Effects of artificial depletion of the bile acid pool in man

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SUMMARY In order to elucidate the relationship between bile acid pool size and cholesterol saturation index of fasting state gall bladder bile, we artificially depleted the bile acid pool in 12 healthy volunteers. Bile acid pool size decreased from 7.6 ± 0.9 to 5.8 ± 0.7 mmol (mean $\pm$ SEM, $p < 0.01$), and saturation index of fasting state gall bladder bile increased from 0.93 ± 0.07 to 1.18 ± 0.07 ($p < 0.001$). There was no alteration in saturation index of basal or stimulated hepatic bile. There was no change in gall bladder storage of basal hepatic bile, nor in the proportion of the bile acid pool stored in the gall bladder. The bile acid mass in the gall bladder fell from 4.9 ± 0.5 to 3.4 ± 0.4 mmol ($p < 0.05$) and phospholipid mass from 1.6 ± 0.3 to 1.2 ± 0.2 mmol ($p < 0.05$), but there was no change in cholesterol mass. The gall bladder volume fell from 30 ± 4 to 18 ± 2 ml ($p < 0.01$). These results suggest that artificial depletion of the bile acid pool increased saturation index of fasting state gall bladder bile without altering saturation index of basal or stimulated hepatic bile; it probably increased the ratio of basal: stimulated hepatic bile within the gall bladder by decreasing gall bladder storage of stimulated hepatic bile.

Fasting state gall bladder bile of cholesterol gall stone patients differs from that of normal controls by being more saturated with cholesterol.¹ Excess cholesterol appears to be the cause of this in obese gall stone patients, because biliary cholesterol secretion is increased in obesity.² Bile acid deficiency is the probable cause in non-obese gall stone patients, as bile acid pool size is reduced.³ There is an association between a low bile acid pool size and an increased cholesterol saturation index of fasting gall bladder bile,^{3,4} but the nature of this relationship has not been clearly defined. It has been widely assumed that the connecting link is a reduced hepatic bile acid secretion rate, and results using the constant infusion method of measuring biliary lipid secretion have supported this assumption.⁴ On the other hand, duodenal perfusion studies using the intermittent feeding method, in which the gall bladder is able to contract in response to individual meals, suggest that in subjects with a small bile acid pool this pool recycles more frequently, so that bile acid secretion rate is unchanged.^{5,6} If this latter concept is correct, it implies that the gall bladder

may play a role in the pathogenesis of cholesterol gall stone disease by increasing recycling frequency, and appropriate abnormalities of gall bladder motor function have been reported in gall stone patients.^{7,8} A key question is – can gall bladder storage functions alone explain the association between a reduced bile acid pool size and an increased cholesterol saturation index of fasting gall bladder bile without the need to invoke differences in secretion rate?

On the basis of experiments carried out in the baboon, McSherry and colleagues⁹ have pointed out that gall bladder bile comprises a combination of basal and stimulated hepatic bile. Because basal hepatic bile is supersaturated with cholesterol, and stimulated hepatic bile is unsaturated, the net saturation index of gall bladder bile will depend on the relative ratio of basal hepatic bile:stimulated hepatic bile. We speculate that the quantity of stimulated hepatic bile entering the gall bladder after the last meal of the day is likely to depend on the bile acid pool size, so that a reduction in pool size should cause a reduction in the quantity of stimulated hepatic bile in the gall bladder. The gall bladder could respond to this in one of two ways. If gall bladder filling was kept constant so that the quantity of basal hepatic bile entering the gall

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bladder during overnight fasting was unchanged, the net effect of a reduction in bile acid pool size would be to decrease the ratio of stimulated hepatic bile:basal hepatic bile within the gall bladder, and thus to increase saturation index of fasting gall bladder bile and reduce gall bladder volume (Fig. 1b). If gall bladder volume were kept constant, by increasing the volume of basal hepatic bile entering gall bladder so as to compensate for the reduced quantity of stimulated hepatic bile, an even greater effect on saturation index would be obtained (Fig. 1c). In the absence of an alteration in bile acid secretion rate, cholesterol saturation index of basal hepatic bile and stimulated hepatic bile should be unaltered for both models.

