Lipid composition of bile in diabetics and obesity-matched controls

G. B. HABER AND K. W. HEATON
With the technical assistance of J. Yeates

From the University of Bristol Department of Medicine, Bristol Royal Infirmary, Bristol

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 gallbladder 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)² (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. Pre-menopausal 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-
Lipid composition of bile in diabetics and obesity-matched controls

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mean, range)</th>
<th>Treatment*</th>
<th>Body mass index†</th>
<th>Plasma triglycerides (mmol l⁻¹)‡</th>
<th>Plasma cholesterol (mmol l⁻¹)§</th>
<th>Bile lipid composition</th>
<th>Percent of total moles</th>
<th>Saturation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male juvenile onset</td>
<td>34 (21-55)</td>
<td>All insulin</td>
<td>21.6 (0-7)</td>
<td>1.03 (0-12)</td>
<td>5.50 (0-32)</td>
<td>44.5 (10-2)</td>
<td>70.4 (1-4)</td>
<td>Bile acids</td>
</tr>
<tr>
<td>Controls</td>
<td>34 (23-54)</td>
<td>—</td>
<td>22.4 (0-7)</td>
<td>1.16 (0-11)</td>
<td>5.76 (0-28)</td>
<td>46.4 (5-2)</td>
<td>69.7 (1-4)</td>
<td>22.7 (0-8)</td>
</tr>
<tr>
<td>Female maturity onset</td>
<td>55 (46-60)</td>
<td>1 insulin 2 oral 4 diet</td>
<td>26.0 (1-6)</td>
<td>1.28 (0-18)</td>
<td>5.65 (0-33)</td>
<td>57.4 (17-0)</td>
<td>66.4 (2-4)</td>
<td>21.9 (1-5)</td>
</tr>
<tr>
<td>Controls</td>
<td>53 (45-66)</td>
<td>—</td>
<td>26.2 (1-2)</td>
<td>1.23 (0-19)</td>
<td>5.81 (0-45)</td>
<td>57.8 (17-8)</td>
<td>69.5 (2-5)</td>
<td>21.7 (2-3)</td>
</tr>
<tr>
<td>Male maturity onset</td>
<td>60 (47-68)</td>
<td>1 insulin 2 oral 4 diet</td>
<td>29.2 (2-1)</td>
<td>1.69 (0-20)</td>
<td>6.54 (0-48)</td>
<td>24.2 (5-7)</td>
<td>66.3 (1-1)</td>
<td>23.7 (0-7)</td>
</tr>
<tr>
<td>Controls</td>
<td>60 (47-68)</td>
<td>—</td>
<td>29.8 (2-1)</td>
<td>1.66 (0-20)</td>
<td>6.54 (0-48)</td>
<td>24.2 (5-7)</td>
<td>66.3 (1-1)</td>
<td>23.7 (0-7)</td>
</tr>
</tbody>
</table>

†Weight in kg/(height in m)‡.
§Normal range 0.5-1.7.
¶Normal range 3-17.3.
||Vs controls † < 0.05.
††Vs controls † < 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)

<table>
<thead>
<tr>
<th></th>
<th>Diabetics (n = 16)</th>
<th>Controls (n = 16)</th>
<th>Diabetics (n = 12)</th>
<th>Controls (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholic</td>
<td>42.0 (2.7)</td>
<td>41.8 (2.0)</td>
<td>42.7 (3.4)</td>
<td>38.2 (2.8)</td>
</tr>
<tr>
<td>Glycochenodeoxycholic</td>
<td>32.7 (1.9)</td>
<td>32.2 (2.1)</td>
<td>32.3 (3.2)</td>
<td>40.0 (3.4)</td>
</tr>
<tr>
<td>Glycodeoxycholic</td>
<td>25.3 (3.4)</td>
<td>26.0 (2.8)</td>
<td>26.0 (4.0)</td>
<td>21.8 (3.0)</td>
</tr>
<tr>
<td>Glycine/taurine conjugation ratio</td>
<td>2.8 (0.3)</td>
<td>3.3 (0.4)</td>
<td>5.3 (1.2)</td>
<td>4.3 (0.7)</td>
</tr>
</tbody>
</table>

*Versus controls † < 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.

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 3α- and 7α-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/H²) 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 ± 0.20 and 1.25 ± 0.08 mmol l⁻¹ 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⁻¹) 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 ± 5.4 and 54.1 ± 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 and females (diabetics 1.42 ± 0.12, controls 1.25 ± 0.11) and for the three subgroups of diabetics, as well as for all 27 diabetics combined (1.40 ± 0.09, controls 1.23 ± 0.08; t = 1.746, 2p = 0.092). There was still no difference 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, 1943), 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
Lipid composition of bile in diabetics and obesity-matched controls


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

G B Haber and K W Heaton

_Gut_ 1979 20: 518-522
doi: 10.1136/gut.20.6.518

Updated information and services can be found at:
http://gut.bmj.com/content/20/6/518

_Email alerting service_

_These include:_
Receive free email alerts when new articles cite this article.
Sign up in the box at the top right corner of the online article.

_Topic Collections_
Articles on similar topics can be found in the following collections
Pancreas and biliary tract (1949)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/