induced cholesterol and pigment gallstones. The prairie dog develops cholesterol gallstones when fed a synthetic diet containing cholesterol, has biliary lipid secretory kinetics similar to that in human subjects, and provides a model in which the discrete events in the evolution of cholesterol gallstones may be studied (10-12). Additionally, recent studies from our laboratory indicate that prairie dogs maintained on a high carbohydrate, iron deficient diet, have increased biliary concentrations of calcium, phospholipids, and cholesterol, and develop pigment gallstones (13).

MATERIAL AND METHODS

Experimental design. Adult male prairie dogs *(Cynomys ludovicianus),* trapped in the wild state and obtained from Otto Marten Locke, New Braunfels, TX, were caged in thermo-regulated (23°C) rooms with 12 hour light cycles. Four groups of animals were maintained on either a control non-lithogenic chow (N = 12, Purina Laboratory Chow®, Ralston Purina, St. Louis, MO), a 1.2% cholesterol enriched diet (N = 6, Teklad Test Diets®, Harlan Sprague-Dawley, Inc., Madison, W) for two weeks, or a high carbohydrate diet deficient in iron (N = 6, Teklad), or a high carbohydrate diet with normal iron levels (N = 6, Teklad) for eight weeks. The precise composition of the four diets is summarized in Table 1. The 1.2% cholesterol enriched diet has been previously shown to induce cholesterol crystals and gallstones within ten and fourteen days, respectively (14). Prairie dogs maintained on the high carbohydrate, iron deficient diet for eight weeks developed calcium bilirubinate crystals and microscopic stones (13). Iron supplementation has been shown to significantly reduce the incidence of stones in this model (13). All diets contain ample essential fatty acids, minerals, and fiber. All animals tolerated the diets, with maintenance of weight and fur, and had only minimal diarrhea.

After a 16 hour fast with water *ad libitum,* animals were anesthetized with ketamine (100 mg/kg of body weight) and diazepam (0.15 mg/kg of body weight) (15). After a midline laparotomy, the cystic duct was ligated and the gallbladder was carefully removed. The distal common bile duct was ligated, and a 25 cm long silastic catheter (i.d. 0.02 in., o.d. 0.032 in.) was placed into the common bile duct. Hourly hepatic bile (HB) samples were collected on ice in tared tubes wrapped with aluminum foil for

*To whom correspondence should be addressed at Division of General Surgery, UCLA School of Medicine, Room #72-215 CHS, Los Angeles, CA 90024-1749.

Abbreviations: CA, cholic acid; CDCA, chenodexycholic acid; CHO, high carbohydrate diet; CHO-FeD, high carbohydrate diet, deficient in iron; CSI, cholesterol saturation index; GB, gallbladder bile; HB, hepatic bile; HPLC, high performance liquid chromatography; i.d., inner diameter; TBA, total bile acids; TCA, taurocholic acid; TCDCA, taurochenodexycholic acid; XOL, cholesterol-enriched diet.

TABLE 1

Composition of Diets

	Control (%)	XOL (%)	CHO (%)	CHO-FeD (%)
Carbohydrate	51	58	63	63
Corn	37	13	0	0
Wheat	13	0	0	0
Sucrose	1	45	35	35
Rice	0	0	28	28
Protein	23	20	18	18
Fat	5	9	8	8
Fiber	5	3	1	1
Mineral mix[a]	—	4	3.5	3.5
Vitamin mix[a]	—	1	0.5	0.5
Water	7	2.8	5	5
Misc. (ASH, etc.)	9	1	1	1
Cholesterol	Trace	1.2	Trace	Trace
Iron (ppm)	198	195	220	56

XOL = 1.2% cholesterol enriched chow; CHO = carbohydrate, normal iron diet; CHO-FeD = carbohydrate, iron deficient.

[a]AIN-76 mineral and vitamin mix (32).

protection against light and saved for further analysis. Gallbladders were opened and their mucosal surfaces carefully inspected (using a dissecting microscope) for the presence of sludge or stones. Gallbladder (GB) and HB bile were aliquoted and then stored at −20°C until analyzed for calcium, cholesterol, phospholipids, bile acids, and individual bile acid composition.

