Gut, 1983, **24**, 2–6

Diet and gall stones: effects of refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism

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SUMMARY It has been suggested that consumption of refined carbohydrate foods (notably sugar and white flour) increases bile cholesterol saturation and hence the risk of cholesterol gall stone formation. To test this hypothesis, 13 subjects with probable cholesterol gall stones ate refined and unrefined carbohydrate diets, each for six weeks in random order. On the refined carbohydrate diet, subjects ate more refined sugar (mean = SEM: 106±7 *vs* 6±1 g/day, $p<0.001$), less dietary fibre (13±1 *vs* 27±3 g/day, $p<0.001$), and had a higher energy intake (9·17±0·66 *vs* 7·16±0·64 MJ/day, $p<0.001$). After each diet, the lipid composition of duodenal bile and bile acid kinetics was determined. The cholesterol saturation index of bile was higher on the refined carbohydrate diet in all but one subject, with a mean value of 1·50±0·10 compared with 1·20±0·12 on the unrefined diet ($p<0.005$). On the refined carbohydrate diet, bile contained relatively less cholic acid and slightly more deoxycholic acid. There were, however, no significant differences in total or individual bile acid pool sizes. There were also no differences in the rates of primary bile acid synthesis or fractional turnover on the two diets. Consumption of carbohydrate in refined form increases bile cholesterol saturation. The risk of gall stones might be reduced by avoidance of refined carbohydrate foods.

Cholesterol-rich gall stones are commonly associated with overweight[1] and a dietary factor has long been suspected in their aetiology. As long ago as 1892, Osler suggested a role for sweet foods.[2] More recently, the hypothesis has been put forward that refined carbohydrates are the main dietary culprit,[3 4] their pathogenicity being blamed on their lack of dietary fibre as well as on their tendency to cause overnutrition.[5] In animals, experimental diets which induce cholesterol gall stones are generally rich in refined carbohydrates and their effect can be lessened by adding fibre to the diet.[4] In man, however, direct evidence of the lithogenicity of refined, fibre-depleted diets is limited to the beneficial effect of bran on bile cholesterol saturation.[6–8] In order to test the hypothesis directly, we have determined the effects of refined and unrefined carbohydrate diets on the bile of 13 subjects with probable cholesterol gall stones.

Rcceived for publication 7 April 1982

Methods

SUBJECTS

Thirteen subjects (10 women, three men) with radiolucent gall stones were studied, with ethical committee approval. All had normal plasma lipids and standard liver function tests. None was taking oral contraceptives or other medication. Their mean age was 46 years (range 26–64 years) and their mean weight 120% of ideal (median 117%, range 86–149%).

The subjects were instructed to eat refined and unrefined carbohydrate diets, each for a six-week period in random order. This period was chosen as previous work with bran suggested it is the minimum necessary to effect a significant change in biliary bile acid composition.[9] On both diets the subjects were allowed to eat as much as they wished of animal foods and permitted plant foods. On the refined carbohydrate diet, the permitted plant foods were those containing refined sugar, white flour, and white rice, whereas on the unrefined carbohydrate

diet these foods were forbidden and only wholegrain products were allowed. The intake of vegetables and fruit was unlimited on the unrefined diet, but was restricted on the refined diet to three helpings of vegetables per day and 10 items of fruit per week. The subjects were not instructed to eat additional dietary fibre in the form of wheat bran when consuming the unrefined diet. Thus, in one diet, the carbohydrate was unrefined and fibre-rich, and in the other it was mostly refined and fibre-depleted.

Dietary intakes were assessed by the same dietitian throughout the study, by means of personal interviews and also by detailed dietary records completed by the subjects on every sixth day. This latter method provided a check on compliance with the diets. Dietary data were analysed by computer, using a programme compiled from standard food tables.[10] Refined sugars were defined as sugars separated from their natural fibrous matrix and were taken to include honey but not lactose.

Bile was sampled after the six-week period on each diet. After an overnight fast, bile-rich duodenal fluid was collected by means of duodenal intubation and intravenous injection of cholecystokinin. The composition of bile obtained in this manner reflects accurately that of gall-bladder bile.[11] In six subjects, 5 μcuries each of ^{14}C-cholic acid and ^{14}C-chenodeoxycholic acid (Radiochemical Centre, Amersham, England) were given intravenously after each diet and bile was collected on the following four mornings in order to determine bile acid pool sizes and the rates of primary bile acid synthesis and fractional turnover.[12] In one further subject, bile acid pool sizes alone were determined by isotope injection and a single duodenal intubation.[13]

Total bile salt, phospholipid and biliary cholesterol concentrations were measured.[14] These values were used to calculate bile cholesterol saturation indices by the method of Thomas and Hofmann[15] using the criteria of Hegardt and Dam.[16] Bile samples with a total lipid concentration of <20 mmol/l were rejected as being too weak for accurate analysis.

