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Diurnal variation in cholesterol saturation of gall-bladder bile

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SUMMARY In order to determine whether a diurnal variation in cholesterol saturation index is present in gall-bladder bile, samples of bile were taken by nasoduodenal intubation and cholecystokinin infusion at 9 am after the conventional 12 hour fast, and also at 5 pm five hours after a meal containing no cholesterol or phospholipid. In healthy controls saturation index (mean±SEM) fell from 1·02±0·08 at 9 am to 0·86±0·08 at 5 pm ($n=8$, $p<0·05$). In untreated cholesterol gall-stone patients saturation index fell from 1·30±0·07 to 1·04±0·07 ($n=8$, $p<0·05$); on chenodeoxycholic acid 15 mg/kg/day it fell from 0·91±0·06 to 0·78±0·07 ($n=16$, $p<0·01$). The degree of diurnal variation was similar in those taking chenodeoxycholic acid at bedtime and in those taking it at mealtimes. The 9 am sample was supersaturated in three non-responders (showing no evidence of gall stone dissolution on oral cholecystogram after at least six months treatment) and in four responders. The 5 pm sample was a better predictor of treatment failure, being supersaturated in all four non-responders but in only one out of the 12 responders ($p<0·01$).

Cholesterol crystal formation in model bile solutions which are moderately supersaturated with cholesterol is slow, probably taking in the region of four to 28 hours.[1] Dissolution of cholesterol crystals in model bile solutions, however, is rapid at physiological biliary lipid compositions.[2] If there were a marked diurnal variation in the cholesterol saturation index of gall-bladder bile, the slow induction and rapid dissolution of cholesterol crystals would tend to prevent formation of gall stones if the gall-bladder bile became unsaturated with cholesterol at some point during the day. A diurnal variation has been established for hepatic bile,[3] but not for gall-bladder bile. We have, therefore, compared the saturation index of gall-bladder bile obtained after the conventional overnight fast with that obtained at 5 pm only five hours after a meal. We have studied cholesterol gall-stone patients before and during treatment; and also control subjects without gall stones.

Methods

PATIENTS

We studied eight patients with radiolucent gall stones before treatment with chenodeoxycholic (chenic) acid, eight patients on chenic acid 15 mg/kg/day given in three divided doses at mealtimes, eight patients on chenic acid 15 mg/kg/day in a single bedtime dose and eight control subjects with normal gall-bladder ultrasound examinations. Mean weight of the gall-stone patients was 63 kg (range 55–89 kg) and of the normal controls 67 kg (61–79 kg). Mean age of the gall-stone patients was 55 years (27–70 years) and of the normal controls 38 years (25–58 years). Mean height of the gall-stone patients was 163·8 cm (155–183 cm) and of normal controls 164·1 cm (158–173 cm). There were seven men and 17 women in the gall-stone group, four men and four women in the control group. One of the gall-stone patients was taking propranolol. No other subject was taking additional medication at the time of the study.

Bile-rich duodenal fluid was obtained from gall-stone and control subjects by nasoduodenal intubation with cholecystokinin infusion at 9 am after a 12 hour fast, and at 5 pm five hours after a liquid meal containing 15 g amino acid and 40 g glucose. Fasting duodenal fluid (hepatic bile) was also obtained before gall bladder contraction in the normal controls. The liquid meal contained only 0·3 mm/l inorganic phosphate and no detectable phospholipid or cholesterol, thus avoiding contamination of the bile samples with these lipids.

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The two intubations were undertaken in random order at least one week apart. Mean interval between intubations was 11 days (range seven–28 days). On the night preceding the 9 am sample the subject took his normal supper at 9 pm. On the morning of the 5 pm sample he took his normal breakfast but no other food until 12 noon when he took the liquid formula meal.

Total bile acid concentration was measured by the 3α hydroxy steroid dehydrogenase enzyme assay as described by Talalay[4] and modified by Admirand and Small.[5] Cholesterol was measured by the cholesterol oxidase method in an aliquot of bile that was diluted at the bedside in isopropanol (1 in 10).[6] Phospholipid was measured enzymatically by the method described by Qureshi, Murphy, and Dowling.[7] Saturation index was calculated using the polynomial equation developed by Thomas and Hofmann,[8] based on the limits of cholesterol solubility described by Hegardt and Dam[9] and Holzbach *et al.*[10]

Results were compared statistically using Wilcoxon's test for paired samples; and by Chi-square test, using Yates' correction for small numbers.

