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Liver and biliary

Effects of dietary sucrose on factors influencing cholesterol gall stone formation

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SUMMARY Twelve subjects with radiolucent gall stones and bile supersaturated with cholesterol were studied after six weeks each on diets which contained 112 g and 16 g respectively of refined (fibre depleted) sucrose but which allowed free access to other foods. Energy intake was 24·5% higher on the high sugar diet and body weight ended 1·4 kg higher than on the low sugar diet. Biliary secretion rates of cholesterol, phospholipid and bile acid, measured by a perfusion technique, were similar on the two diets, as were the bile acid pool sizes measured by isotope dilution. Cholesterol saturation index of fasting 'gall bladder' bile was 1·30±0·11 and 1·37±0·14 on high and low sugar respectively (NS). Plasma triglycerides were 36% higher and plasma high density lipoprotein cholesterol concentrations were 9% lower on the high sugar diet. These findings indicate that over a six week period refined sugar in normally consumed amounts does not adversely affect the lipid composition of bile.

On epidemiological and experimental grounds one of us postulated some years ago that the consumption of refined carbohydrates (better described as fibre depleted foods) favours the secretion of bile supersaturated with cholesterol and hence increases the risk of gall stones.[1 2] It has also been argued that the nature of refined foods, especially refined sugars, is such that they inevitably inflate the intake of energy.[3 4] These hypotheses were put to the test by Thornton *et al*[5] in an experiment in which subjects with radiolucent gall stones were asked to eat *ad libitum* of a diet rich in refined carbohydrate in one study period while in the other they ate *ad libitum* of a diet which excluded refined foods and replaced them with unrefined or fibre rich foods. As predicted, energy intake was higher on the refined diet while 'gall bladder' bile aspirated from the duodenum was more saturated with cholesterol. Interpretation of these findings was complicated by the fact that, on the refined diet, there was not only a much greater intake of sucrose but also a much lower intake of fibre and it was not clear which of these was responsible for the deleterious effect on bile. A beneficial effect of wheat fibre, in the form of bran, on the cholesterol saturation of bile has been observed by most [6–8] but not all[9] workers when the bile is initially supersaturated, so lack of dietary fibre could perhaps have been responsible. On the other hand, when bran has had this beneficial effect it has usually changed the bile acid composition of bile, reducing the proportion of the secondary bile acid deoxycholic acid,[6–8] whereas in the experiment of Thornton *et al*[5] there was minimal change in bile acid composition. We therefore wondered if a high intake of sucrose was deleterious to bile[5] and the present study was designed to test this possibility.

Previous studies of bile composition on high and low intakes of sucrose are scanty and hard to interpret.[10 11] In one study,[10] a large amount of sucrose (200g/day) was given to 10 gall stone patients for 14 days before their operation, at which bile was taken from the common hepatic duct. There was no difference in biliary lipid concentrations and cholesterol saturation index between these patients and 22 controls who were studied after 14 days on the hospital diet. Limitations of this study are the use of hepatic rather than gall bladder bile samples and the apparent failure to match patients and controls. Furthermore, sucrose was exchanged isocalorically with ward food which, we consider, is artificial as in real life sucrose is added to foods and drinks, not substituted for them. Yet another limitation is the short duration of the experimental periods. Experience with bran suggests that dietary change may take six weeks to have a noticeable effect on the bile acid composition of bile.[12]

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The other study[11] also used an isocaloric design and 14 day experimental periods. In a crossover study, hyperlipoproteinaemic patients ate two diets, one deriving 60% of its energy from carbohydrate including much sucrose and the other deriving 60% of its energy from fat. The molar percentage of cholesterol in duodenal bile after cholecystokinin was lower on the high carbohydrate diet. A further problem in interpreting this study is the complex way in which the diet was changed. Intakes of dietary fibre were not analysed but probably rose on the high carbohydrate diet.

In designing the present study, sucrose was treated in what we hoped was a realistic manner, as a sweetening agent rather than as a food. On the low sucrose diet, subjects were allowed to use artificial sweeteners. No attempt was made to control total food intake but, rather, subjects ate *ad libitum* on both diets, apart from sucrose. Hence, we were able again to test the hypothesis that refined carbohydrate, but this time specifically refined sugar, inevitably inflates energy intake by comparing total energy intake on the high and low sucrose diets.