Analytic methods. Frozen bile was warmed to room temperature in a water bath at 37°C and biochemical determinations were performed on the entire volume of the aliquot. Biliary total calcium was measured by spectrophotometric methods as described by Anderegg *et al.* (16), and as modified by Connerty and Briggs (17). Unpublished data from our laboratory has shown that this analysis is not significantly altered by the bilirubin level. Bile cholesterol was assayed enzymatically by the method of Röschlau *et al.* (18). Biliary phospholipids were quantified by measuring lipid phosphorus as described by Dryer and co-workers (19). Total bile acids were determined spectrophotometrically using the steroid dehydrogenase method of Iwata (20). The cholesterol saturation index (CSI) was calculated using Carey's critical tables for cholesterol saturation, based on the total lipid concentration (21). Individual bile acids were analyzed by a modification of the high performance liquid chromatographic (HPLC) method described by Nakayama *et al.* (22). The changes made in methodology were necessary to adapt to our system. All changes were validated with authentic standards. Modifications improved resolution and accuracy of the originally described methodology. The HPLC system consisted of a Perkin-Elmer 400 series solvent delivery pump with a fixed loop Rheodyne Model 7125-S bypass injector, a LC-95 high sensitivity variable wave length UV-VIS detector and a LCI-100 laboratory computing integrator (Perkin-Elmer Corporation, Irvine, CA). The analytical column used was a 10-micron μBondapak® C18, 30 cm × 3.9 mm i.d. (Waters Associates, Milford, MA). To prepare bile samples for chromatographic analysis, an aliquot of 50 μl of the bile was mixed with 50 μl of internal standard (testosterone acetate in ethanol, 2 mg/ml). The mixture was extracted with nine times its volume of ethanol, boiled in a water bath for 10 minutes, cooled to room temperature, and then filtered through a 0.45 μm Alpha Metricel® membrane filter (Gelman Sciences, Inc., Ann Arbor, MI). An aliquot of 5–10 μl of the filtrate was then injected into the column through the sampling valve and chromatographed with a solvent system of methanol acetonitrile and water (40:30:30, v/v/v), acidified to pH 3.4 with phosphoric acid. The flow rate was 1 ml/min and detection was made at 210 nm. Individual bile acids were identified by comparison of retention times with those of bile acid reference standards (Calbiochem, La Jolla, CA), and quantitated using relative response factors determined by known amounts of bile salt standards and the same amount of internal standard added to the sample. All bile salt standards were obtained from Calbiochem. They were in the form of sodium salts of either taurine or glycine conjugates of respective bile acids, and their purity was better than 97%. The internal standard (testosterone acetate) was purchased from Sigma Chemicals and its purity was better than 99%.

Statistical analysis. All data are presented as the mean ± standard deviation. Fisher's exact test was used to compare the incidences of stones. Statistical comparisons between the dietary groups were made using analysis of variance and the Student's t-test for unpaired variables.

RESULTS

Gallstone formation. As expected, no control animals had either crystals or gallstones. All cholesterol-fed animals had cholesterol crystals, and five of the six had typical yellow cholesterol gallstones ($p < 0.002$ vs. controls). Of the six animals maintained on a high carbohydrate, normal iron diet (CHO), only one had calcium bilirubinate crystals. In the high carbohydrate iron deficient group (CHO-FeD), three of six animals had both stones ($p < 0.04$ vs. controls) and crystals, and one animal had crystals alone. The microscopic appearances of the crystals in carbohydrate-fed animals were quite different from cholesterol gallstone animals and were brown-yellowish, comparable to calcium bilirubinate crystals previously reported by other investigators.

