Liver and biliary

Intestinal transit, deoxycholic acid and the cholesterol saturation of bile – three inter-related factors

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SUMMARY   There is considerable evidence that the level of deoxycholic acid in the bile influences biliary cholesterol saturation. Deoxycholic acid is formed in the colon and absorbed slowly. Hence changes in colonic transit rate might influence biliary deoxycholic acid and the cholesterol saturation of bile. When 14 constipated subjects took standardised senna tablets for six weeks in a dose sufficient to lower mean whole gut transit time from 134 to 54 hours, deoxycholic acid as a proportion of biliary bile acids fell from $25\cdot9\pm8\cdot6$ to $17\cdot2\pm8\cdot3\%$ (p<0·0001) and deoxycholic acid pool measured by isotope dilution fell from $0\cdot64\pm0\cdot34$ to $0\cdot45\pm0\cdot29$ g (p<0·0001). In those subjects (n=8) whose bile was initially supersaturated with cholesterol, the saturation index fell from $1\cdot40\pm0\cdot22$ to $1\cdot20\pm0\cdot19$ (p=0·02). Conversely, when 12 normal volunteers took loperamide capsules sufficient to cause symptomatic constipation and to prolong mean transit-time from 48 to 103 hours, the deoxycholic acid pool increased from $0\cdot40\pm0\cdot24$ to $0\cdot57\pm0\cdot17$ g (p=0·008). The percentage deoxycholic acid did not alter significantly, because the estimated total bile acid pool expanded (from $1\cdot98\pm0\cdot61$ to $2\cdot81\pm0\cdot48$ g; p<0·001), presumably because of loperamide slowing down small bowel transit. Despite this expansion of the bile acid pool, loperamide increased the cholesterol saturation index from $1\cdot10\pm0\cdot31$ to $1\cdot20\pm0\cdot32$ (p=0·01). Changes in colonic transit rate alter the size of the deoxycholic acid pool and bile cholesterol saturation. These findings suggest that constipation or slow colonic transit might increase the chance of supersaturated bile and hence of gall stones.

Several lines of evidence suggest that a raised level of deoxycholic acid in the bile is a risk factor for gall stones. Patients with gall stones tend to have raised levels of deoxycholic acid in relation to the two other major bile acids, cholic, and cheno-deoxycholic,[1–6] though this is not a universal finding.[7–10] In patients with gall stones a correlation has been shown between % deoxycholic acid in the bile acid pool and the cholesterol saturation index of bile.[11 12] When the proportion of deoxycholic acid in the bile was raised in normal subjects by feeding physiological amounts of this bile acid, 100–150 mg/day for two weeks, there was a significant rise in the molar percentage of cholesterol in bile.[13] The same results can follow the feeding of cholic acid, the precursor of deoxycholic acid.[14] When deoxycholic acid has been fed in pharmacological doses, 750–1000 mg/day, there has been no rise in the` cholesterol saturation of bile[15–17] but at this

dosage deoxycholic acid has toxic effects on the liver, raising serum transaminase concentrations, and possibly on the intestine. The percentage of deoxycholic acid in the bile can be reduced by diverse measures – by administering metronidazole[18] or ampicillin,[14] by feeding bran,[19–21] lactulose[22] or a preparation of *Streptococcus faecium*,[23] or by treating hypothyroidism with thyroxine.[24] With all six of these manoeuvres there is a simultaneous reduction in the cholesterol saturation of bile.

