

Intestinal bile acid malabsorption in cystic fibrosis

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Abstract

This study aimed at examining the mechanisms participating in excessive faecal bile acid loss in cystic fibrosis. The study was designed to define the relation between faecal fat and faecal bile acid loss in patients with and without cystic fibrosis related liver disease; to assess terminal ileal bile acid absorption by a seven day whole body retention of selenium labelled homotaurocholic acid (SeHCAT); and to determine if small intestinal bacterial overgrowth contributes to faecal bile acid loss. The study population comprised 40 patients (27 men; median age 18 years) with cystic fibrosis (n=8) and without (n=32) liver disease and eight control subjects. Faecal bile acid excretion was significantly higher in cystic fibrosis patients without liver disease compared with control subjects (mean (SEM) 21.5 (2.4) and 7.3 (1.2) $\mu\text{mol/kg/24 hours}$ respectively; $p < 0.01$) and patients with liver disease (7.9 (1.3) $\mu\text{mol/kg/24 hours}$; $p < 0.01$). No correlation was found between faecal fat (g fat/24 hours) and faecal bile acid ($\mu\text{mol/24 hours}$) excretion. Eight (33%) of cystic fibrosis patients had seven day SeHCAT retention $< 10\%$ (normal retention $> 20\%$). SeHCAT retention in cystic fibrosis patients with liver disease was comparable with control subjects (30.0 (SEM) 8.3% v 36.8 (5.9)%; $p = \text{NS}$) while SeHCAT retention in cystic fibrosis patients who did not have liver disease was significantly reduced (19.9 (3.8); $p < 0.05$). Although evidence of small bowel bacterial overgrowth was present in 40% of patients no relation was found between breath hydrogen excretion, faecal fat, and faecal bile acid loss. The results are consistent with the presence of an abnormality in terminal ileal function in patients with cystic fibrosis who do not have liver disease and that a defect in the ileal absorption of bile acids may be a contributory factor to excessive faecal bile acid loss. Faecal bile acid loss in cystic fibrosis is unrelated to the presence of intraluminal fat or intestinal bacterial overgrowth.

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Excessive faecal bile acid loss is well recognised in patients with cystic fibrosis¹⁻⁶ and has been attributed to an inhibitory effect of intraluminal unhydrolysed triglycerides on the intestinal absorption of bile acids. Some studies, however, have shown no correlation between faecal fat excretion and faecal bile acid loss suggesting that additional factors are responsible for bile acid malabsorption in cystic fibrosis.³⁻⁸ Thus in vitro studies have shown a defect in the terminal ileal bile acid active transport mechanisms in patients with cystic fibrosis, which if present in vivo, would also contribute to excessive faecal bile acid

loss.^{9,10} In addition, intestinal bacterial overgrowth, resulting from prolonged intestinal transit and stasis,¹¹ may result in deconjugation and dehydroxylation of bile salts and contribute to impaired bile acid absorption and faecal bile acid loss.

Excessive faecal bile acid losses have not been found in all studies in adult patients with cystic fibrosis.^{3,12} This finding has been attributed to the increased prevalence of hepatic dysfunction, with associated impairment of bile acid synthesis and contraction of the total bile acid pool, in adults with cystic fibrosis.³

The objectives of this study in an adult population of patients with cystic fibrosis were to: (1) define the relation between faecal fat and faecal bile acid excretion in patients with and without liver disease; (2) to discover if a defect in the ileal bile acid active transport mechanism contributes to faecal bile acid loss; and (3) to assess the contribution of small bowel bacterial overgrowth to faecal fat and faecal bile acid losses.

Methods

PATIENTS

The study population comprised of 40 patients with cystic fibrosis admitted to the Adult Cystic Fibrosis Centre for evaluation of their respiratory or hepatic state, or both. Most patients were receiving standard treatment with bronchodilators, antibiotic prophylaxis against pseudomonas or staphylococcal infections, or both, and pancreatic enzyme supplements. The presence of hepatic disease was determined by abnormal liver biochemistry tests (gammaglutamyl transferase $> 50 \text{ IU/l}$, 5' nucleotidase $> 15 \text{ IU/l}$) of at least six months duration, histological evidence of fibrosis/cirrhosis on liver biopsy and the presence of portal hypertension, or both. Eight patients in whom there was no evidence of gastrointestinal, pancreatic, or hepatobiliary disease served as a control population.