Hepatic bile composition. The data summarizing the effects of cholesterol and carbohydrate feeding on HB composition are listed in Table 2. The HB concentrations of total bile acids (TBA) and calcium were similar in all four experimental groups. However, animals fed either the cholesterol or the high carbohydrate diets, demonstrated a significant increase in HB phospholipid concentration ($p < 0.001$ vs. control). In addition, there was a significant increase in cholesterol concentration as compared to control, in both cholesterol fed (XOL) ($p < 0.001$) and iron deficient carbohydrate-fed (CHO-FeD) animals ($p < 0.005$). Prairie dogs maintained on the high carbohydrate (CHO), normal iron diet had similar cholesterol concentrations to controls. The cholesterol-fed animals had cholesterol saturated bile as reflected by a CSI of 1.14 ± 0.35, in contrast to the control (0.52 ± 0.22), CHO (0.38 ± 0.09) and CHO-FeD (0.64 ± 0.20) animals.

The effects of cholesterol and carbohydrate feeding on individual bile acid composition are summarized in

TABLE 2

The Effects of Cholesterol and Carbohydrate Feeding on Hepatic Bile Composition in Prairie Dogs[a]

Dietary group	Total bile acids (μM/ml)	Phospholipids (μM/ml)	Cholesterol (μM/ml)	Total lipids (g/dl)	CSI	Total C_a^{++} (mg/dl)
Control	38.4 ± 12.1	4.9 ± 2.0	0.7 ± 0.2	2.3 ± 0.6	0.52 ± 0.22	9.3 ± 1.6
XOL	31.8 ± 14.5	8.9 ± 3.5[c]	2.2 ± 1.0[c]	2.6 ± 0.9	1.14 ± 0.35[c]	9.4 ± 1.6
CHO	39.3 ± 10.4	8.8 ± 2.3[c]	0.8 ± 0.2[d]	2.4 ± 0.5	0.38 ± 0.09[e]	10.4 ± 1.8
CHO-FeD	35.3 ± 19.6	9.1 ± 2.6[c]	1.5 ± 0.8[b]	2.5 ± 1.2	0.64 ± 0.20	10.1 ± 1.6

[a] A portion of this data has been previously published in Reference 13.

[b] $p < 0.005$, [c] $p < 0.001$, vs. corresponding control.

[d] $p < 0.005$, [e] $p < 0.001$, vs. corresponding XOL.

XOL = 1.2% cholesterol-enriched diet; CHO = high-carbohydrate, normal iron diet; CHO-FeD = high-carbohydrate, iron deficient diet.

TABLE 3

Effect of Diets on Bile Acid Composition of Hepatic Bile

	Biliary bile acids (% TBA)										
	Taurine conjugates						Glycine conjugates				
Diet	UDCA	CA	CDCA	DCA	LCA	Total	CA	CDCA	DCA	LCA	Total
Control	1.2 ± 1.1	75.0 ± 3.5	11.3 ± 2.6	3.5 ± 2.7	1.0 ± 0.5	92.0 ± 2.1	0.8 ± 0.4	1.7 ± 1.0	2.4 ± 0.7	3.1 ± 1.3	8.0 ± 2.1
XOL	1.1 ± 0.5	52.1 ± 1.9[b]	32.8 ± 3.2[b]	6.6 ± 3.4	0.6 ± 0.3	93.2 ± 2.7	0.9 ± 0.5	1.4 ± 0.9	1.1 ± 0.9	3.4 ± 2.8	6.8 ± 2.7
CHO	0.8 ± 0.3	51.7 ± 7.3[b]	34.2 ± 6.7[b]	3.4 ± 2.8	1.1 ± 0.3	91.2 ± 2.3	0.8 ± 0.4	2.3 ± 1.3	1.1 ± 0.9	4.6 ± 0.8	8.8 ± 2.3
CHO-FeD	0.6 ± 0.3	54.6 ± 11.5[b]	34.5 ± 9.9[b]	2.2 ± 1.1	1.2 ± 0.4	93.1 ± 2.9	1.1 ± 0.5	2.2 ± 1.1	1.1 ± 0.3[a]	3.5 ± 1.2	6.9 ± 2.4

[a] $p < 0.005$, [b] $p < 0.001$ vs. corresponding controls.

UDCA = Ursodeoxycholic acid; CA = cholic acid; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; and LCA = lithocholic acid.

XOL = 1.2% cholesterol-enriched diet; CHO = high-carbohydrate, normal iron diet; CHO-FeD = high-carbohydrate, iron-deficient diet.