Deoxycholic acid is the main secondary bile acid in bile. It is produced in the colon when anaerobic bacteria remove the 7α-hydroxyl group from any cholic acid which has escaped absorption in the terminal ileum. This dehydroxylation process is almost complete as faeces normally contain minimal amounts of cholic acid.[25 26] About 30% of newly formed deoxycholic acid is absorbed and transported to the liver[27] together with any previously formed and recirculated deoxycholic acid which enters the colon. In the liver, deoxycholic acid is conjugated with glycine and taurine and secreted into the bile alongside glyco- and taurochenodeoxy-

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cholate. Conjugated deoxycholates are handled by the liver and small intestine in the same way as the other dihydroxy bile acids, the chenodeoxy-cholates.[27] In particular, they are efficiently re-absorbed with less than 5% escaping into the colon at each turn of the enterohepatic circulation. The circulating deoxycholic acid pool is about 0·66 g (1·7 mmol)[27 28] and about 30% of the pool is turned over each day (fractional turnover rate 0·3, equivalent to a half-life of 2·8 days). Input into the pool consists of newly formed deoxycholic acid absorbed from the colon.

Absorption of deoxycholic acid from the colon is probably slow, occurring only by passive diffusion. When radioactive cholic acid is injected into the colon at laparotomy most of it is absorbed and re-secreted in the bile (largely as deoxycholate) during the first 24 hours but absorption continues for several days.[29 30]

If deoxycholic acid is absorbed slowly and in-completely then slowing down colonic transit might well increase its absorption and cause expansion of its circulating pool. This in turn would be expected to lead to increased saturation of bile with cholesterol. Conversely, speeding up colonic transit, at least in subjects with slow transit, should reduce the absorption of deoxycholic acid and lead to a reduced pool of this bile acid and perhaps to reduced cholesterol saturation of bile. This study was designed to test these predictions by examining the results of administering a constipating agent in effective doses to normal subjects and of administering a chemical laxative in effective doses to people with spontaneous constipation. Because the risk of forming cholesterol gall stones depends in part on the saturation of bile with cholesterol these studies also test the hypothesis that constipation or, at any rate, slow colonic transit, is a risk factor for gall stones.

Methods

SUBJECTS AND DESIGN OF STUDY
Constipated and normal subjects were recruited in response to posters in local chemist shops and health centres, in the waiting areas of the Bristol Royal Infirmary and Dental Hospital and in WRVS stalls. Advertisements were inserted in the university newsletter and health authority gazette. Letters of appeal were sent to members of the hospital voluntary service, St. John's Ambulance Brigade and Red Cross Society.

The constipated group consisted of 12 women and two men aged 38–69 years (mean 48), with body mass or Quetelet index 20·3–27·3 (mean 23·6), who admitted to three bowel actions per week or less or

who often strained to pass scybalous faeces and whose whole gut transit time was around three days or longer. Volunteers admitting to alternating constipation and diarrhoea were excluded. Those who were already taking laxatives or bran-containing cereals were instructed to stop them for at least four weeks before entering the study. Six subjects were taking other drugs (two diuretics, two hypnotics, one each lithium, ranitidine, oestrogen replacement and non-cyclical progestogen) but the dosages remained unaltered during the study. Baseline measurements of transit time, bile composition, and deoxycholic pool size and kinetics were carried out. Dietary intake was assessed by an experienced dietitian using a questionnaire and a 48 hour weighed record. Subjects were then provided with sennoside B tablets (Senokot) and instructed to take them in a single daily dose sufficient to relieve their constipation (15–60 mg sennoside B daily) for six weeks, the effect being monitored by one of us (SNM) by regular telephone calls. At the end of this period the transit time, biliary studies and dietary assessment were repeated.

The group with normal bowel function consisted of 10 women and two men aged 38–62 years (mean 45), with body mass index 20·9–27·7 (mean 24·0) who claimed to have a normal bowel habit and whose whole gut transit time was shorter than three days. Baseline measurements of transit time, bile composition, and deoxycholic acid pool size and kinetics were carried out. Subjects were then provided with loperamide capsules and instructed to take them in divided doses sufficient to induce constipation (4–20 mg daily) for six weeks, the effect again being monitored by regular telephone calls. No other drugs were taken by subjects in this group. Dietary intake was monitored as above. Two additional women who entered this group were later excluded because they could not tolerate an effective dose of loperamide. At the end of this period the transit time, biliary studies, and dietary assessment were repeated.