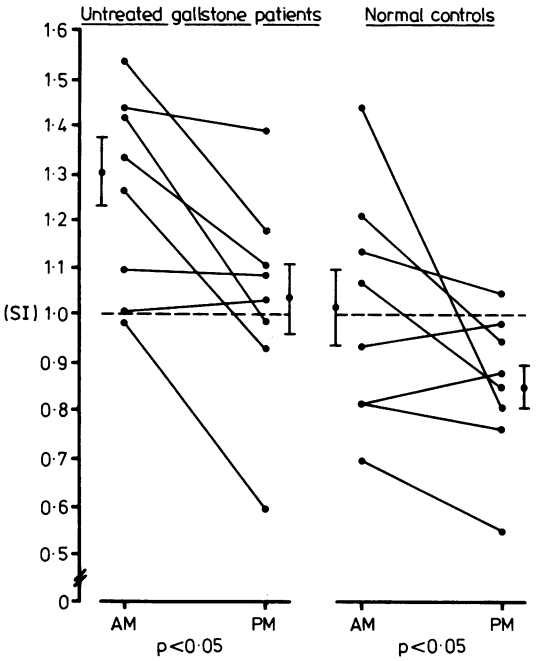

Fig. 1 *Saturation index of 9 am and 5 pm gall-bladder bile samples in untreated gall-stone patients and normal controls.*

Results

In normal controls saturation index of gall-bladder bile fell from 1·02±0·08 (mean±SEM) at 9 am to 0·86±0·08 at 5 pm (Fig. 1; $p<0·05$). Diurnal variation for hepatic bile was greater quantitatively than for gall-bladder bile, as mean saturation index fell from 2·06 at 9 am to 1·30 at 5 pm, but the difference was not significant because these measurements were only carried out in five of the eight controls. In untreated cholesterol gall-stone patients saturation index of gall-bladder bile fell from 1·30±0·07 to 1·04±0·07 (Fig. 1; $p<0·05$). Saturation index of gall-stone patients was higher than that of controls at both 9 am and 5 pm ($p<0·05$). In the normal controls only one patient had a supersaturated 5 pm gall-bladder sample, whereas in the gall-stone group before treatment five patients had a supersaturated 5 pm sample (the corresponding figures for 9 am samples being four and seven).

On chenic acid 15 mg/kg/day given at mealtimes gall-bladder bile saturation index fell from 0·90±0·09 at 9 am to 0·79±0·09 at 5 pm (Fig. 2; $p<0·05$); and on chenic acid 15 mg/kg/day given at bedtime saturation index fell from 0·95±0·09 at 9 am to 0·80±0·10 at 5 pm (Fig. 3; $p<0·05$). The 5 pm sample discriminated better between responders and non-responders during chenic acid treatment. In the sixteen patients on chenic acid at mealtimes or at bedtime there were four patients who did not respond to treatment in that there was no measureable reduction in gall-stone size assessed from carefully standardised oral cholecystogram after six months treatment. All four of these patients had supersaturated 5 pm samples, whereas of the 12 patients who did show a reduction in stone size only one had a supersaturated 5 pm sample (saturation index 1·03; $\chi^2=7·86$, $p<0·01$). Three of the four non-responders had a supersaturated 9 am sample, compared with four out of 12 responders, ($\chi^2=0·76$, ns).

Discussion

We have shown a significant diurnal variation in cholesterol saturation of gall-bladder bile in healthy subjects, and in cholesterol gall-stone patients before and during treatment with chenic acid. This has not been shown previously for gall-bladder bile, but has been shown for hepatic bile. A diurnal variation for hepatic bile is present because cholesterol saturation is related to hepatic bile acid flux,[11 12] and saturation is therefore higher for fasting than for postprandial hepatic bile.[3] As gall-bladder bile is likely at all times to represent a composite of different proportions of fasting and

