ORIGINAL ARTICLE

Sex differences in the bile acid composition of human bile: studies in patients with and without gallstones

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Summary: The bile acid composition of human gallbladder bile was studied in 83 subjects, 20 of each sex without discernible hepatobiliary disease, and 20 men and 23 women with cholelithiasis. The bile acids were measured by combined thin-layer and gas-liquid chromatography.

In the bile of patients without cholelithiasis the molar percent of cholic acid was significantly greater in men while that of chenodeoxycholic acid was significantly greater in women.

In the bile of patients with cholelithiasis the concentration of total bile acids was reduced in both sexes but there was no sex difference in the molar percent of any of the bile acids. The molar percent of CDCA (both glycine and taurine conjugates) was reduced in women, while the molar percent of CA (only the glycine conjugate) was reduced in men.

Résumé: *Différences entre les sexes dans la composition de l'acide biliaire chez l'homme: étude de malades porteurs ou non de calculs biliaires*

La composition en acide biliaire de la bile vésiculaire a été étudiée chez 83 sujets: chez 20 sujets de chaque sexe, exempts de pathologie hépatobiliaire discernable, et chez 20 hommes et chez 23 femmes souffrant de cholélithiase. La mesure des sels biliaires a été faite par la chromatographie en couche mince et par la chromatographie en phase gazeuse-liquide.

Dans la bile des malades exempts de lithiase, le pourcentage molaire de l'acide cholique était nettement plus élevé chez l'homme tandis que, chez la femme, c'était l'acide chénodésoxycholique qui prédominait.

Dans la bile des lithiasiques, la concentration des acides biliaires globaux était réduite dans les deux sexes, mais on ne notait aucune différence entre les sexes quant au pourcentage molaire de l'un quelconque des acides biliaires. Le pourcentage molaire d'ACDC (à la fois glyco- et tauro-conjugués) était diminué chez la femme, tandis que le pourcentage molaire d'AC (seulement le glyco-conjugué) était réduit chez l'homme.

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The pathogenesis of cholesterol cholelithiasis remains obscure. On the basis of *in vitro* studies it seemed that the condition was due to the hepatic secretion of bile supersaturated with cholesterol.[1] However, it is now apparent that normal individuals may secrete bile supersaturated with cholesterol[2] and that patients with cholesterol gallstones can secrete bile which is normal on the basis of cholesterol-bile salt-phospholipid coordinates.[3-6] These observations, along with those that have documented the return of lithogenic bile to normal following cholecystectomy,[7] have once again raised the possibility that the primary abnormality in this disease may reside in the gallbladder and not in the liver. Because it has been shown that the bile acid pool is lower in patients with cholesterol cholelithiasis,[8,9] it seemed pertinent to examine the bile acid composition of the gallbladder bile of patients with and without cholesterol gallstones.

Patients

Gallbladder bile was obtained from 83 subjects during the course of abdominal operations. The subjects were patients in five general hospitals in the area of Metropolitan Toronto. All subjects fasted overnight and none had received antibiotics prior to operation.

Forty subjects, 20 men and 20 women, had no discernible disease of the liver or biliary tract. Most were operated on for diseases of the upper gastrointestinal tract and had not undergone "routine liver function studies". Sixteen men and nine women had peptic ulcers; two men and seven women had hiatus hernias. There is no evidence that these differences influenced our results significantly. None of these patients had gallstones detectable by palpation of the gallbladder at operation.

The other 43 subjects, 20 men and 23 women, had cholelithiasis. Their clinical status varied but all were subjected to an elective operative procedure and did not have marked aberration in liver function at the time of operation. Oral cholecystography demonstrated a functioning gallbladder in 16 men and 22 women. All subjects had a tissue diagnosis of cholelithiasis and chronic cholecystitis. Although some patients had more severe disease than others, none had a gangrenous gallbladder and on the basis of the pathology reports there was no sex difference in the degree of cholecystitis found. Stones were available for analysis

from 17 subjects, 11 women and 6 men. Three subjects had black stones but these were not pure pigment stones. None of the 17 stones had a cholesterol content of less than 50% as measured by gas chromatography. According to the pathology reports none of the remaining patients had pure pigment stones.