A third group consisted of six healthy members of staff (two women and four men aged 30–60 years, mean 43) in whom mouth-caecum transit time and gastric emptying time were measured after two doses of placebo or loperamide (2 mg) in a random-ised, double blind comparison. On a separate occasion, mouth-caecum transit time was measured in the same six subjects after two doses of sennoside B 7·5 mg. Test and placebo capsules were taken at midnight and four hours before the test breakfast.

Volunteers in the first two groups had blood taken for liver function tests and these were normal. Fasting lipids were also measured and these were normal or only slightly raised. Ultrasound scan of

the gall bladder showed the absence of gall stones in all subjects except for one non-constipated volunteer who had stones in a functioning gall bladder on oral cholecystogram.

WHOLE GUT TRANSIT TIME

Transit time was measured by the single stool method[31] with two modifications. Subjects with constipation (spontaneous or drug induced) ingested a gelatine capsule containing 20 radio-opaque Portex markers at breakfast time on alternate days for one week (rather than on three consecutive days) and then collected their next two stools. Each day the markers were of a different shape and were taken in a predetermined order. Stools were excreted directly into gussetted polyethylene bags which were sealed and stored at $-20°C$ until radiographed. Whole gut transit time was calculated from each stool by counting the two most abundant markers and applying the formula:

$$\text{Whole gut transit time (h)} = \frac{t1s1+t2s2}{s1+s2}$$

where t1 and t2=time in hours from the ingestion of the two markers and s1 and s2=the number of each marker present. The mean value from the two stools was taken.

BILE COMPOSITION AND THE POOL SIZE AND KINETICS OF DEOXYCHOLIC ACID

Three hours after the last meal of the day 0·37MBq [10 µCi] 24-^{14}C-deoxycholic acid (Radiochemical Centre, Amersham) was injected intravenously and, after an overnight fast, bile-rich duodenal fluid was collected by means of duodenal intubation and cholecystokinin injection. The period of fasting was standardised for each volunteer.[32 33] In seven constipated volunteers and in nine normal ones 5 ml aliquots of duodenal bile were collected on four consecutive mornings to enable determination of deoxycholic acid pool size, input and fractional turnover.[34] In the other 10 subjects who were unwilling to have repeated intubations, deoxycholic acid pool size was determined by a slightly modified version of the 'one shot' technique.[35]

Total bile salt, phospholipid and cholesterol concentrations were measured[36] and the cholesterol saturation index of bile was determined by the method of Thomas and Hofmann[37] according to the criteria of Hegardt and Dam.[38] In the small number of samples in which the total lipid concentration was <20mmol/l phospholipid concentration was measured using an enzymatic method.[39]

After enzymatic deconjugation and thin layer chromatographic separation of the dihydroxy bile acids,[40] deoxycholic acid mass was determined by subtracting the 7α- from the 3α-hydroxysteroid dehydrogenase results (Sigma Chemical Co.).[41] The mass of the separated cholic acid was also determined using 3α-hydroxysteroid dehydrogenase. Radioactivity was measured by liquid scintillation counting. The counts were corrected for any effects due to quenching using quench curves calculated from external standards of ^{14}C.

From these data the pool size, half-life and input of deoxycholic acid were determined as well as the relative proportions of the three major bile acids. By combining these data the size of the total bile acid pool was estimated, accepting that a slight systematic underestimate of the cholate pool is likely using this method.

GASTRIC EMPTYING TIME

The half-life of gastric emptying was determined by adding 12MBq [324 µCi] of ^{111}In to a liquid test meal and measuring the disappearance of radioactive counts over the surface of the stomach every 10 minutes for up to three hours using a gamma camera.[42 43] The test meal consisted of 30 g lactulose, 40 g glucose, 20 g corn oil and 15 g Casilan made up to 300 ml with water[44] and flavoured with vanilla essence.