Fig. 1 Hypothetical models of alternative effects of bile acid pool depletion. A represents the relative proportion of basal hepatic bile (BHB; stippled) and of stimulated hepatic bile (SHB; clear) within the gallbladder prior to depletion. In (b) and (c), the amount of SHB entering the gall bladder is reduced due to the smaller bile acid pool size. In (b) gall bladder filling and therefore the amount of BHB entering the gall bladder are unchanged, so that gall bladder volume is reduced. In (c), gall bladder volume is unchanged, SHB is reduced as in (b), and BHB is increased because of a compensatory increase in fasting state gall bladder filling. In (b) and (c), saturation index of BHB and SHB is unchanged, but the ratio BHB:SHB is increased relative to (a). As BHB is supersaturated with cholesterol and SHB is unsaturated, net cholesterol saturation index is increased in both (b) and (c) relative to (a).

The aim of the present study has been to answer the following questions: does artificial depletion of the bile acid pool in man cause an increase in cholesterol saturation index of fasting gall bladder bile? If so, what is the mechanism? In order to answer these questions we have studied the effect of artificial depletion of the bile acid pool on cholesterol saturation index and on gall bladder storage functions in 12 healthy volunteers.

Methods

SUBJECTS

Twelve healthy non-obese male volunteers were studied, aged (mean $\pm$ SEM) 39 $\pm$ 4 years (range 21–60 years), weight 72 $\pm$ 4 kg (range 62–100 kg), height 171 $\pm$ 2 cm (range 160–183 cm) and % ideal body weight 99 $\pm$ 3% (range 86–120%). They were all seen in a gastroenterology outpatient clinic complaining of abdominal discomfort, but no evidence of organic disease was found on thorough gastrointestinal investigation, including upper gastrointestinal endoscopy and either ultrasound examination or an oral cholecystogram for exclusion of gall stones. All subjects gave written informed consent for these studies, which were approved by the local hospital ethical committee (April 1980).

Clinical procedures were carried out on two successive days after a 12 hour overnight fast. On day 1 the subjects had an intravenous injection of 1 mCi of ^{99m}Tc HIDA and of 5 μCi carboxyl- ^{14}C -chenodeoxycholic acid (Radiochemical Centre, Amersham, Bucks). The gall bladder volume was then determined using ultrasound (Unirad, EDP; 1000 static scanner) and 90 minutes after the initial Tc HIDA injection an abdominal cholescintigram was carried out using a double headed rectilinear scanner with area of interest facility to determine Tc HIDA radioactivity over the gall bladder and gut areas. We have previously validated this as an index of gall bladder filling.¹¹ A double lumen polyvinyl nasoduodenal tube was then passed and directed under radiograph screening to the duodenal-jejunal flexure. Gall bladder samples were collected after gall bladder contraction was stimulated by a slow intravenous infusion of 100 Ivy Dog Units of cholecystokinin over 30 minutes (Pancreozymin, Boots Ltd.). A sample of basal hepatic bile was taken before the start of gall bladder contraction. The whole sample of gall bladder bile, followed by five samples of stimulated hepatic bile collected at half hour intervals after the cessation of gall bladder contraction (as determined by disappearance of Tc HIDA counts), were retained in order to deplete the bile acid pool. Tc HIDA radioactivity was counted in one gall bladder bile sample in the same way as

for the subjects' gall bladder *in vivo*. This sample was used later to determine biliary lipid composition. From this determination and from the radioactivity measurement, it was possible to calculate biliary lipid mass in the gall bladder. All samples were retained for further laboratory analysis. On day 2, the same procedure was carried out, except that (1) 5 μCi of carboxyl- ^{14}C cholic acid was used instead of ^{14}C -chenodeoxycholic acid and (2) only one 5 ml sample of gall bladder bile was retained together with the basal hepatic bile and stimulated hepatic bile samples. The radiation exposure to the whole body and to the critical organ was 1.4 mrad and 180 mrad respectively for each of the ^{14}C -bile acids, and was 40 mrad and 1430 mrad respectively for $^{99\text{m}}\text{Tc}$ HIDA.