Bile samples were deproteinised by brief boiling with alcohol, deconjugated using the enzyme cholylglycine hydrolase (Sigma Chemical Co), methylated by the use of diazomethane and subsequently converted to trifluoroacetates by the addition of trifluoroacetic anhydride.[17] Mean recovery of bile acids was 91±4%. These bile acid derivatives were used for the gas-liquid chromatographic determination of the proportions of cholic, chenodeoxycholic, deoxycholic and unsulphated lithocholic acids in relation to an internal standard of 7-ketolithocholic acid. The analyses were performed using a Pye-Unicam GCD chromatograph and 3% QF-1 columns. The column temperature was 230°C, injection port temperature 245°C, and flame-ionisation detector temperature 260°C. The carrier gas (nitrogen) flow rate was 60 ml/min, hydrogen 65 ml/min, and air 300 ml/min. The percentage coefficients of variation of this method are shown in Table 1.

In bile samples containing ^{14}C-isotopes, the deconjugated bile acids were separated by thin-layer chromatography using a solvent system of ethyl acetate:2,4-trimethylpentane:glacial acetic acid, 5:5:1.[18] Specific activities of the separated bile acids were determined after enzymatic assay for mass[14] and liquid scintillation counting of radioactivity. From these data, the pool sizes, daily synthesis, and fractional turnover rates of the primary bile acids were calculated.[12] Deoxycholic acid pool size was estimated from the relative proportions of deoxycholic and chenodeoxycholic acids as measured by gas-liquid chromatography. Total bile acid pool size was taken as the sum of the three major individual bile acid pools (the trivial contribution of lithocholic acid being ignored). The statistical significance of differences was determined by Student's *t* test.

Results

As expected, the subjects ate substantially more refined sugar and less dietary fibre on the refined carbohydrate diet. They also had a higher energy intake (Table 2). On this diet, body weight rose 1·6±0·4 kg. Weight fell 1·5±0·3 kg on the unrefined carbohydrate diet without any of the subjects complaining of being hungry.

On the refined carbohydrate diet, all the subjects had bile which was supersaturated with cholesterol. The saturation index was higher on the refined carbohydrate diet in all but one subject (Figure), the mean being 1·50±0·10 on the refined diet compared with 1·20±0·12 on the unrefined diet (<0·005). The mean molar percentages of total bile acids, phospholipids, and cholesterol on the two diets are shown in Table 3.

Analysis of individual bile acid composition

Table 1 *Percentage co-efficients of variation for individual bile acid analysis by gas liquid chromatography*

	Cholic acid	*Chenodeoxycholic acid*	*Deoxycholic acid*	*Lithocholic acid*
Within-assay	5·8	4·7	3·4	11·4
Between-assay	7·9	5·7	6·1	13·3

Table 2 *Dietary intake (g/day) on refined and unrefined carbohydrate diets*

	Diet		
	Refined	*Unrefined*	*p*
Refined sugar	106±7	6±1	<0·001
Dietary fibre	13±1	27±3	<0·001
Cereal	6±1	15±2	<0·001
Non-cereal	7±1	12±1	<0·001
Carbohydrate	266±19	158±15	<0·001
Protein	75±5	75±6	NS
Fat	93±8	86±8	NS
Cholesterol (mg/day)	406±52	363±50	NS
Energy (MJ/day)*	9·17±0·66	7·16±0·64	<0·001

* 1 MJ = 239 kcal.

showed a lower percentage of cholic acid and slightly more deoxycholic acid on the refined diet (Table 3). However, there were no significant differences in bile acid pool sizes (Table 4). There were also no differences in the rates of primary bile acid synthesis or fractional turnover (Table 5). Apart from the four major bile acids, only trace amounts of other bile acids were detected on either of the two diets.

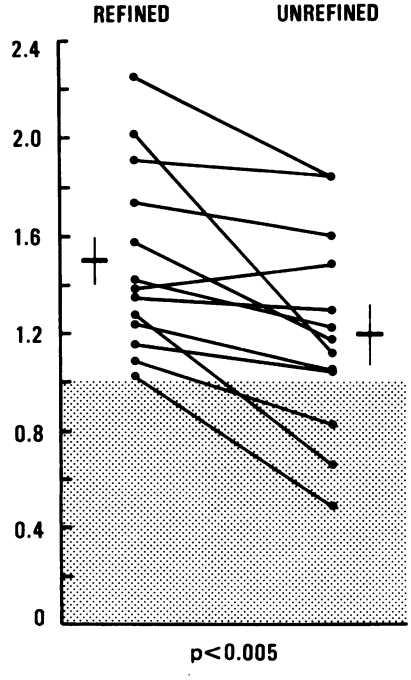

Figure *Cholesterol saturation index of bile in 13 subjects on refined and unrefined carbohydrate diets*