Dietary advice to achieve a total energy intake of 120-150% of the recommended daily allowance for age,¹³ with approximately 40% of the total energy intake derived from fat, was given to each patient. All patients received fat and water soluble vitamin supplements and none were taking taurine supplementation.

FAECAL FAT AND BILE ACID ANALYSIS

Faecal fat and faecal bile acid analysis was performed on 17 patients with cystic fibrosis (five with liver disease) and eight control subjects. Faecal samples were collected over three days during which at least 50 grams of fat were ingested per day. The dietary intake over the 72 hour collection period was noted and fat intake

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TABLE I Clinical features of cystic fibrosis patients with and without liver disease

	No liver disease (n=32)	Liver disease (n=8)	p Value
Median age in years (range)	20 (13-32)	15 (14-21)	NS
Men:women	22:10	5:3	NS
Pancreatic supplements	30/32	6/8*	NS
Meconium ileus equivalent	17/32	5/8	NS
Body weight (%IBW (SEM))	84.2 (2.8)	84.5 (2.8)	NS

IBW=Ideal body weight; NS=not significant; *=one patient pancreas sufficient.

calculated in grams/day. Three day stool collection were stored at -20°C and were subsequently analysed for fat and bile acid content.

FAECAL FAT MEASUREMENT

Faecal fat analysis was performed using the method of van de Kamer¹⁴ and expressed as g fat/g stool/24 hours. The coefficient of fat absorption (CFA) was calculated from the daily dietary fat intake and the daily stool fat output and expressed as a percentage of the daily fat intake.

$$\text{CFA} = \frac{\text{Daily fat intake (g)} - \text{daily stool output (g)}}{\text{Daily fat intake (g)}}$$

FAECAL BILE ACID MEASUREMENT

Bile acids were extracted from a 100 mg aliquot of dried homogenate of faeces. Fifty microlitres of [¹⁴C]-sodium cholate was added as an internal standard to allow assessment of recovery rates at the end of the extraction procedure. Bile acids were hydrolysed under alkaline conditions at 220°C and were subsequently neutralised with hydrochloric acid and extracted with diethyl-ether. Total faecal bile acids were measured using a 3a-hydroxysteroid dehydrogenase assay¹⁵ and expressed as μmol bile acid/g stool/24 hours. The results were also expressed as mg glycocholate equivalent bile acid/g stool/24 hours by adding a glycocholate standard to the stool specimen before analysis.

SELENIUM HOMOTAUROCHOLIC ACID (SeHCAT) RETENTION

The seven day retention of ⁷⁵selenium labelled homotaurocholic acid (SeHCAT), a bile acid specifically absorbed by an ileal active transport mechanism,^{16,17} was measured in 21 cystic fibrosis patients (six with cystic fibrosis related liver disease) and eight control subjects. One

TABLE II Faecal fat and faecal bile acid excretion in cystic fibrosis patients with and without liver disease

	No liver disease (n=12)	Liver disease (n=5)	Control (n=8)
FF (g fat/g stool/24 hours) × 10 ⁻³	45.9 (8.6)	50.0 (15.1)	26.9 (3.5)
CFA (%)	85.7 (3.9)	81.3 (9.1)	97.1 (0.5)*
FBA (μmol/kg body wt/24 hours)	21.5 (2.4)†	7.9 (1.3)	7.3 (1.2)
FBA (mg bile acid/g stool/24 hours) × 10 ⁻²	175.4 (32.5)‡	86.5 (28.6)	100.3 (12)
Seven day SeHCAT retention (%)	19.9 (3.8)§	30.0 (8.3)	36.8 (5.9)

*=p<0.01 compared with cystic fibrosis patients; †=p<0.01 compared with liver disease and controls; ‡=p<0.05 compared with liver disease and controls; §=p<0.05 compared with controls. FF=faecal fat, CFA=coefficient of fat absorption, FBA=faecal bile acids.