TABLE 4

Effect of Diets on Individual Bile Acids of Gallbladder Bile

	Biliary bile acids (% TBA)										
	Taurine conjugates						Glycine conjugates				
Diet	UDCA	CA	CDCA	DCA	LCA	Total	CA	CDCA	DCA	LCA	Total
Control	1.3 ± 0.7	77.4 ± 2.9	15.4 ± 4.4	2.2 ± 1.7	0.5 ± 0.2	96.8 ± 1.5	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.4	1.7 ± 0.9	3.2 − 1.5
XOL	0.8 ± 0.5	62.4 ± 8.4[d]	32.7 ± 10.2[d]	1.7 ± 1.1	0.6 ± 0.3	98.0 ± 1.0[a]	0.2 ± 0.1[b]	0.5 ± 0.2	0.2 ± 0.1	0.9 ± 0.5	1.8 ± 0.9[c]
CHO	2.8 ± 1.7	57.7 ± 5.6[d]	35.8 ± 6.8[d]	1.1 ± 0.9	0.2 ± 0.1	97.6 ± 1.5	0.2 ± 0.1[b]	0.3 ± 0.2	0.3 ± 0.2	1.6 ± 1.2	2.4 ± 1.5
CHO-FeD	1.8 ± 1.1	65.1 ± 12.0[c]	30.7 ± 11.7[d]	0.8 ± 0.6	0.2 ± 0.1	98.6 ± 1.6[a]	0.1 ± 0.1[b]	0.2 ± 0.1	0.3 ± 0.3	0.8 ± 0.3	1.4 ± 0.5[b]

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.005$, [d] $p < 0.001$ vs. corresponding control.

UDCA = Ursodeoxycholic acid; CA = cholic acid; CDCA = chenodeoxycholic acid; DCA = deoxycholic acid; and LCA = lithocholic acid.

XOL = 1.2% cholesterol-enriched diet; CHO = high-carbohydrate, normal iron diet; CHO-FeD = high-carbohydrate, iron-deficient diet.

Table 3. In each of the four groups, taurine conjugates accounted for approximately 90% of the TBA. In the control group, cholic acid (CA) was the predominant bile acid and comprised 76% of the TBA. The other primary bile acid, chenodeoxycholic acid (CDCA), accounted for approximately 13% of the total. The secondary bile acids, deoxycholic and lithocholic acid, accounted for 5% and 4%, respectively. Cholesterol feeding caused a significant alteration in the relative concentration of the individual bile acids such that CA decreased significantly ($p < 0.001$), while CDCA increased by 300% ($p < 0.001$). The changes in the secondary bile acids were insignificant. There was an identical shift in individual bile acid composition noted in animals maintained on the high carbohydrate diet, irrespective of iron content. Once again, CA decreased significantly ($p < 0.001$) while there was a three-fold increase in CDCA.

Gallbladder bile composition. The effects of diet on GB composition are displayed in Figure 1. Cholesterol feeding resulted in a significant increase in TBA concentration ($p < 0.02$), phospholipids, cholesterol, and total calcium ($p < 0.001$) as compared to controls. Animals maintained on the high carbohydrate diets also had significantly increased GB concentrations of phospholipids and calcium. However, biliary cholesterol was significantly elevated only in the CHO-FeD animals. The CSI was significantly higher ($p < 0.001$) in cholesterol-fed animals compared to controls and the carbohydrate animals. The values were similar for controls and CHO-FeD animals, but significantly lower in CHO animals ($P < 0.01$ vs. controls).

Data summarizing the effects of the diet on GB concentrations of individual bile acids are listed in Table 4. Taurine conjugates accounted for 95 to 98% of the total bile acids in each of the four groups. As was noted in HB, CA was the predominant bile acid in control animals, with CDCA comprising 15% of the total. Once again, feeding either cholesterol or high carbohydrate diets caused significant alterations in the relative concentrations of bile acids. All three of the experimental groups had a significant decrease in the percentage of CA, which was associated with a concomitant doubling of CDCA.