At the same time, the opportunity was taken to study the effect of sucrose on plasma lipids. This seemed relevant as plasma triglyceride concentrations rise with increased carbohydrate intake[13] and especially with sucrose[14] while, in large amounts, sucrose can lower plasma high density lipoprotein cholesterol[15] and as bile cholesterol saturation is correlated with plasma triglyceride levels[16 17] and, inversely, with high density lipoprotein cholesterol.[17]

Methods

SUBJECTS

Twelve subjects (eight women and four men) with radiolucent gall stones and with normal liver function tests, plasma lipids and plasma glucose concentrations volunteered for this study, which had been approved by the district ethical committee. All were shown to have bile supersaturated with cholesterol at an initial duodenal intubation which immediately preceded the study in most cases but in a few had been performed 6–24 months before as part of another study. All the women were postmenopausal except one who had had a tubal ligation. The subjects' mean age was 48 years (range 26–69 years) and their mean body weight was 119% of ideal (range 96–151%).[18] Five were frankly obese (relative weight >120%). None of the subjects was taking any medication.

DESIGN OF STUDY

The subjects were instructed to eat two diets, one high and one low in refined (fibre depleted) sucrose, each for a six week period in random order. Otherwise, the subjects' normal daily routine remained unchanged. In the high sucrose period they were asked to add household sugar to drinks, and to eat sweets, cakes, biscuits and other prepared foods sweetened with sucrose. To ensure that their daily sucrose intake exceeded 100 g they were asked to drink every day one or two cans of proprietary soft drink each containing 29–39 g sugar. During the low sucrose period subjects were instructed to add saccharine (if desired) to food or drinks, to avoid prepared foods sweetened with sugar, and to drink one or two cans per day of soft drink of the same flavour as the sugar containing one but sweetened with saccharine. Cans of soft drink were supplied free of charge. Other qualitative dietary changes were discouraged but subjects were told not to go hungry and were allowed to eat *ad libitum* of foods free of added sucrose.

Every sixth day the subjects recorded on a specially designed form everything they ate or drank. This produced seven record forms for each six week period, providing information on dietary intake for every day of the week. Subjects were interviewed by the same experienced dietitian to clarify any ambiguities on their forms. Amounts were recorded in household measures and converted to grams. To aid these estimates, samples of representative food items were bought and weighed. Mean daily intakes of energy, nutrients and dietary fibre were calculated by computer using a program compiled from standard food tables.[19]

At the end of each six week period the subjects attended early in the morning after their usual overnight fast. Fasting blood samples were obtained for plasma lipid and glucose estimation and the duodenum was intubated. In two subjects who were unwilling to undergo the perfusion study, bile rich duodenal contents were obtained after intravenous injection of Pancreozymin (Boots) 95 units for analysis of bile lipid and bile acid composition. In the other 10 subjects, five μCi each of 24-^{14}C-chenodeoxycholic acid and 24-^{14}C-cholic acid (Radiochemical Centre, Amersham) were given intravenously. Intestinal perfusion was then carried out by the method of Grundy and Metzger[20] with minor modifications. The subjects were intubated with a triple lumen tube having a proximal aspiration port 1 cm cephalad to the proximal infusion port and a 12 cm mixing segment. The proximal aspiration port was positioned in the middle of the duodenal loop under fluoroscopic control. Pancreozymin 95 units was given intravenously and 8–10 ml bile rich duodenal juice collected for estimation of cholesterol saturation index. Perfusion was then

carried out for 10 hours at a rate of 2 ml/min using a Harvard peristaltic pump. The perfusing fluid was prepared by taking 1 litre of a mixture of synthetic L-amino acids (Synthamin 9, Travenol Laboratories, Thetford) and adding to it 50 g D-glucose, water to 1·5 litre and bromsulphthalein as a non-absorbable marker in a final concentration of 0·1 mg/ml. Intestinal contents were aspirated continuously by suction pump at a rate of 12·5–15 ml per minute per port and the aspirate from each hour was pooled. Aliquots of each hourly sample were immediately pipetted out and frozen at −20°C until analysis.

ANALYTICAL PROCEDURES AND CALCULATIONS

Cholesterol in intestinal aspirates was measured by gas liquid chromatography and total bile acids by an enzymatic method.[21] Phospholipids were measured in the concentrated initial samples using a digestion method[22] but with the perfused samples an enzymatic method (Phospholipid B-Test, Wako Chemicals, Osaka, Japan) was used because it is unaffected by the phosphate ions in the perfusing solution.[23] Bromsulphthalein was measured by the method of Seligson *et al.*[24] The sizes of the cholic and chenodeoxycholic acid pools were measured by isotope dilution. To allow time for the injected radioactive bile acid to mix with the pool, bile acid specific radioactivity was measured only in the last six hourly collections of aspirate, in which specific activity was relatively constant. Samples were deproteinised with ethanol and the bile acids deconjugated with cholylglycine hydrolase (Sigma Chemicals, London). The deconjugated bile acids were extracted with ether and separated by thin layer chromatography using iso-octane:ethylacetate:glacial acetic acid 5:5:1. Cholic and chenodeoxycholic acids were assayed for mass enzymatically and for radioactivity by liquid scintillation counting.[5]