Table 3 *Mean molar percentages of cholesterol, phospholipids and total bile acids, and percentage bile acid composition on refined and unrefined carbohydrate diets*

	Diet		
	Refined	*Unrefined*	*p*
Cholesterol	9·1±0·8	6·8±0·6	<0·01
Phospholipids	17·8±0·7	18·4±0·9	NS
Total bile acids	70·9±2·6	75·8±2·2	<0·05
Cholic acid	36·9±1·9	42·1±1·9	<0·002
Chenodeoxycholic acid	32·1±2·1	31·0±2·1	NS
Deoxycholic acid	28·7±2·8	25·1±2·4	<0·02
Lithocholic acid	2·2±0·3	1·8±0·1	NS

Discussion

This study has demonstrated that bile is significantly more saturated with cholesterol after six weeks on a refined carbohydrate diet than after a similar period on an unrefined carbohydrate diet. This effect of refined carbohydrate might be expected to increase the risk of cholesterol gall stone formation, as the degree of bile cholesterol supersaturation is considered to be the main factor affecting cholesterol precipitation.[19]

This dietary effect is presumably due to changes either in bile acid pool size or in biliary cholesterol secretion. As there was no significant difference in bile acid pool size on the two diets, it seems likely that there was a change in cholesterol secretion.

Several explanations are possible for the observed difference in bile cholesterol saturation on the two diets. Theoretically, it might be due to the different intakes of refined sugar, dietary fibre, or energy, or to the changes in weight, or to a combination of these factors.

The beneficial effect on bile of weight reduction has been reported only with substantial weight loss (mean 25 kg) and after a weight-maintaining diet has been resumed.[20] The small changes in weight of our subjects (1–2 kg) seem insufficient to account for their changes in bile saturation. Another possible

Table 4 *Bile acid pool sizes (mmol) on refined and unrefined carbohydrate diets*

	Diet		
	Refined	*Unrefined*	*p*
Cholic	1·97±0·23	2·18±0·11	NS
Chenodeoxycholic	1·18±0·15	1·04±0·12	NS
Deoxycholic	1·83±0·33	1·44±0·18	NS
Total	4·98±0·57	4·67±0·33	NS

Table 5 *Primary bile acid synthesis (mmol/day) and fractional turnover (pools/day) on refined and unrefined carbohydrate diets*

	Diet				
	Refined		*Unrefined*		
	Synthesis	*Frac. turn.*	*Synthesis*	*Frac. turn.*	*p*
Cholic	0·54±0·05	0·30±0·04	0·59±0·13	0·26±0·05	NS
Chenodeoxycholic	0·28±0·04	0·25±0·02	0·25±0·04	0·25±0·04	NS

explanation is that the 28% greater intake of energy on the refined carbohydrate diet resulted in a greater secretion of biliary cholesterol. Reduction of energy intake from about 4000 to 1000 kcal/day has been associated with a fall in bile cholesterol secretion (though not in bile saturation).[20] The effects of lesser changes in energy intake, however, have not been reported.

The greater intake of energy on the refined diet was largely attributable to the much greater intake of refined sugar (chiefly sucrose). This raises the possibility that sucrose, as such, has an effect on bile cholesterol saturation.

Another possible explanation for more saturated bile on the refined carbohydrate diet is the lower content of dietary fibre of this diet. Raising fibre intake by addition of wheat bran to the diet reduces the cholesterol saturation of bile, provided that the bile was initially supersaturated.[6–8] Bran, however, is thought to act by altering bile acid metabolism, initially by reducing secondary bile acid formation as manifest by a lower percentage of deoxycholic acid in bile. The present study demonstrated only a 3·6% fall in the mean percentage of deoxycholic acid on the unrefined carbohydrate diet, which was much less than that shown in bran feeding studies.[6–8] Hence, it is unlikely that the difference in fibre intake on the two diets fully explains the observed difference in bile cholesterol saturation. The percentage of the primary bile acid, cholic acid, was lower on the refined carbohydrate diet, but the biological significance of this is uncertain. The percentage of chenodeoxycholic acid, a bile acid known to be capable of lowering bile cholesterol saturation and dissolving cholesterol gall stones, was not significantly different on the two diets.

Whatever the mechanism involved, this study shows that consumption of carbohydrate in refined form increases the cholesterol saturation of bile. The amounts of refined sugar (106 g/day) and dietary fibre (13 g/day) eaten by our subjects during the refined carbohydrate diet are commonly eaten in Britain.[21 22] Thus, in susceptible individuals, the avoidance of refined carbohydrate foods and their replacement by wholegrain products and by fruit and vegetables might reduce the risk of cholesterol gall stone formation.

This study was supported by a grant from the Medical Research Council. We thank Mrs J S Nicholls for technical assistance and Mr J C A Emmett for compiling the computer programme.

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