Biliary calcium and bile acids. The relationships between the concentrations of biliary calcium and bile acids in both hepatic and gallbladder bile were analyzed irrespective of stone formation. There was no significant correlation noted between HB concentrations of calcium and either total or individual bile acids. Data summarizing the relationships between GB concentrations of calcium and bile acids are displayed in Figure 2. There was a linear correlation ($y = 4.35 + 0.14x$, $p < 0.001$) when the gallbladder concentration of TBA was compared to calcium. The finding can be explained on the basis of the observed correlation ($y = 15.04 + 0.46x$, $p < 0.001$) between GB calcium concentration and the amount of taurochenodeoxycholic acid (TCDCA), expressed as a percentage of the total bile acids. In contrast, there was an inverse linear relationship ($Y = 55.16 - 0.41X$, $p < 0.008$) when taurocholic acid (TCA) was measured instead of TCDCA. Although the presence of linearity between concentrations of bile and calcium is noteworthy, a cause and effect relationship has not yet been established.

DISCUSSION

Data from this study indicates that in the prairie dog, consumption of diets rich in cholesterol or carbohydrates results in a significant alteration in the hepatic metabolism and secretion of individual bile acids. These changes occur despite the apparent absence of any significant change in the TBA concentration. Prairie dogs

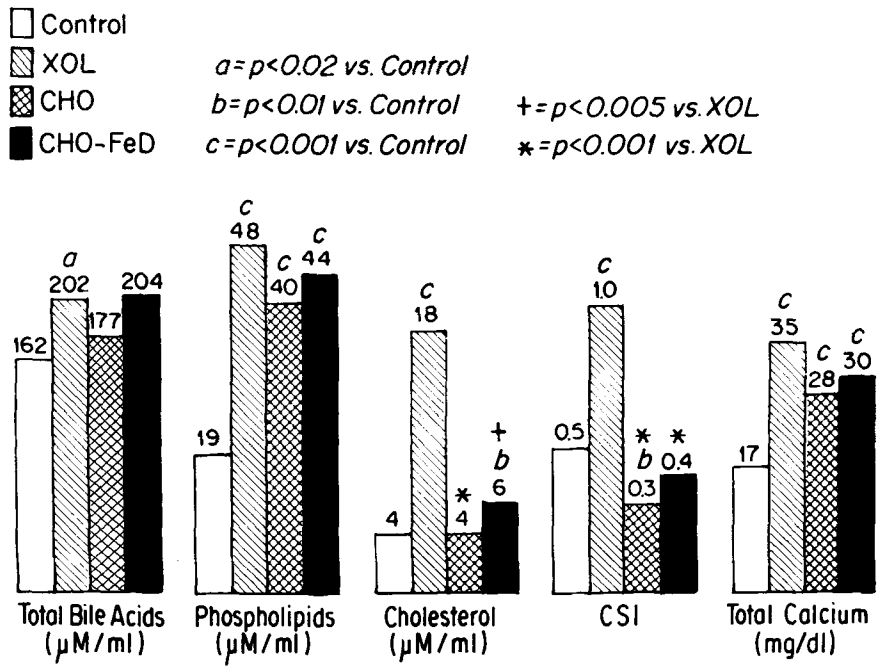

FIG. 1. The effects of diet on gallbladder bile composition. XOL = 1.2% cholesterol enriched diet; CHO = high carbohydrate, normal iron diet; CHO-FeD = high carbohydrate, iron-deficient diet. A portion of this data has been previously published in Reference 13.