TABLE III Hydrogen breath tests in cystic fibrosis patients with and without liver disease

	No liver disease (n=28)	Liver disease (n=5)	p Value
Fasting breath H ₂ (ppm)			
>12<75	5	3	NS
>75	8	0	NS
Fasting H ₂ <12 ppm × 100% post sucrose	3	1	NS
H ₂ breath test			
Positive	16/28	4/5	NS
Negative	12/28	1/5	NS

H₂=hydrogen; ppm=parts per million; NS=not significant.

microcurie (37 kBq) of SeHCAT was given orally in capsule form to each subject. Whole body retention (% of dose) of selenium radioactivity was measured on two occasions using a shallow shield whole body counter (Cammerra Accuscan) three hours and seven days after ingestion of the radiolabelled isotope.

HYDROGEN BREATH TEST ANALYSIS

Increased breath hydrogen excretion after the ingestion of glucose and increased breath hydrogen excretion in the fasting state, is a useful indicator of intestinal bacterial overgrowth.^{18,19} Hydrogen breath tests were performed on 33 patients with cystic fibrosis including five patients with cystic fibrosis related liver disease and eight control subjects. None of the patients had ingested lactulose in the preceding 36 hours. Breath hydrogen concentration was measured using an exhaled hydrogen monitor (Keymed). After an overnight fast (minimum 10 hours) two 20 ml aliquots of end expiratory breath samples were obtained using a modified Haldane-Priestly tube. Breath hydrogen was measured immediately and if less than 12 parts per million (ppm) 50 g of sucrose was given orally. After ingestion of sucrose, breath hydrogen was measured at 20 minute intervals for 160 minutes. If during this period breath hydrogen increased by 100% or more the test was considered abnormal. If the fasting breath hydrogen was greater than 12 ppm fasting was continued for up to 14 hours and a breath hydrogen persistently greater than 12 ppm at the end of this period was considered abnormal.

EFFECT OF ANTIMICROBIAL TREATMENT

To find out if small bowel bacterial overgrowth contributes to faecal fat and faecal bile acid losses four patients with positive hydrogen breath tests and excessive faecal fat and faecal bile acid losses were treated with a seven day course of oral metronidazole (400 mg three times daily). Hydrogen breath tests and three day stool collections for faecal fat were repeated at the end of the course of treatment.

STATISTICS

All data are expressed as mean (SEM). Differences between groups were compared using a Wilcoxon rank sum test. Faecal fat and faecal bile acid values before and after metronidazole

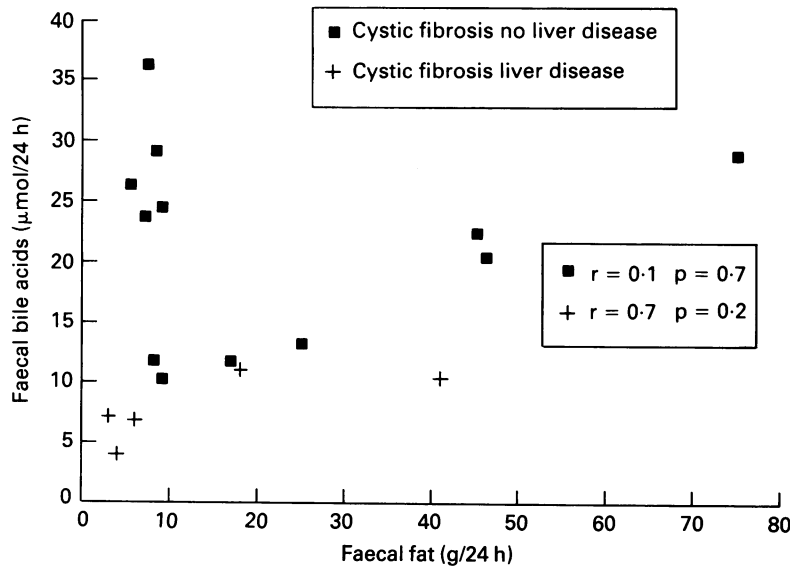


Figure 1: Correlation between faecal fat and faecal bile acid excretion in cystic fibrosis patients with and without liver disease.