MOUTH-CAECUM TRANSIT TIME

Mouth-caecum transit time was measured by analysing expired air samples for hydrogen concentration at 15 minute intervals using a breath hydrogen machine (Moogstraat Medische Techniek NU, Kampen, Holland) and was defined as the time from taking the above liquid meal to the first sustained rise in breath hydrogen concentration.[45]

STATISTICAL ANALYSIS

Student's paired *t* test was used for determining the significance of differences. A p value of <0·05 was taken as significant. Results are expressed as mean±SD.

Results

DIETARY INTAKE AND BODY WEIGHT

The dietary intake of all volunteers remained unaltered during the study, in particular there was no change in the fibre content of their diet. In the constipated group treated with senna laxative there was a slight increase in body weight from 63·9±9·6 to 64·3±9·9 kg (p=0·02) but this was of doubtful biological importance. The increase in body weight in the normal group prescribed loperamide, from 63·9±15·6 to 64·4±15·3 kg, was not significant.

WHOLE GUT TRANSIT TIME (Fig. 1)

With senna laxative the transit time fell in all subjects from a mean of 134±55 to 51±21 hours (p<0·0001). With loperamide the transit time rose in all subjects from a mean of 48±11 to 103±31 hours (p<0·0001).

DEOXYCHOLIC ACID POOL (Fig. 2)

With senna laxative deoxycholic acid pool fell in all but one subject, the mean decreasing by 30% from 0·64±0·34 to 0·45±0·29 g (p<0·0001). Conversely, with loperamide deoxycholic acid pool rose in all but three subjects, the mean increasing by 43% from 0·40±0·24 to 0·57±0·17 g (p=0·008).

RELATIVE PROPORTIONS OF BILE ACIDS IN BILE

The proportion of deoxycholic acid fell in all senna treated subjects (Fig. 3), from a mean of 25·9±8·6 to 17·2±8·3% (p<0·0001), whereas in the loperamide group (Fig. 3) there was no consistent change (18·9±10·3 to 19·7±6·9%).

The proportion of chenodeoxycholic acid rose with senna laxative in all but one subject (Fig. 4),

Fig. 2 *Deoxycholic acid (DCA) pool in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide.*

the mean increasing from 36·5±7·7 to 43·7±8·0% (p<0·0001), while in the loperamide group there was no consistent change (44·0±10·7 to 43·5±5·5%).

The percentage of cholic acid did not alter significantly with senna laxative (37·6±6·5 to 38·7±6·0%) nor with loperamide (37·6±6·1 to 36·8±4·8%).

TOTAL BILE ACID POOL (Fig. 5)

Senna laxative had no significant effect on the total bile acid pool (2·38±0·90 to 2·47±0·77 g), whereas the pool increased in all subjects treated with loperamide, the mean increasing by 42% from 1·98±0·61 to 2·81±0·48 g (p<0·001).

CHOLESTEROL SATURATION INDEX (CSI) (Fig. 6)