The ages of the patients ranged from 20 to 75 years. The mean age of the patients without cholelithiasis was 40 years for women and 47 years for men. The mean age of the patients with cholelithiasis was 50 years for women and 52 years for men.

Methods

As much bile as possible was obtained by aspiration of the gallbladder at a time during the operative procedure convenient to the surgeon. The bile was frozen immediately and kept so until analysis. No samples of white bile were analysed. The bile was thawed to room temperature, vortexed vigorously and then centrifuged at 1000 r.p.m. for 5 minutes. The supernatant was vortexed again and then analysed.

Total solids were determined on the basis of dry weight. Cholesterol was measured by the method of Abell *et al*[10] and phospholipid phosphorus by the method of Bartlett.[11] The cholesterol content was subsequently determined by gas chromatography.[12] Although the results obtained were about 5% lower than those obtained by the method of Abell *et al*, the differences were not statistically significant. Quantitative analysis of the bile acid composition of bile and of the conjugation of the bile acids with glycine and taurine was performed according to the thin-layer and gas chromatographic techniques published by this laboratory.[13] Our recovery rates for standard mixtures of bile acids carried through the entire preparative procedure are 90 to 93%. These recoveries have been obtained with mixtures of the glycine and taurine conjugates of cholic, chenodeoxycholic and lithocholic acids. The concentration of each bile acid in an unknown mixture was obtained by calculating its peak area and relating this to its own standard.

Significant differences were determined by Student's t-test with no significant difference, NS, = $P>0.05$.

The following abbreviations have been used for the bile acids referred to in this paper:
CA=cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid);
CDCA=chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid);
DOCA=deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid);
LCA=lithocholic acid (3α-hydroxy-5β-cholanoic acid).
Glycine conjugates are prefaced by the letter G-.
Taurine conjugates are prefaced by the letter T-.

Results

There was no sex difference in the concentration of total solids in the gallbladder bile (GBB) of patients without or with gallstones (Table I). However, female GBB had a greater concentration of total lipids in patients without and with stones. Furthermore, in both sexes there was a

Table I—Composition of gallbladder bile*

	Without gallstones			With gallstones			Without *vs* with gallstones	
	Female†	Male†	P	Female‡	Male†	P	Female	Male
Total solids (mg./ml.)	138.10 ±12.20	116.50 ±11.10	NS	112.80 ±12.50	110.40 ±7.90	NS	NS	NS
Total lipids (μmole/ml.)	200.04 ±16.10	143.90 ±9.91	<0.01	134.89 ±9.59	85.69 ±6.90	<0.001	<0.001	<0.001
% Bile acids	72.40 ±4.99	69.20 ±3.80	NS	67.70 ±4.21	64.10 ±2.88	NS	NS	NS
% Phospholipids	20.50 ±2.12	21.10 ±1.97	NS	24.80 ±1.90	24.60 ±3.55	NS	NS	NS
% Cholesterol	7.10 ±0.93	9.70 ±1.11	NS	7.50 ±0.99	11.30 ±1.62	NS	NS	NS

*Values represent mean ± standard error
† n = 20
‡ n = 23
NS = no significant difference (P > 0.05)

Table II—Bile composition of gallbladder bile*

	Without gallstones			With gallstones			Without *vs* with gallstones	
	Female†	Male†	P	Female‡	Male†	P	Female	Male
Total bile acids (μmole/ml.)	144.77 ±9.99	99.50 ±5.47	<0.001	91.26 ±5.68	54.96 ±2.47	<0.001	<0.001	<0.001
% Cholic	33.44 ±2.75	52.69 ±3.20	<0.001	35.93 ±6.16	27.26 ±5.97	NS	NS	<0.001
% Chenodeoxycholic	46.92 ±4.66	32.98 ±1.98	<0.02	31.32 ±3.80	44.34 ±7.99	NS	<0.02	NS
% Deoxycholic	14.30 ±2.18	9.48 ±1.58	NS	25.72 ±3.89	20.91 ±4.22	NS	<0.05	<0.02
% Lithocholic	1.66 ±0.21	1.47 ±0.16	NS	3.25 ±0.30	3.35 ±0.71	NS	<0.001	<0.002