LABORATORY METHODS

Biliary lipid composition was determined using enzymatic analysis of the bile samples for determination of bile acid¹² and cholesterol¹³ and the perchlorate method for phospholipid determination.¹⁴ Saturation index was determined according to the criteria of Hegardt and Dam¹⁵ and of Holzbach *et al*¹⁶ using the polynomial equation of Thomas and Hofmann.¹⁷

For determination of the bile acid pool size, the bile samples were extracted with ethanol ammonia 1% solution and subjected to thin layer chromatography to separate the glycine and taurine conjugates of chenodeoxycholic and cholic acid (using chloroform:ethanol:glacial acetic acid:water: 12:8:4:1 v/v as solvent system). ^{14}C radioactivity for chenodeoxycholic acid or cholic acid was determined by liquid scintillation counting, and mass by enzymatic assay in order to obtain the specific activity. This 'one shot' method for measuring bile acid pool size has been shown by Pomare and Low-Beer¹⁸ to correlate well with the conventional Lindstedt method.¹⁹

Calculation:

$$(1) \text{ Gallbladder filling (\%)} = \frac{\text{GBc} \times 100}{\text{GBc} + \text{gutc}}$$

Where GBc is the Tc HIDA radioactivity over the gall bladder area, and gutc is the Tc HIDA radioactivity over the gut area.

(2) Biliary lipid mass in the gall bladder was determined using the following formula:

$$\frac{\text{GBm}}{\text{GBc}} = \frac{\text{Sm}}{\text{Sc}}$$

Where GBc is the Tc HIDA radioactivity over the

gall bladder area and GBm is the mass of bile acid phospholipid or cholesterol in the gall bladder. Sm is the mass of bile acid, phospholipid or cholesterol in sample, and Sc is the Tc HIDA radioactivity in the sample.

(3) Bile acid concentration in the gall bladder was obtained by dividing bile acid mass in the gall bladder by gall bladder volume.

We have previously described more fully and validated the above measurements of gall bladder filling, biliary lipid mass in the gall bladder and bile acid concentration.¹¹

In the present study there was a good agreement between day 1 and day 2 for gall bladder filling (coefficient of variation 12%) and for gall bladder emptying (coefficient of variation 9%).

STATISTICAL ANALYSIS

All results are expressed as comparisons of the mean $\pm$ standard error of the mean (SEM). Student's paired *t* test for significance was calculated where appropriate.

Results

EFFECT ON POOL SIZE AND BILE ACID COMPOSITION (Table 1)

Artificial depletion of the bile acid pool was obtained by removal of 2.8 ± 0.4 mmol of bile acid (mean $\pm$ SEM; $n=5$). The measured bile acid pool size fell from 7.6 ± 0.9 mmol on day 1 to 5.8 ± 0.7 mmol on day 2 ($p < 0.001$; $n=11$). This reduction was manifest mainly as a reduction in the mass of the secondary bile acid dexoycholic acid from 1.7 ± 0.4 mmol to 0.9 ± 0.3 mmol ($p < 0.005$). There was a smaller reduction in the mass of the primary bile acids, cholic acid falling from 2.7 ± 0.4 mmol to 2.3 ± 0.3 mmol (NS) and chenodeoxycholic acid from 3.1 ± 0.2 mmol to 2.6 ± 0.3 mmol ($p < 0.05$).

EFFECT ON CHOLESTEROL SATURATION INDEX (Table 2 and Fig. 2)

Artificial depletion of the bile acid pool resulted in an increase in saturation index of fasting gall bladder bile from 0.93 ± 0.07 on day 1 to 1.18 ± 0.07 on day 2 ($p < 0.005$). There was no change in saturation index of basal hepatic bile collected in the fasting state before initiation of gall bladder contraction (SI of 1.46 ± 0.15 and of 1.44 ± 0.11 respectively). After cessation of gall bladder contraction (indicated by change in colour of bile aspirated from dark green to yellow, confirmed by disappearance of Tc HIDA radioactivity) five samples of stimulated hepatic bile were collected (Fig. 2) at half-hour intervals. There was no significant difference between day 1 and day 2 at any time interval. A steady plateau level was

Table 1 Pool sizes for total and individual bile acids (mmol) before and after depletion procedure.