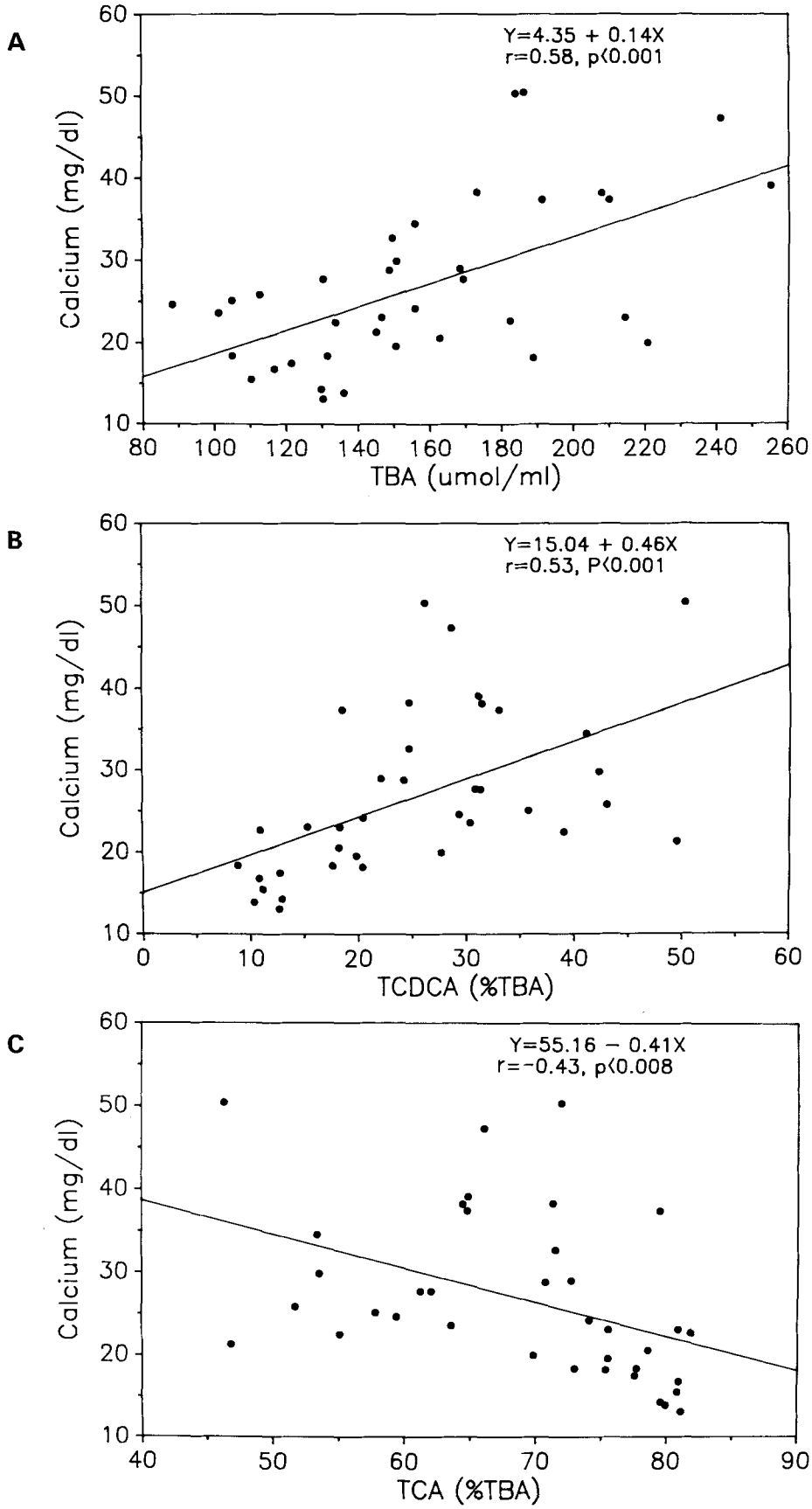

FIG. 2. The linear relationships between gallbladder bile total calcium and biliary bile acid concentrations. (A) Total bile acids (TBA), correlation coefficient (r) is 0.58, $p < 0.001$; (B) taurochenodeoxycholic acid (TCDCA), $r = 0.53$, $p < 0.001$; (C) taurocholic acid (TCA), $r = -0.53$, $p < 0.008$.

maintained on XOL, which is known to induce cholesterol gallstones in this model (10-12), were noted to have a significant shift from TA to TCDCA. Similar changes were noted in animals maintained on the CHO-FeD diet. This latter diet has recently been shown to induce calcium bilirubinate sludge and microscopic pigment gallstones in the prairie dog model (13,23). Therefore, our findings indicate that both cholesterol and pigment gallstone formation are characterized by comparable changes in biliary lipid metabolism and individual bile acid concentrations.