*Values represent mean ± standard error
† n = 20
‡ n = 23
Two other bile acids tentatively identified as 3α, 7β- and 3β, 12α-dihydroxycholanoic acids made up the balance. P values were not significant for either.
NS = no significant difference (P > 0.05)

highly significant reduction in the concentration of total lipids in the GBB associated with gallstones compared to that in GBB unassociated with gallstones.

Although the concentration of total lipids was greater in the GBB of women, Table I demonstrates that there was no significant sex difference in the molar percent contribution to the total lipids of the bile acids, phospholipids and cholesterol. This was the case for patients without and with cholelithiasis. Furthermore, although the concentration of total lipids was significantly reduced in the lithogenic GBB of both sexes, there was no significant difference in either sex in the molar percent contribution to the total lipids of the bile acids, phospholipids and cholesterol when findings in subjects without and with cholelithiasis were compared.

In patients without cholelithiasis the concentration of total bile acids was significantly greater in the GBB of women (Table II). On a molar basis the percent contribution to the total bile acids of CA was significantly greater in male GBB ($P<0.001$), while that of CDCA was significantly greater in female GBB ($P<0.02$). There was no significant sex difference in the molar percent of DOCA, LCA or the other two bile acids which have been tentatively identified as $3\alpha,7\beta$- and $3\beta,12\alpha$-dihydroxycholanoic acids.

In patients with cholelithiasis the concentration of total bile acids was again significantly greater in the GBB of women. However, there was no significant sex difference in the molar percent of any of the six bile acids.

A feature of the lithogenic GBB of both sexes was a lesser concentration of total bile acids (Table II). In women this reduction in the concentration of total bile acids in lithogenic GBB was associated with a reduction in the molar percent of CDCA but not of CA. In men the reduction in the concentration of total bile acids in lithogenic bile was associated with a reduction in the molar percent of CA but not of CDCA. The molar percent of DOCA and LCA was significantly increased in the lithogenic GBB of both sexes.

Analysis of biliary bile acids as their glycine and taurine conjugates demonstrated that in the GBB of patients without cholelithiasis the molar percent of G-CA and T-CA was greater in men while the molar percent of G-CDCA and T-DOCA was greater in women (Table III). There was no significant sex difference in the molar percent of any bile acid in either the glycine or taurine fractions of the GBB of patients with cholelithiasis.

Table III also presents a comparison of the results obtained in patients without and with gallstones but of the same sex. The only significant difference in female lithogenic GBB was an increase in the molar percent of G-DOCA. The reduction in the molar percent of CDCA in female lithogenic GBB (Table II) involved both glycine- and taurine-conjugated CDCA but neither conjugate in an individually significant way. Male lithogenic GBB featured a significant increase in the molar percent of both glycine- and taurine-conjugated DOCA. The reduction in CA in male lithogenic GBB (Table II) involved a significant reduction of the glycine conjugate but not of the taurine conjugate.

Discussion

There have been numerous studies on the chemical composition of the GBB obtained from human patients with and without cholelithiasis, but none has addressed itself specifically to the question of the possible sex differences involved.[3,14-18] Our studies have documented a significantly higher concentration of total lipids in the GBB of women without and with cholelithiasis.

