

# Lipid composition of bile in diabetics and obesity-matched controls

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**SUMMARY** Duodenal bile from 27 diabetics was compared with samples from healthy subjects matched for age, sex, and body mass index. Cholesterol saturation and the molar percentages of bile acids, phospholipids, and cholesterol were not significantly different. Most bile samples were supersaturated in both groups. The maturity onset diabetics who were almost all obese had more saturated bile than the slimmer juvenile onset patients. Body fatness and plasma triglyceride levels were both positively correlated with the cholesterol saturation of bile in the controls but not in the diabetics. Bile was less concentrated in female diabetics than in controls, which is consistent with impaired gall-bladder emptying. It is possible that the increased prevalence of gallstones in diabetics is due not so much to diabetes itself as to the frequently associated obesity.

There is abundant evidence for increased prevalence of gallstones in patients with diabetes mellitus (Gross, 1929; Robertson, 1945; Lieber, 1952; Twiss and Carter, 1952; Newman and Northup, 1959; Mundth, 1962; Goldstein and Schein, 1963; Watkinson, 1967). The chemical composition of these stones has not been reported but it is generally accepted that they are rich in cholesterol. Diabetics tend to be obese and to have hypertriglyceridaemia. Both of these disorders are associated with an increased risk of gallstones and increased cholesterol saturation of bile (Mentzer, 1926; Gross, 1929; Friedman *et al.*, 1966; Záhoř *et al.*, 1974; Bennion and Grundy, 1975; Einarsson *et al.*, 1975; Freeman *et al.*, 1975; Angelin, 1977; Shaffer and Small, 1977). Supersaturation of bile is believed to be the key factor, or at least the essential first stage, in cholesterol gallstone formation. Surprisingly, there is no published study in which the lipid composition of diabetic bile has been compared with that of an adequate control population. The purpose of the present study was to make such a comparison in the hope of determining whether the increased incidence of cholelithiasis in diabetics is related to the diabetic state itself or to the associated lipid and weight disturbances.

## Methods

### SUBJECTS

Sixteen male and 11 post-menopausal female diabetics were recruited from an outpatient clinic. All the female and seven of the male patients were judged to have the maturity-onset type of diabetes; 10 were being treated by diet alone, six were receiving oral hypoglycaemic agents (four biguanides, two metformin and glibenclamide), and two were on insulin. Nine male patients were judged to have the juvenile-onset type of diabetes and all were receiving insulin (16-60, mean 41, units daily). In all patients the diabetes was well or adequately controlled; their prescribed diets followed the traditional low (40%) carbohydrate pattern but were otherwise normal. Degree of body fatness was determined by a body mass index—weight in kg/ (height in m)<sup>2</sup> (Florey, 1970). The normal range for this is about 20-25. Each diabetic was matched for age, sex, and obesity index with a healthy volunteer recruited from hospital staff and eating a normal British diet. Premenopausal women were excluded because the cholesterol saturation of bile is believed to change during the menstrual cycle (Low-Beer *et al.*, 1977).

After a 12-hour overnight fast venous blood samples were taken and analysed for plasma glucose, triglyceride, and cholesterol by routine autoanalyser methods. Fasting bile-rich duodenal juice was aspirated after intubation with a mercury-weighted polyethylene catheter and intravenous administra-

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Table 1 Clinical data, plasma lipids, and bile lipid composition in 27 diabetics and 27 matched controls (mean, SEM)