Although the data were scattered, our results demonstrate a significant correlation between the changes in bile acids induced during gallstone formation, and increases in GB concentrations of calcium. The conspicuous absence of these relationships in HB provides further evidence for the hypothesis that the observed increase in GB calcium is a gallbladder, rather than a hepatic phenomenon (7). Furthermore, it is conceivable that the diet induced changes in bile acid composition predisposed to calcium precipitation either by altering the buffering capacity of bile (8) or by modulating gallbladder absorptive function and ion transport (24,25).

A shift from cholic to chenodeoxycholic acid during cholesterol gallstone formation has been previously reported in the prairie dog model (12,26,27). These changes are noted within five days of initiation of cholesterol feeding (14). Similar changes have been observed in humans with cholesterol gallstones (28). It has been previously suggested that the observed changes in individual bile acids may facilitate the formation of cholesterol gallstones (12). Early reports suggested that micelles containing dihydroxy bile acids are larger and less stable than those containing trihydroxy bile acids (29). The implication is that the predominance of the less stable dihydroxy micelles might provide a milieu in which cholesterol precipitation could be facilitated, thereby initiating the cascade of events ultimately resulting in cholesterol gallstones. This suggestion is particularly interesting given the fact that CDCA has been well documented to be effective in cholesterol gallstone dissolution. The apparent dichotomy in terms of potential effects remains unexplained, but may be related to changes in physical structure as opposed to inhibition of cholesterol synthesis. Recent studies have implicated increases in biliary concentrations of cholesterol in the pathogenesis of mixed or pigment gallstone disease as well (13,23,30). Although bile is not saturated with cholesterol in these models, it has been suggested that biliary concentrations of cholesterol may be critical to the formation of non-cholesterol gallstones.

The factors responsible for the shift from CA to CDCA remain unclear. It is conceivable that the relative activities of 12 α-hydroxylase and 26 hydroxylase may act as regulators of these changes. However, the factors responsible for the preferential initiation of 26 hydroxylase and the associated inhibition of 12 α-hydroxylase are not well defined. However, it is interesting to speculate that cholesterol may play a role in this mechanism. It has previously been suggested that the alterations in bile acid composition represent a compensatory response to cholesterol overloading in the hepatocytes (27). Delivery of an increased amount of cholesterol to the liver may induce a subtle alteration in cholesterol metabolism, favoring the synthesis of CDCA. This, in turn, would tend to limit cholesterol production via the negative feedback of CDCA on HMG-CoA reductase, the rate limiting enzyme for cholesterol synthesis (31). The potential interrelationship between cholesterol metabolism and bile acid composition would appear to warrant further investigation. The finding that changes in bile composition were noted despite the presence of normal biliary cholesterol levels (animals fed the CHO diet) suggest that other factors as well may play an important role in the alteration of bile acid compostion.

Increasing evidence suggests that the precipitation of calcium in bile with either carbonate, bilirubinate, phosphate, or palmitate may be a common factor in the pathogenesis of both cholesterol and pigment gallstones. Theoretically, calcium precipitation is thermodynamically possible when the ion-product of calcium and any one of the above mentioned ions exceeds the solubility product of the calcium salt of corresponding ions. In the current study, gallbladder biliary concentrations of total calcium and phospholipids were increased in animals developing either pigment or cholesterol stones. The observation that hepatic biliary calcium levels are unchanged in these animals suggests that a gallbladder mechanism may be ultimately responsible for the increase in gallbladder calcium, as opposed to alterations in hepatic secretion of calcium. One possible explanation is that the changes in the relative concentrations of individual bile acids may alter the phospholipid-bile acid micelles, resulting in a reduction in calcium solubility in bile. Further studies are clearly needed to elucidate the factors in bile which determine calcium solubility.