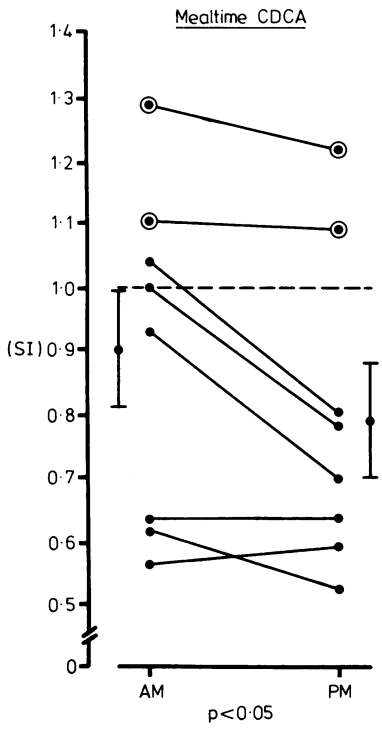

Fig. 2 *Saturation index of 9 am and 5 pm gall-bladder bile samples in gall-stone patients treated with chenodeoxycholic acid 15 mg/kg/day given in divided doses at mealtimes. Non-responders, whose gall stones did not dissolve during treatment, are indicated by circled data points.*

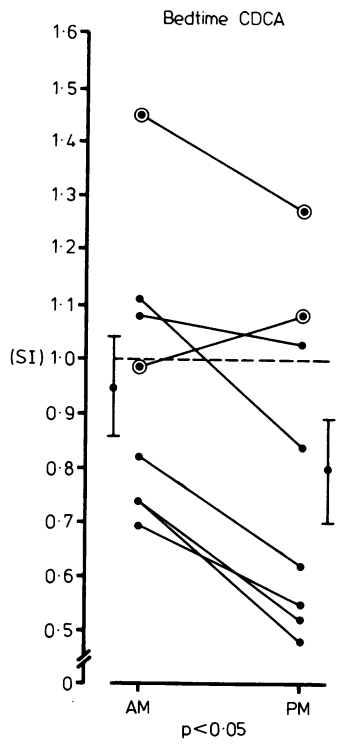

Fig. 3 *Saturation index of 9 am and 5 pm gall-bladder bile samples in gall-stone patients on chenodeoxycholic acid 15 mg/kg/day as a single bedtime dose (symbols as in Fig. 2).*

postprandial hepatic bile, a damping effect on diurnal variation would be expected for gall-bladder bile, and this is supported by our finding of a much smaller diurnal variation for gall-bladder bile than for hepatic bile in our normal subjects. One limitation of our study is the use of an artificial liquid formula meal at lunchtime. It is clearly not possible to say whether the same results would have been obtained using a more conventional meal, but the almost universal presence of phospholipid and cholesterol in 'natural' food makes the use of a formula meal mandatory.

Only one of the eight normal control subjects had supersaturated evening samples. This may provide one explanation for the failure of normal controls to form gall stones, despite the presence of supersaturated bile in the morning; if the bile becomes unsaturated later in the day microcrystals of cholesterol may dissolve or be prevented from forming. This finding suggests that when studying methods of gall-stone prophylaxis, it may be more important to check whether these produce unsaturated gall-bladder bile at some time in the day than to check their effect on fasting gall-bladder bile in the morning.

Evening samples in all four gall-stone patients who did not dissolve their stones on treatment were supersaturated, whereas in only one out of the 12 patients who showed gall stone dissolution was the evening sample supersaturated ($p<0{\cdot}01$). The morning sample was not such a good predictor of response to treatment in that three of the morning samples were supersaturated with cholesterol in the non-responders and four in the responders.

We have previously shown that bedtime administration of chenic acid has a greater effect on saturation index of fasting gall-bladder bile than mealtime administration in the same subjects.[13] We have suggested that this is because bedtime administration prevents overnight interruption of the enterohepatic circulation of bile acids and thus reduces secretion of supersaturated nocturnal hepatic bile. An alternative explanation is the shorter time interval between bile acid administra-

tion and sampling of the gall-bladder bile after the bedtime dose. The latter explanation implies that if gall-bladder bile was sampled later in the day in those receiving bedtime bile acid the difference in saturation index between the two treatment regimens would no longer be apparent. This latter explanation is contradicted by the finding in the present study that the degree of diurnal variation is at least as great in those receiving bedtime chenic acid (mean difference in saturation index 0·15) as those receiving mealtime chenic acid (mean 0·11). The fact that the actual saturation index on the bedtime regimen was not lower than on the mealtime regimen is attributable to the fact that two different groups of subjects were studied on this occasion, and that those receiving the bedtime regimen had a higher pretreatment saturation index (mean±SEM 1·21±0·07) than those receiving the mealtime regimen (1·02±0·10).

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