Deoxycholic acid pool size was determined from the proportion of this bile acid relative to chenodeoxycholic acid in the concentrated initial bile sample as measured by gas-liquid chromatography. Samples for gas-liquid chromatographic analysis were deconjugated enzymatically and methylated using diazomethane before injection on to a Pye GCD dual flame ionisation detector chromatograph fitted with 1·52 m × 2 mm id glass columns packed with 2AQ/3% SP 2250 (Pye Unicam Ltd). The column temperature was 275°C and the carrier gas was N_2 at a rate of 40 ml/min.

For plasma lipid and lipoprotein estimations, serum was centrifuged at 150 000 g for three to four hours in a Beckman 'Airfuge' to isolate very low density lipoprotein. High density lipoprotein was separated by precipitation of very low density lipoprotein and low density lipoprotein with sodium phosphotungstate. HDL_2 and HDL_3 were isolated as supernatant and infranatant respectively after ultracentrifugation at d 1·125. Cholesterol in serum and lipoprotein fractions was measured using the Boehringer C-system (CHOD/PAP) method and triglycerides by a fully enzymatic kinetic method (Boehringer). Low density lipoprotein cholesterol and triglycerides were found by difference.

The secretion rate of cholesterol was calculated from the relationship

$$\frac{\text{Chol secretion rate}}{\text{BSP infusion rate}} = \frac{\text{Chol concn of distal aspirate}}{\text{BSP concn of distal aspirate}}$$

The secretion rate of bile acid was derived from the cholesterol secretion rate as follows:

$$\frac{\text{BA secretion rate}}{\text{Cholesterol secretion rate}} = \frac{\text{BA concn in prox aspirate}}{\text{Chol concn in prox aspirate}}$$

and the secretion rate of phospholipid was calculated on the same principle.

Biliary cholesterol saturation index was calculated by the method of Thomas and Hofmann[25] using the criteria of Hegardt and Dam.[26]

The statistical significance of differences was determined by Student's *t* test for paired data.

Results

As planned, there was a mean difference of 96 g/day in refined sugar intake between the low and high sugar periods (Table 1). Energy intake was 1·55 MJ/day (370 kcals/day) higher during the high sugar period, a difference of 24·5%, and was higher in all 12 subjects. Body weight was 1·4 kg higher at the end of the high sugar period than at the end of the

Table 1 *Dietary intakes and body weight on the two diets (mean ± SEM)*

	Low sugar	*High sugar*	*p value*
Energy (MJ/day)*	6·33±0·46	7·88±0·45	<0·001
Refined sugar (g/day)	15·7±2·4	111·5±9·5	<0·001
Other carbohydrate (g/day)	139·2±13·9	136·9±8·2	NS
Protein (g/day)	64·2±3·7	62·4±4·1	NS
Fat (g/day)	72·3±6·9	75·5±6·1	NS
Vit C (mg/day)	45·7±3·9	36·7±2·0	<0·05
Folic acid (mg/day)	175±11·5	143±7·2	<0·001
Dietary fibre (g/day)	18·7±1·3	15·5±0·7	<0·01
Body wt (kg)	72·0±5·0	73·4±5·1	<0·005

* 1 MJ = 239 kcal·

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low sugar period.

All other dietary differences were minor and in particular there was no difference in non-sugar carbohydrate, protein, fat or cholesterol intake. There were slight but statistically significant differences in dietary fibre, vitamin C and folic acid intakes (Table 1) which are attributable to the consumption of more fruit on the low sugar diet.

The cholesterol saturation index of bile was 1·30±0·11 on the high sugar and 1·37±0·14 on the low sugar diet (NS). The biliary secretion rates of cholesterol, bile acid and phospholipid were not significantly different after the two dietary periods (Table 2). There were also no differences in bile acid pool sizes (Table 3), the percentage molar biliary lipid composition and the relative proportions of chenodeoxycholic, deoxycholic and cholic acids (Table 4).

Total plasma triglyceride and low density lipoprotein triglyceride levels were 36% and 33% higher respectively on the sugar rich diet, while high density lipoprotein cholesterol levels were 9·0% lower (Table 5).

Fasting plasma glucose levels were the same on both diets.