Subjects	Total bile acids		Chenodeoxycholic acid		Cholic acid		Deoxycholic acid	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	8.1	5.2	3.4	2.7	3.1	2.2	1.6	0.5
2	11.8	9.2	3.7	3.7	2.7	2.9	5.4	2.6
3	10.0	8.9	3.3	2.8	3.8	3.4	2.9	2.7
4	6.5	5.6	3.7	2.3	1.4	2.3	1.4	1.0
5	7.5	5.4	4.0	3.3	2.1	1.5	1.4	0.6
6	9.0	8.2	3.8	3.5	3.6	3.6	1.6	1.1
7	4.1	3.0	2.1	1.6	1.4	1.2	0.6	0.2
8	4.7	3.5	1.9	1.4	1.3	1.4	1.5	0.7
9	7.6	4.5	3.1	2.0	3.2	2.1	1.3	0.4
10	3.9	2.1	1.8	1.2	1.3	0.6	0.8	0.3
11	9.9	8.0	3.2	3.8	5.9	4.0	0.8	0.2
Mean±SEM	7.6±0.9	5.8±0.7	3.1±0.2	2.6±0.3	2.7±0.4	2.3±0.3	1.8±0.4	0.9±0.3
% change		24%		16%		15%		50%
significance		p<0.001		p<0.05		NS		p<0.001

Table 2 Cholesterol saturation index of fasting-state gall bladder bile and of hepatic bile (basal and stimulated) before and after depletion of bile acid pool

Subjects	Gall bladder bile		Basal hepatic bile		Stimulated hepatic bile	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	1.07	1.35	—	—	—	—
2	1.08	1.77	—	—	—	—
3	0.79	1.02	1.40	1.40	0.62	0.47
4	0.90	1.40	1.30	1.50	0.80	0.86
5	0.72	1.09	1.50	1.58	0.45	0.76
6	1.06	1.30	1.12	1.12	0.49	0.39
7	0.84	1.37	1.43	1.14	0.46	0.75
8	0.84	0.95	1.23	1.35	0.74	0.71
9	1.40	1.42	2.30	2.18	1.25	0.77
10	0.55	0.66	0.96	1.10	0.52	0.34
11	0.76	1.20	1.90	1.60	0.27	0.62
Mean±SEM	0.93±0.07	1.18±0.07	1.46±0.15	1.44±0.11	0.62±0.09	0.63±0.06
significance		p<0.005		NS		NS

Fig. 2 Saturation index of successive bile samples on day 1 and day 2, representing in sequence basal hepatic bile, fasting gall bladder bile and sequential aliquots of stimulated hepatic bile obtained at 30 minute intervals.

reached for the last three samples (130, 160 and 190 minutes), suggesting a maximum effect on saturation index due to increased hepatic bile acid flux on both days, and the value for stimulated hepatic bile used in Table 2 is the mean value for these three successive half-hourly measurements.

EFFECT ON GALLBLADDER STORAGE FUNCTION (Tables 3 and 4)

(a) On day 1, gall bladder filling with basal hepatic bile was $55 \pm 8\%$ and on day 2 it was $47 \pm 6\%$ (NS).

(b) After depletion of the bile acid pool, the gall bladder volume was reduced in all subjects from 30 ± 4 ml on day 1 to 18 ± 2 ml on day 2 ($p < 0.05$).

(c) There was a reduction in the bile acid mass within the gall bladder from 4.9 ± 0.5 mmol on day 1 to 3.4 ± 0.4 mmol on day 2 ($p < 0.05$), and of phospholipid mass from 1.6 ± 0.2 mmol to 1.1 ± 0.2 mmol, ($p < 0.05$). There was no significant reduction in cholesterol mass (0.47 ± 0.09 mmol vs 0.43 ± 0.06 mmol).