Group	Age (mean, range)		Treatment*	Body mass index†	Plasma triglycerides (mmol l <sup>-1</sup> )‡	Plasma cholesterol (mmol l <sup>-1</sup> )§	Bile lipid composition				Saturation index
	(yr)	At onset (yr)					Total lipids (mmol l <sup>-1</sup> )	Percent of total moles			
								Bile acids	Phospholipids	Cholesterol	
Male juvenile onset (n = 9)	34 (21-55)	24 (10-42)	All insulin	21.6 (0.7)	1.03 (0.12)	5.50 (0.32)	44.5 (10.2)	70.4 (1.4)	21.7 (1.2)	7.9 (0.9)	1.20 (0.14)
Controls (n = 9)	34 (23-54)	—	—	22.4 (0.7)	1.16 (0.11)	5.76 (0.28)	46.4 (5.2)	69.7 (1.4)	22.7 (0.8)	7.7 (1.0)	1.10 (0.13)
Male maturity onset (n = 7)	55 (46-70)	53 (44-69)	1 insulin 2 oral 4 diet	26.0 (1.6)	1.28 (0.18)	5.65 (0.33)	57.4 (17.0)	66.4 (2.4)	21.9 (1.5)	11.7 (1.2)	1.69 (0.15)
Controls (n = 7)	53 (46-66)	—	—	26.2 (1.2)	1.23 (0.19)	5.81 (0.45)	57.8 (17.8)	69.5 (2.5)	21.7 (2.3)	8.8 (1.1)	1.35 (0.20)
Female maturity onset (n = 11)	60 (47-68)	53 (36-66)	1 insulin 4 oral 6 diet	29.2 (2.1)	1.69 (0.20)	6.54 (0.48)	24.2 (5.7)¶	66.3 (1.1)	23.7 (0.7)	10.0 (1.0)	1.38 (0.13)
Controls (n = 11)	58 (47-68)	—	—	27.8 (1.6)	1.25 (0.08)	6.36 (0.29)	54.1 (6.8)	64.3 (1.7)	26.1 (1.4)	9.6 (0.6)	1.27 (0.09)

\*Oral: oral hypoglycaemic agent. Diet: diet alone.

†Weight in kg/(height in m)<sup>2</sup>.

‡Normal range 0.5-1.7.

§Normal range 3.1-7.3.

||Vs controls P &lt; 0.05.

¶Vs controls P &lt; 0.005.

Table 2 Relative proportions of the three major glycine-conjugated bile acids and the glycine/taurine conjugation ratio in male and female diabetics and matched controls (mean, SEM)

	Males		Females	
	Diabetics (n = 16)	Controls (n = 16)	Diabetics (n = 12)	Controls (n = 12)
Glycocholic	42.0 (2.7)	41.8 (2.0)	42.7 (3.4)	38.2 (2.8)
Glycochenodeoxycholic	32.7 (1.9)	32.2 (2.1)	32.3 (3.2)*	40.0 (3.4)
Glycodeoxycholic	25.3 (3.4)	26.0 (2.8)	26.0 (4.0)	21.8 (3.0)
Glycine/taurine conjugation ratio	2.8 (0.3)	3.3 (0.4)	5.3 (1.2)	4.3 (0.7)

\*Versus controls P &lt; 0.05.

This table includes a female patient and control who were omitted from Table 1 because the control's bile was too weak for cholesterol estimation.

tion of cholecystokinin, 0.5 Ivy dog units/kg. Samples were immediately stored at -20°C and analysed for cholesterol, phospholipid, and bile acids—cholesterol by gas liquid chromatography, phospholipids by the method of King (1932), and total bile acids by 3 $\alpha$ -hydroxysteroid dehydrogenase (Bolton *et al.*, 1978). The ratio of the glycine conjugates of cholic, chenodeoxycholic, and deoxycholic acids was determined by thin layer chromatographic separation of the trihydroxy and dihydroxy fractions followed by 3 $\alpha$ - and 7 $\alpha$ -hydroxysteroid dehydrogenase enzymatic assay (Wicks *et al.*, 1978). The lipid composition of bile was expressed as the molar percentages of cholesterol, phospholipid, and bile acids, and as the cholesterol saturation index calculated by the Dam-Holzbach criteria according to Thomas and Hofmann (1973). Saturation indices were not corrected for total lipid concentration as suggested by Carey and Small (1978) because the samples were obtained by duodenal

aspiration and there was no way of deducing the concentration of bile within the biliary tract. An oral cholecystogram was not permitted by the ethics committee of the hospital, but all subjects were free of biliary symptoms. The statistical tests used were Student's *t* test and the Wilcoxon rank sum test as appropriate.