In the eight constipated subjects whose bile was initially supersaturated with cholesterol (CSI>1·0), the index fell significantly with senna laxative from a mean of 1·40±0·22 to 1·20±0·19 (p=0·02). Out of the whole group, the CSI fell in 10 of the 14 subjects, yet the reduction was not significant (1·1±0·39 to 1·0±0·33). This was because of one subject, Mrs OM, whose pretreatment CSI of 0·55 was surprisingly low for a multiparous middle
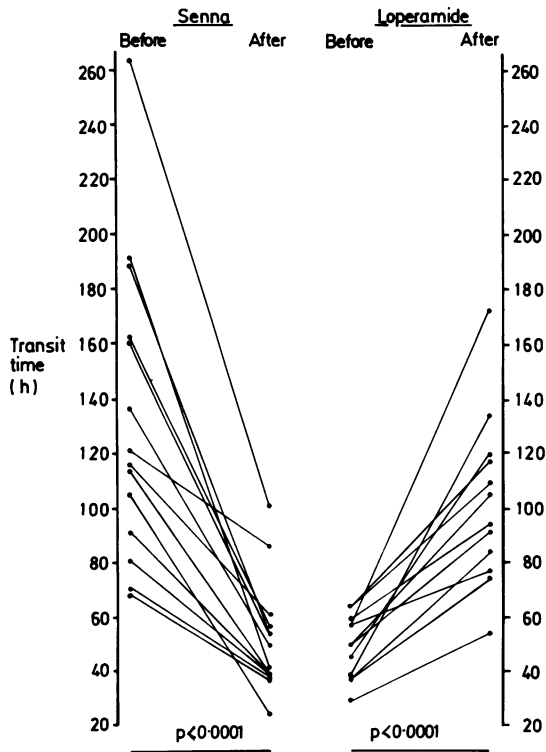
Fig. 1 *Whole gut transit time in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide.*
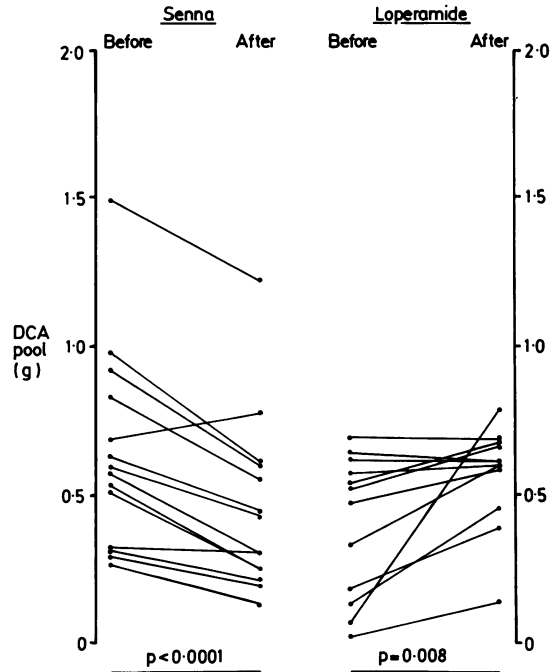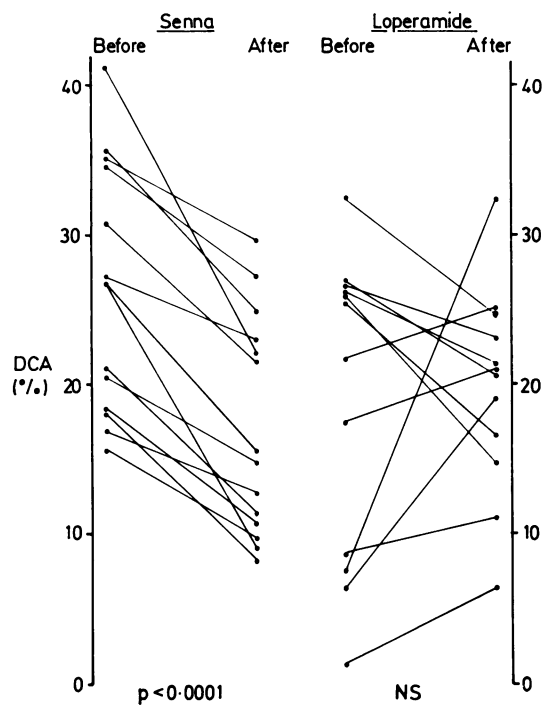
Fig. 3 *Percentage of deoxycholic acid (DCA) in the bile acid pool in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide.*
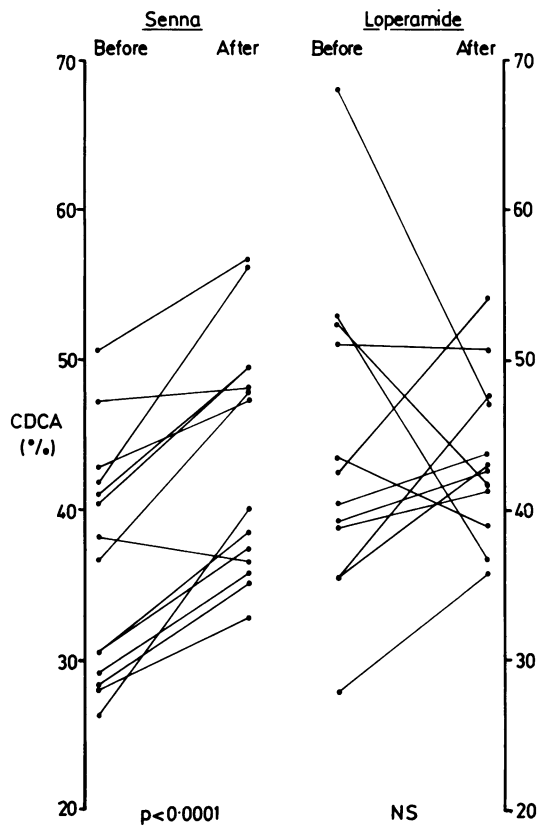