Discussion

This study shows that, over a six week period, the consumption of refined sucrose, in amounts which are frequently taken in the UK[27] and which appreciably increase energy intake, does not adversely affect bile lipid composition nor alter the secretion rate of cholesterol. This implies that our previous finding of an adverse effect on bile after six weeks on a diet rich in refined sugar and starch[5] was due not to the sugar content of the diet nor to its higher energy value but to some other factor. Perhaps the low intake of dietary fibre on the refined diet was the responsible factor, despite our earlier doubts.

At face value, the present findings either contradict the refined carbohydrate hypothesis for the aetiology of cholesterol gall stones[1,2] or, taken in conjunction with our previous study,[5] suggest that a refined diet is pathogenic only inasmuch as it is deficient in dietary fibre. The high sucrose diet, however, was taken for only six weeks and an effect on bile might become apparent over a longer period. This possibility is rendered more likely by the clear cut effects of the high sucrose diet on energy intake and plasma lipids.

This study shows that when sucrose is included in the diet in realistic amounts most individuals do not compensate for the increased energy intake by eating less food, and most gain weight. It remains to be shown that this energy increment and weight gain continue over a long period. Nevertheless, the data support the view that sucrose consumption is important in the causation of overnutrition and obesity.[3,4] Obesity in turn is a major risk factor for gall stones.[28]

Hypertriglyceridaemia is strongly associated with cholesterol gall stones[16,29] and, even in normolipidaemic subjects, there is a correlation between the plasma triglyceride concentration and the cholesterol-saturation index of bile.[17] Hence, the well-documented ability of dietary sucrose to raise plasma triglyceride concentrations, which was confirmed in this study, suggests that dietary sucrose will predispose at least some individuals to gall stones. Similarly, there is an inverse correlation between plasma high density lipoprotein cholesterol and the cholesterol saturation index of bile[17] and an increased risk of gall stones in people with low high density lipoprotein cholesterol levels.[30] Thus, the present finding of a lower high density lipoprotein cholesterol level on the high sucrose diet (previously shown only with very high intakes of sucrose[15]) once

Table 2 *Secretion rates of the biliary lipids on the two diets (μmol/h)*

	Low sugar	*High sugar*	*p*
Cholesterol	61±5	59±9	NS
Bile acid	1010±146	897±222	NS
Phospholipid	125±30	91±15	NS

Table 3 *Bile acid pool sizes on the two diets (mmol)*

	Low sugar	*High sugar*	*p*
Cholic acid	2·96±0.31	3·24±0·61	NS
Chenodeoxycholic acid	2·01±0·28	2·44±0·47	NS
Deoxycholic acid	1·07±0·26	1·09±0·17	NS
Total*	6·04±0·69	6·77±1·11	NS

* Ignoring lithocholic and trace bile acids which were not measured.

Table 4 *Mean molar percentages of biliary lipids and percentage bile acid composition* in fasting duodenal bile*

	Low sugar	*High sugar*	*p*
Cholesterol	7·6±0·8	7·6±0·9	NS
Phospholipid	16·9±1·0	16·8±1·4	NS
Total bile acids	75·5±1·7	75·6±2·0	NS
Cholic acid	27·0±2·2	26·6±3·3	NS
Chenodeoxycholic acid	48·3±2·2	49·1±2·0	NS
Deoxycholic acid	25·1±3·7	24·3±4·3	NS

* In nine subjects; samples insufficient in three.

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Table 5 *Fasting plasma cholesterol and triglyceride concentrations on the two diets (mmol/l)*

	Low sugar	*High sugar*	*p*
Cholesterol			
Total	5·34±0·38	5·49±0·34	NS
HDL	1·22±0·07	1·11±0·05	<0·02
HDL_2	0·28±0·06	0·33±0·07	NS
HDL_3	0·95±0·09	0·86±0·09	NS
LDL	3·63±0·28	3·66±0·35	NS
Triglyceride			
Total	0·98±0·15	1·33±0·27	<0·05
VLDL	0·40±0·11	0·57±0·19	NS
HDL	0·22±0·02	0·26±0·02	NS
LDL	0·39±0·06	0·52±0·07	<0·05

again suggests that dietary sucrose will favour gall stone formation in some people.

The lower intakes of vitamin C, folic acid and dietary fibre on the high sugar diet are of uncertain significance. Nothing is known of the effects of these two vitamins on bile composition. The difference in dietary fibre intake was probably too small (3 g/day) to affect bile acid metabolism but there have been no reports on the effects of fruit fibre on bile. When bran, which is usually about 40% fibre, has been shown to alter bile composition the daily dose has generally been 30 g or more.[6–8]

In conclusion, this study indicates that refined sugar in normally consumed amounts does not adversely affect the lipid composition of bile over a six week period. The effects of sugar on energy intake and plasma lipids, however, suggest that, in the long term, it might increase the risk of gall stones.

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