## Results (Tables 1 and 2)

As designed, the body mass index (W/H<sup>2</sup>) was closely similar in the three subgroups of diabetics and their paired controls. The fasting plasma triglyceride level was higher in female maturity-onset diabetics than in their controls (1.69  $\pm$  0.20 and 1.25  $\pm$  0.08 mmol l<sup>-1</sup> respectively, P < 0.05), but there was no significant difference between male diabetics of either group and their controls. A raised plasma triglyceride level (> 1.70 mmol l<sup>-1</sup>) was found in six

maturity-onset diabetics (four female, two male) and borderline levels were present in two male controls.

The total bile lipid concentration (the sum of bile acids, phospholipid and cholesterol) was substantially less in female diabetics than their controls ( $24.2 \pm 5.4$  and  $54.1 \pm 6.8$  mmol  $l^{-1}$  respectively,  $P < 0.005$ ), but there was no difference in the males. The molar percentages of cholesterol, phospholipid, and bile acids were not significantly different in diabetics and controls.

The cholesterol saturation index of bile was not significantly different in diabetics and their matched controls (Figure). This was true for both males

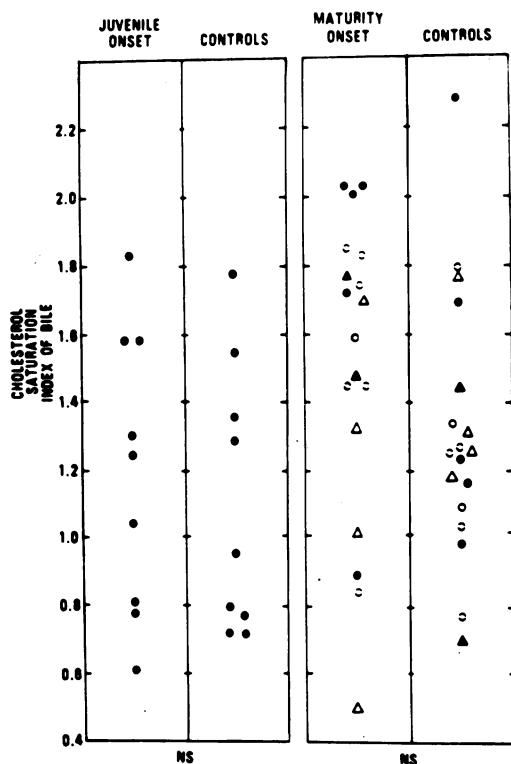


Figure The cholesterol saturation index of gallbladder bile aspirated from the duodenum of juvenile- and maturity-onset diabetics and of controls matched for age, sex, and obesity index (closed circles and triangles males, open circles and triangles females; triangles indicate diabetics with hypertriglyceridaemia and their controls).

(diabetics  $1.42 \pm 0.12$ , controls  $1.25 \pm 0.11$ ) and females ( $1.38 \pm 0.13$  and  $1.27 \pm 0.09$ ), and for the three subgroups of diabetics, as well as for all 27 diabetics combined ( $1.40 \pm 0.09$ , controls  $1.23 \pm 0.08$ ;  $t = 1.746$ ,  $2p = 0.092$ ). There was still no dif-

ference when the few weak bile samples (total lipid concentrations  $< 15$  or  $< 20$  mmol/ $l^{-1}$ ) were omitted from the calculations. Bile was found to be supersaturated (saturation index  $> 1.0$ ) in 21 (78%) of the diabetics and in 19 (70%) of the controls.