Fig. 4 *Percentage of chenodeoxycholic acid (CDCA) in the bile acid pool in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide.*

aged woman on oestrogen replacement therapy. To verify this, a further sample of bile was obtained three months after completion of the experiment and on this occasion the CSI was 0·99. If the data of subject OM are excluded from analysis as a 'rogue' result the CSI of the group is significantly reduced by senna laxative, falling from 1·20±0·37 to 0·99±0·33 (p=0·004).

In the normal subjects made constipated with loperamide the CSI rose in all but two, the mean increasing from 1·10±0·31 to 1·20±0·32 (p=0·01).

DEOXYCHOLIC ACID KINETIC STUDIES
The half-life of deoxycholic acid fell with senna laxative in all seven subjects in whom it was measured, from a mean of 4·4±2·1 to 2·4±0·72 days (p=0·02) and was lengthened in all but one of the nine loperamide treated subjects, increasing from a mean of 2·1±0·76 to 5·1±2·9 days (p=0·006).

Deoxycholic acid input was not significantly altered by senna laxative (0·14±0·08 to 0·17±0·08 g/day) nor by loperamide (0·11±0·08 to 0·10±0·05 g/day).

CORRELATIONS (Table)
Correlations between changes in transit time and changes in bile composition with senna laxative and loperamide were determined. There was a significant positive correlation between ΔCSI and Δwhole-gut transit time (WGTT) which became stronger when subject OM was excluded. Similarly there was a significant positive correlation between ΔCSI and Δdeoxycholic acid pool, again becoming stronger when this subject was omitted. A significant positive correlation between ΔCSI and Δdeoxycholic acid% and between ΔCSI and Δtotal bile acid pool existed only when subject OM was removed from analysis. Significant positive correlations also occurred between ΔWGTT and Δdeoxycholic acid pool, between ΔWGTT and Δdeoxycholic acid% and between ΔWGTT and Δtotal bile acid pool. There was a significant negative correlation between Δchenodeoxycholic acid% and Δdeoxycholic acid
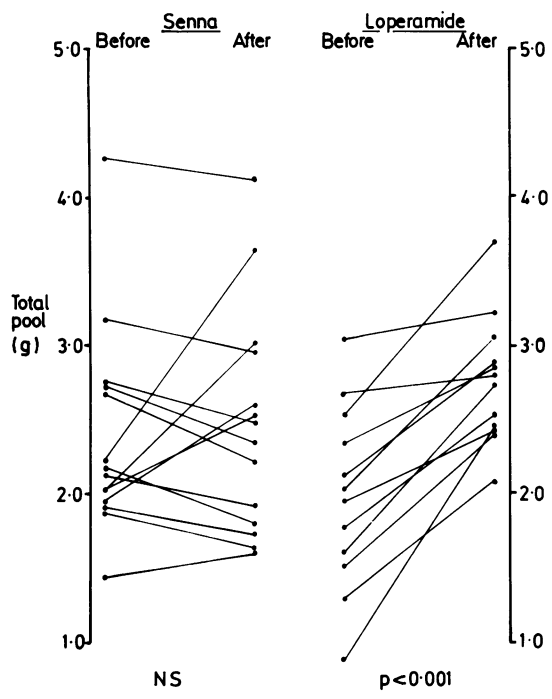
Fig. 5 *Total bile acid pool in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide.*