Table 3 Mass of bile acid, phospholipid and cholesterol in the fasting state gall bladder before and after depletion of the bile acid pool

Subjects	Bile acids (mmol)		Phospholipid (mmol)		Cholesterol (mmol)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	5.16	3.48	1.20	0.64	0.43	0.23
2	8.73	4.88	1.35	1.39	0.96	0.64
3	6.64	5.62	2.81	2.34	0.49	0.55
4	5.35	4.00	3.00	0.98	0.97	0.68
5	3.79	2.80	0.85	0.80	0.14	0.27
6	4.59	4.06	1.14	1.16	0.40	0.54
7	3.67	2.52	0.94	0.85	0.23	0.31
8	3.74	2.98	2.07	1.65	0.33	0.45
9	5.44	2.44	1.54	1.32	0.73	0.58
10	1.80	1.08	0.53	0.34	0.81	0.72
Mean±SEM	4.93±0.51	3.39±0.42	1.61±0.25	1.15±0.18	0.47±0.09	0.43±0.06
significance	p<0.005		p<0.05		NS	

Table 4 Effect of bile acid (BA) pool depletion of gall bladder storage functions

Subjects	Gall bladder filling (%)		Gall bladder volume (ml)		BA concentration in gall bladder (mmol/l)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	67	64	35.8	21.7	144.2	160.4
2	57	36	45.8	26.6	190.6	183.4
3	24	41	33.5	19.5	198.2	288.3
4	71	48	—	—	—	—
5	25	33	36.8	15.2	103.0	183.9
6	31	31	—	—	—	—
7	91	68	15.4	11.4	238.5	221.2
8	64	71	21.5	16.3	173.8	182.8
9	84	5	36.2	15.0	150.1	154.6
10	13	6	17.6	15.8	102.3	68.2
11	45	—	—	—	—	—
12	77	67	26.1	18.2	—	—
Mean±SEM	55±8	47±6	30±4	18±2	163±16	180±21
significance	NS		p<0.05		NS	

mmol). Bile acid mass in the gall bladder correlated well with gall bladder volume on both days ($r=0.81$, $p<0.01$; $r=0.73$, $p<0.05$ on day 1 and day 2 respectively).

(d) There was no difference in per cent bile acid pool in the gall bladder after depletion of the pool ($66.5\pm4.6\%$, and $69.7\pm4.3\%$ on days 1 and 2 respectively).

(e) Bile acid concentration within the gall bladder was 163 ± 16 mmol/l on day 1 and 180 ± 21 mmol/l on day 2 (NS).

Some of the results are missing on Table 4, mainly because the rectilinear scanner was out of action on day 2 for subject number 11 and the ultrasound being out of action for subjects 4 and 6. Also subject number 12 failed to give a bile sample on day 2.

Discussion

In our study, depletion of the bile acid pool was followed by an increase in cholesterol saturation

index of gall bladder bile from 0.93 ± 0.07 to 1.18 ± 0.07 ($p<0.005$). This increase in saturation index was because of a reduction in bile acid and phospholipid mass within the gall bladder without an equivalent reduction in cholesterol mass (Table 3). It was not the result of an increase in saturation index of basal hepatic bile or of stimulated hepatic bile, which was unchanged (Fig. 2 and Table 2). These findings would fit for either model B or model C in Figure 1. The fact that gall bladder volume was significantly reduced, whereas fasting state gall bladder filling and the proportion of the bile acid pool stored in the gall bladder overnight were unchanged, is consistent with model B but not with model C. The mean depletion in bile acid pool obtained in this study was 24%, whereas Vlahevic *et al*³ found that the mean reduction in bile acid pool size in eight male gall stone patients was 46% compared with nine male controls. The increase in gall bladder bile saturation index in our non-obese

male subjects (27%) was similar to the degree of depletion of the bile acid pool (24%) in our study. In obese gall stone patients, increased cholesterol secretion, not reduced bile acid pool size, is the mechanism for the increased saturation index of gall bladder bile.