REFERENCES

1. Maki, T. (1966) *Ann. Surg. 164,* 90-100.
2. Sutor, D.J., and Wilkie, L.I. (1977) *Clin. Sci. Mol. Med. 53,* 101-103.
3. Been, J.M., Bills, P.M., and Lewis, D. (1979) *Gastroenterology 76,* 548-555.
4. Wosiewitz, U. (1980) *Path. Res. Pract. 167,* 273-286.
5. Muller, E.L., Grace, P.A., and Pitt, H.A. (1986) *J. Surg. Res. 40,* 55-62.
6. Strichartz, S.D., Abedin, M.Z., Abdou, M.S., and Roslyn, J.J. (1987) *Surg. Forum 38,* 167-169.
7. Strichartz, S.D., Abedin, M.Z., Abdou, M.S., and Roslyn, J.J. (1988) *Am. J. Surg. 155,* 131-137.
8. Moore, E.W., Celic, L., and Ostrow, J.D. (1982) *Gastroenterology 83,* 1079-1089.
9. Cummings, S.A., and Hofmann, A.F. (1984) *Gastroenterology 87,* 664-673.
10. Gurll, N., and DenBesten, L. (1978) *Lab. Anim. Sci. 28,* 428-432.
11. Holzbach, R.T. (1984) *Hepatology 4,* 1915-1985.
12. Brenneman, D.E., Conner, W.E., Forker, E.L., and DenBesten, L. (1972) *J. Clin. Invest. 51,* 1495-1503.
13. Roslyn, J.J., Conter, R.L., Julian, E., and Abedin, M.Z. (1987) *Surg. 102,* 327-333.
14. DenBesten, L., Safaie-Shirazi, S., Connor, W.E., and Bell, S. (1974) *Gastroenterology 66,* 1036-1045.
15. Roslyn, J.J., Thompson, J.E., Jr., and DenBesten, L. (1979) *Lab. Anim. Sci. 29,* 542-544.
16. Andregg, C., Flaschka, H., Sallman, R., and Schwarzenback, G.M. (1954) *Helu. Chim. Acta 37,* 113-120.
17. Connerty, H., and Briggs, A. (1966) *Am. J. Clin. Path. 45,* 290-296.
18. Röschlau, P., Bernt, E., and Gruber, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H., ed.) Vol. 4, pp. 1890-1893, Academic Press, New York, NY.
19. Dryer, R.L., Tammes, A.R., and Routh, J.I. (1957) *J. Biol. Chem. 225,* 177-183.

20. Iwata, T., and Yamasaki, K. (1969) *J. Biochem. (Tokyo) 56,* 424-429.
21. Carey, M.C. (1978) *J. Lipid Res. 19,* 945-955.
22. Nakayama, F., and Nakagaki, M. (1980) *J. Chromatogr. 183,* 287-293.
23. Conter, R.L., Roslyn, J.J., Pitt, H.A., and DenBesten, L. (1986) *J. Surg. Res. 40,* 580-587.
24. Roslyn, J.J., Conter, R.L., and DenBesten, L. (1984) *Surg. Forum 35,* 221-223.
25. Abdou, M.S., Strichartz, S.D., Abedin, M.Z., Roslyn, J.J. (1988) *J. Surg. Res. 44,* 672-679.
26. Chang, S.H., and Ho, K.J. (1973) *Arch. Pathol. 96,* 417-426.
27. Gardner, B., Chenouda, M., Dennis, L., and Patti, J. (1978) *Am. J. Surg. 135,* 40-47.
28. Burnett, W. (1965) in *The Biliary System* (Taylor, W., ed.), p. 601, Blackwell Scientific Publications, Oxford, England.
29. Carey, M.C., and Small, D.M. (1970) *Am. J. Med. 49,* 590-608.
30. Strichartz, S.D., Abedin, M.Z., Safarian, E.K., and Roslyn, J.J., *Am. J. Surg.,* in press.
31. Moton, P.N., Ellis, H.J., Higgins, M.J.P., and Dowling, H. (1980) *Eur. J. Clin. Invest. 10,* 325-332.
32. Report of the American Institute of Nutrition Ad Hoc Committee on Standards, for Nutritional Studies (1977) *J. Nutrition 107,* 1340-1348.

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