Fig. 6 *Cholesterol saturation index (CSI) in constipated subjects (n=14) before and after senna laxative and in normal subjects (n=12) before and after loperamide. The p value given is that obtained without subject OM (see text). Shaded area represents bile which is unsaturated with cholesterol.*

pool and a negative correlation between Δchenodeoxycholic acid% and ΔCSI.

GASTRIC EMPTYING TIME

In one of the six volunteers the data were inconsistent and uninterpretable and were therefore excluded. In the other five subjects, loperamide had no effect on gastric emptying half-life (126 ± 30 versus 124 ± 34 minutes on placebo).
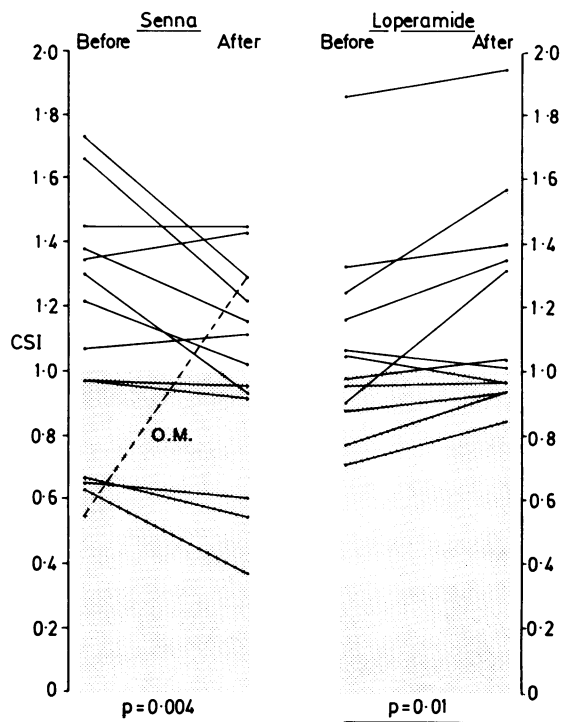
MOUTH-CAECUM TRANSIT TIME

In all six subjects given loperamide mouth-caecum transit time was lengthened, the mean increasing from 88 ± 23 to 160 ± 40 minutes ($p = 0.002$). In contrast, senna laxative had no overall effect (88 ± 23 to 98 ± 50 minutes).

Table *Correlation coefficients*

	$\Delta CDCA\%$	$\Delta CA\%$	ΔDCA pool	$\Delta Total$ pool	ΔCSI	$\Delta DCA\%$
$\Delta CA\%$	-0.263					
ΔDCA pool	$-0.727\ddagger$	-0.216				
$\Delta Total$ pool	-0.403^*	0.078	$0.716\ddagger$			
ΔCSI	-0.483^*	0.403^*	0.401^*	0.380		
	$(-0.616\dagger)$		$(0.666\ddagger)$	(0.422^*)		
$\Delta DCA\%$	$-0.837\ddagger$	-0.296	$0.838\ddagger$	0.342	0.285	
					$(0.595\dagger)$	
$\Delta Transit$ time	-0.447^*	0.058	$0.648\ddagger$	$0.570\dagger$	$0.553\dagger$	0.439^*
					$(0.708\ddagger)$	

* $p < 0.05$
† $p < 0.01$
‡ $p < 0.001$

The results in parentheses are those which apply when subject OM is excluded.