We cannot exclude the possibility that an increase in saturation index of hepatic bile entering the gall bladder on the evening before the second day sample might have contributed to the increased saturation index of fasting gall bladder bile next morning. The fact that in all our subjects basal hepatic bile on the second morning was not altered, and stimulated hepatic bile measured on serial samples on the second day was also unaltered makes this possibility unlikely. Moreover, even if this possibility were correct, it cannot provide an alternative explanation to our model, but only an additional explanation. Further evidence in favour of our model (Fig. 1b) is provided by finding similar mean reductions in total bile acid pool size (24%), gall bladder bile acid mass (31%) and gall bladder volume (40%) after depletion. Although fasting gall bladder volume was not significantly different in 34 gall stone patients and in 34 matched controls, as assessed by carefully standardised oral cholecystography,²⁰ this comparison took no account of the volume occupied by the gall stones. If this volume is subtracted, so as to give the volume occupied by gall bladder bile in both cases, the volume is reduced by a mean value of 14% in the gall stone patients by comparison with the control values ($p < 0.02$, unpublished data).

Our method of measuring gall bladder filling has already been validated in normal subjects,¹¹ and results in 12 healthy controls were found to be similar to those obtained by other authors using a duodenal perfusion technique.^{21, 22} Furthermore, duplicate studies in five subjects gave values which varied by 1–8% with 95% confidence limit of 11%. Gall bladder filling in the present study was comparable on day 1 and day 2, (coefficient of variation 12%). The one exception to this (subject number 9) had a considerable difference in gall bladder filling between the two days, probably because of spontaneous gall bladder contraction at the time of measurement. The detection of very minor gall bladder filling (5% and 6%) in subjects 9 and 10 respectively (Table 4) implies that most of the Tc HIDA radioactivity was not in the gall bladder. Thus, when gall bladder bile was aspirated and its Tc HIDA radioactivity used to estimate biliary lipid mass within the gall bladder, the results might be inaccurate if contaminated with Tc HIDA in the duodenum. The fact that we allowed 90 minutes between the bolus injection of Tc HIDA and gall

bladder contraction should have enabled the Tc HIDA that cleared the liver and was not sequestered into gall bladder to move down the intestine beyond the aspiration site by the time of gall bladder contraction. Furthermore, in both subjects the low value for gall bladder filling was on the second day, and in both subjects the bile acid pool size and bile acid mass in the gall bladder were reduced to the same relative extent as in the other subjects.

Artificial depletion of the bile acid pool in our subjects involved removal on average of 41% of the initial bile acid pool, and deoxycholic acid pool remained depleted by a similar amount on day 2 (mean of 50%, $r = 0.77$, $p < 0.05$). The total bile acid pool was only reduced by a mean of 24% on day 2 and this reduction correlated with the reduction in bile acid mass in the gall bladder on day 2 of 31% on average ($r = 0.72$, $p < 0.01$). The measured reduction in pool size the two primary bile acids (chenodeoxycholic acid and cholic acid) was only 14 and 15% respectively, presumably because of new bile acid synthesis during the 24 hour period. The method we used for measuring bile acid pool size was the one shot dilution technique used by Pomare and Low Beer,¹⁸ which they found to correlate well with the Lindstedt technique. We were unable to use the Lindstedt technique¹⁹ because of the need to carry out independent measurements of the bile acid pool size on the two consecutive days. For the same reason we had to use two different labelled bile acids (¹⁴C chenodeoxycholic acid and ¹⁴C cholic acid) so as to avoid artefact in the day 2 measurement due to radioactivity remaining from day 1.

Mok and colleagues²³ have also shown that artificial depletion of the bile acid pool results in an increase in saturation index of fasting state gall bladder bile in human volunteers. They observed that during their depletion procedure, which involved removal of 40 to 60% of the bile acid pool, there was a significant reduction in the hepatic secretion rate for bile acid and phospholipid but a less marked drop in cholesterol secretion. This is in keeping with the results of Dowling and colleagues in the rhesus monkey,²⁴ where it was clearly shown that artificial interruption of the enterohepatic circulation of bile acids by more than 20% causes hepatic secretion of supersaturated bile. This immediate effect of acute interruption of the enterohepatic circulation of bile acids, as shown by Mok and colleagues,²³ must be distinguished from the delayed effect of depletion of the bile acid pool, as studied by us. Our studies were repeated at an interval of 24 hours after the depletion procedure, and by this time we were unable to detect any alteration in saturation index of hepatic bile in either

the basal or stimulated samples. In this situation, the observed increase in saturation index of fasting state gall bladder bile can only be attributed to an increased ratio of basal:stimulated hepatic bile, as suggested by our other measurements.

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