Analysis of the bile acid composition of the GBB of patients without gallstones revealed that the molar percent contribution of CA was greater in men while that of CDCA was greater in women. There was no sex difference in the molar percent contribution of individual bile acids

Table III—Conjugation of bile acids of gallbladder bile*

	Without gallstones			With gallstones			Without *vs* with gallstones	
	Female†	Male†	P	Female‡	Male†	P	Female	Male
Glyco- bile acids								
Total (μmole/ml.)	101.45 ±6.41	78.97 ±5.31	<0.02	67.04 ±4.96	35.69 ±4.42	<0.001	<0.001	<0.001
% Cholic	30.92 ±2.41	51.70 ±3.38	<0.001	27.30 ±4.31	20.11 ±3.87	NS	NS	<0.001
% Chenodeoxycholic	50.16 ±4.92	33.05 ±2.28	<0.01	37.66 ±5.15	48.39 ±9.72	NS	NS	NS
% Deoxycholic	13.62 ±2.08	10.16 ±1.91	NS	29.91 ±4.28	24.24 ±5.55	NS	<0.01	<0.05
% Others¶	5.29 ±0.77	5.09 ±0.83	NS	5.14 ±0.94	7.26 ±1.00	NS	NS	NS
Tauro- bile acids								
Total (μmole/ml.)	42.89 ±3.47	20.05 ±1.09	<0.001	31.01 ±4.05	19.07 ±3.43	<0.05	<0.05	NS
% Cholic	39.53 ±3.88	56.46 ±3.74	<0.01	49.02 ±10.16	46.30 ±9.23	NS	NS	NS
% Chenodeoxycholic	38.15 ±4.81	31.57 ±2.64	NS	28.28 ±5.45	35.40 ±7.29	NS	NS	NS
% Deoxycholic	16.66 ±2.50	5.94 ±1.00	<0.001	17.61 ±2.93	12.95 ±2.41	NS	NS	<0.02
% Others¶	5.66 ±0.80	6.04 ±0.85	NS	5.09 ±0.77	6.35 ±0.79	NS	NS	NS

*Values represent mean ± standard error
† n = 20
‡ n = 23
¶Lithocholic and two other bile acids tentatively identified as $3\alpha,7\beta$- and $3\beta,12\alpha$-dihydroxycholanoic acids.
NS = no significant difference ($P > 0.05$)

in the GBB of patients with cholelithiasis. Several sex differences in the relative degree of the conjugation of individual bile acids with glycine and taurine were documented in the GBB of patients without gallstones but none in the GBB of patients with gallstones.

When the GBB of patients without and with gallstones, but of the same sex, was compared a highly significant reduction was revealed in the concentration of total lipids and total bile acids in the lithogenic GBB of both sexes. Again, there were significant differences in the bile acid composition of the GBB. On a molar percent basis the lithogenic GBB of women contained less CDCA while that of men contained less CA. The lithogenic GBB of both sexes contained more DOCA and LCA and a keto acid tentatively identified as 7-keto-lithocholic acid.

These studies have not considered the bile of normal individuals. All our subjects were fasted, anesthetized and traumatised, and those who did not have gallstones did have disease of their upper gastrointestinal tracts. However, it appears that CDCA is quantitatively the most important bile acid of the GBB of women and that a reduction in CDCA largely accounts for the reduced bile acids in the GBB of women with gallstones. This is in keeping with the observations of Danzinger *et al*[8] that the pool size of CDCA but not of CA is reduced in women with cholelithiasis. CA is quantitatively the most important bile acid in male GBB and a reduction in cholic acid largely accounts for the reduced bile acids in the GBB of men with gallstones. It is of interest in this regard that Vlahcevic *et al*[9] have reported diminished pools of both CA and CDCA in male patients with cholelithiasis.

On the basis of these studies we are led to believe that there is a characteristic pattern of the bile acids in the GBB of patients with cholelithiasis and that each sex has its own pattern. The cholelithiasis pattern of both sexes features a general reduction in the concentration of total bile acids. But the female pattern features a dramatic reduction in CDCA, both glycine- and taurine-conjugated, while the male pattern features a dramatic reduction in CA and especially G-CA.

It is obvious that further studies will be required in order to determine the biological significance of these observations and to provide an explanation for them. Evaluation of their biological significance may be the easier task because their explanation must await a better understanding of the intestinal metabolism, enterohepatic circulation and hepatic metabolism of bile acids, and of gallbladder function in patients without and with cholelithiasis.

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