Discussion

As predicted, speeding up colonic transit by the administration of senna laxative reduced the deoxycholic acid pool and the cholesterol saturation of bile, while slowing down transit by the administration of loperamide had the opposite effects. Changes in whole gut transit time, deoxycholic acid pool and cholesterol saturation all correlated with each other. These findings support the concept that the amount of deoxycholic acid in circulation influences the cholesterol saturation of bile and hence the risk of forming cholesterol gall stones.[13] They also provide the first objective evidence that slow colonic transit may be a risk factor for gall stones. Many years ago, physicians commented upon the commonness of constipation in patients with gall stones[46][47] but no data were given nor were controls studied.

Recently it was observed that the input of deoxycholic acid varied significantly with gut transit in young people but not in older subjects.[48] These authors did not report on correlations between transit time and deoxycholic acid pool size or per cent deoxycholic acid.

In acute experiments in man, replacing the bile acid pool with deoxycholic acid causes the liver to secrete bile which is more saturated with cholesterol, probably because of the more hydrophobic character and hence greater detergency of this bile acid over the other major bile acids so that it leaches out more cholesterol from the canalicular membrane.[49] If this effect of deoxycholic acid on cholesterol secretion occurs in real life, and if it is sensitive to changes in deoxycholic acid pool of the order we have found, it could explain our findings. Another way in which increased circulating deoxycholic acid could make bile more saturated is by displacing chenodeoxycholic acid from the pool, as increasing the pool of chenodeoxycholic acid by oral administration, as in gall stone dissolution therapy, lowers the cholesterol saturation of bile.[50] We did find a negative correlation between change in per cent chenodeoxycholic acid and change in deoxycholic acid pool. Deoxycholic acid could displace chenodeoxycholic acid by suppressing its synthesis[51] or by competing with it for absorption.[52]

The increase in cholesterol saturation index with loperamide was modest despite a substantial rise in the deoxycholic acid pool. This may be because the pool of chenodeoxycholic acid increased in parallel. The fact that the cholic acid pool expanded too, as judged by the relative proportions of the bile acids, is probably irrelevant because, in another study in which cholic acid was administered, subjects who responded by increasing their deoxycholic acid pool suffered a rise in the cholesterol saturation of their bile even if the cholic acid pool expanded too.[14] Despite the parallel expansion of the chenodeoxycholic acid pool, loperamide did increase the cholesterol saturation of bile. This suggests that the deleterious effect of deoxycholic acid on cholesterol saturation may be greater than the beneficial effect of chenodeoxycholic acid.

The present findings raise the possibility that the beneficial effect of chenodeoxycholic acid treatment on bile cholesterol saturation may in part be due to its laxative effects, with consequent reduction in deoxycholic acid absorption and pool size.

The expansion of the primary bile acid pool by loperamide can be attributed to slowing down of small intestinal transit because both these effects have been noted with administration of propantheline bromide.[53] Conversely, when small intestinal transit was accelerated by administration of sorbitol the total bile acid pool contracted.[54] Slowing of small bowel transit cannot explain our finding of expansion of the deoxycholic acid pool since such an expansion did not occur with propantheline. Loperamide has previously been shown to prolong small bowel transit only in subjects with chronic diarrhoea[55] or with irritable bowel syndrome[56] but a recent preliminary report of a study in normal subjects[57] agrees with our findings.

The prolongation of deoxycholic acid half-life with loperamide can be explained by slower small bowel transit leading to more efficient absorption. The shortening of this half-life with senna laxative is less easy to explain. Senna is normally considered to act only after bacterial formation of active metabolites in the colon.[58] We detected no shortening of small bowel transit time with senna in acute studies of normal subjects but cannot rule out the possibility that it has such an effect with chronic usage or in constipated subjects.

In conclusion, the present study supports the notion that constipation may be a risk factor for gall stones, but epidemiological studies are necessary to prove this.

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