Maturity onset diabetics had more saturated bile than juvenile onset patients ( $P < 0.05$ ) and a higher body mass index ( $P < 0.05$ ). In the 27 control subjects the saturation index of bile was positively correlated with the body mass index ( $r = 0.41$ ,  $P < 0.05$ ) and with the plasma triglyceride concentration ( $r = 0.47$ ,  $P < 0.05$ ). No such correlations were present in the diabetics. In the six diabetics with hypertriglyceridaemia the mean saturation index was the same as in their controls (1.28 and 1.27 respectively), although the latter were all normotriglyceridaemic.

The relative proportions of glycocholate, glycochenodeoxycholate, and glycodeoxycholate, also the glycine/taurine conjugation ratio, were similar in diabetics and controls except that there was a just significant reduction in glycochenodeoxycholate in female diabetics (Table 2).

## Discussion

We have been unable to show any significant difference between the cholesterol saturation of bile of diabetic patients and that of control subjects matched for obesity as well as age and sex. There are several possible explanations. (1) The extra gallstones of diabetes may be cholesterol-poor (so-called pigment) stones. This seems unlikely since bile was supersaturated with cholesterol—at least by conventional criteria—in 78% of the diabetics. (2) Supersaturated bile may crystallise more readily in diabetes. This is pure speculation. (3) Gallstones may lead to diabetes rather than the reverse. This hypothesis was once popular (Robertson, 1945), but now it commands no support. (4) Gallstones may not be caused by diabetes but rather share a major aetiological factor with it. Obesity is a strong risk factor for both cholesterol gallstones and maturity onset diabetes. If, in matching the diabetics and controls for obesity, we inadvertently matched them for the strongest gallstone risk factor, one would expect the two groups to be similar in respect of a measure of the stone-forming tendency—namely, the cholesterol saturation of bile. On the other hand a difference might have emerged if larger numbers had been studied, especially in the maturity onset group, and it cannot be concluded that obesity is the only factor predisposing diabetics to gallstones. Indeed, although there was, in the control subjects, a positive correlation between the body mass index and the saturation index of bile while the maturity onset diabetics, who

tended to be obese, had greater saturation indices than the slimmer, juvenile onset ones, the diabetics as a group showed no relationship between bile saturation and obesity. This suggests the existence of additional factors in diabetics which modify bile secretion.

Hypertriglyceridaemia is another condition which can largely be attributed to obesity, or at least to weight gain since maturity (Blacket *et al.*, 1975; Lewis, 1976). In this study there was a significant if weak correlation between the plasma triglyceride concentration and the body mass index (in the 54 diabetics and controls combined,  $r = 0.266$ ,  $P < 0.05$ ). Hypertriglyceridaemia is particularly prone to occur in diabetics, as found here. It is also associated with an increased proneness to gallstones (Einarsson *et al.*, 1975). However, our data suggest that it does not increase the gallstone risk of diabetics. One third of the maturity-onset diabetics were found to have raised triglycerides, but their bile was no more saturated than that of their obesity-matched controls. A correlation between the plasma triglyceride level and the cholesterol saturation index of bile was present in the controls but not in the diabetics.

There may be other factors which favour gallstone formation in the diabetic. Impaired gallbladder contraction due to autonomic neuropathy is present in some patients (Grodzki *et al.*, 1968), and bile stasis could favour the retention of cholesterol crystals and hence formation of calculi. Impaired gallbladder emptying after exogenous cholecystokin could explain our finding of a lower total lipid concentration in the duodenal bile of maturity-onset female diabetics than in controls.

It was found in one study that insulin treatment increased the cholesterol saturation of bile (Bennion and Grundy, 1977). Our insulin-treated diabetics had near-normal saturation indices. However, our insulin-treated diabetics were male, slim, and insulin dependent, whereas those studied by Bennion and Grundy were female, grossly obese, non-insulin dependent, and from a population with an unusual susceptibility to gallstones.

In conclusion, these data suggest that in diabetic subjects obesity may be the most important factor predisposing them to gallstones. An additional factor, at least in females, may be gallbladder stasis.

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