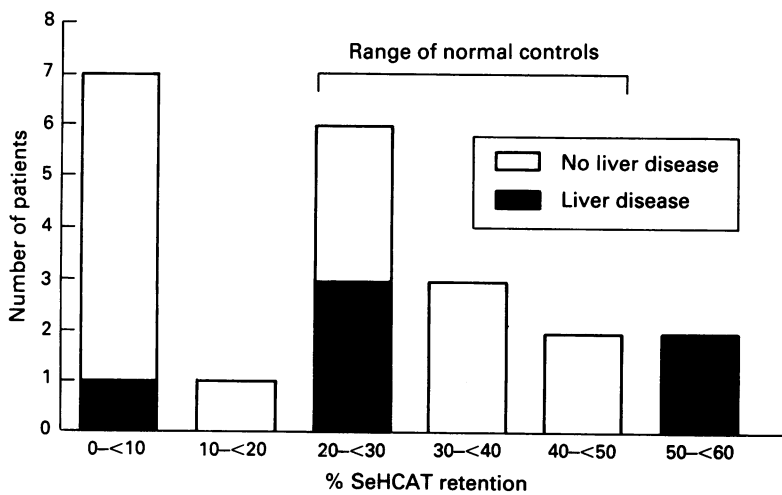


Figure 2: Percentage SeHCAT retention in 21 patients with cystic fibrosis.

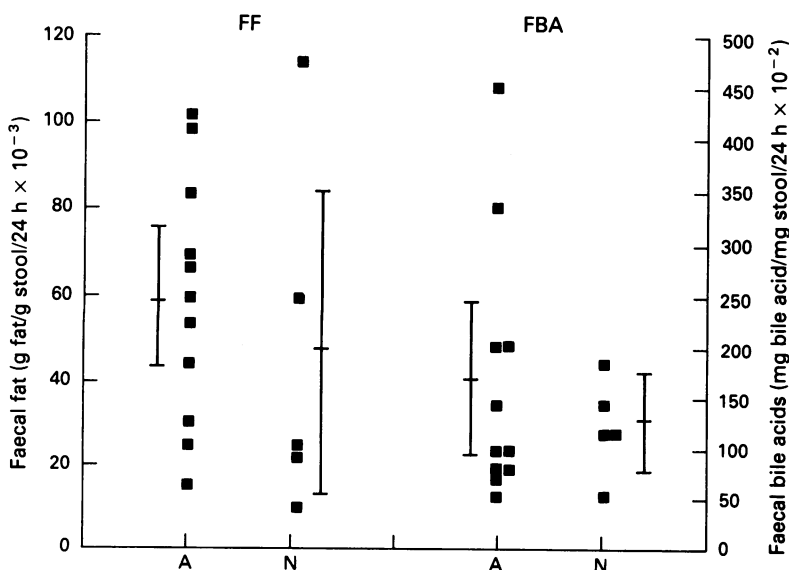


Figure 3: Faecal fat (FF) and faecal bile acid (FBA) excretion in patients with abnormal (A) and normal (N) hydrogen breath tests. Bars indicate mean and standard deviation of the mean.

treatment were compared using a paired Student's *t* test. A *p* value of <0.05 was considered significant.

Results

PATIENTS

The patients with and without liver disease were comparable with regard to age, sex, pancreatic enzyme supplementation, the prevalence of meconium ileus equivalent, and nutritional state (Table I). All patients with liver disease had advanced disease with either fibrosis on liver biopsy examination or clinical or endoscopic evidence of portal hypertension.

FAECAL FAT

Faecal fat excretion, expressed as g fat/g stool/24 hours in patients without liver disease ($45.9 (8.6) \times 10^{-3}$) was similar to that seen in patients with liver disease ($50.0 (15.1) \times 10^{-3}$). The coefficient of fat absorption was 81.3 (9.1)% and 85.7 (3.9)% in the patients with and without liver disease respectively and significantly lower than in control subjects (97.1 (0.5)%; *p*<0.01; Table II).

FAECAL BILE ACID EXCRETION

Faecal bile acid excretion was significantly higher in cystic fibrosis patients without liver disease compared with control subjects (21.5 (2.4) and 7.3 (1.2) µmol/kg/24 hours respectively; *p*<0.01) and patients with liver disease (7.9 (1.3) µmol/kg/24 hours; *p*<0.01). Similar results were obtained when faecal bile acid losses were expressed as mg glycocholate equivalent bile acid/g stool/24 hours × 10⁻² (Table II).

There was no correlation between faecal fat (g fat/24 hours) and faecal bile acid (µmol bile acid/24 hours) excretion in the patient group as a whole or when patients were stratified for the presence or absence of liver disease (Fig 1). Similarly no correlation was seen between faecal fat expressed as g fat/g stool/24 hours and faecal bile acids expressed as mg bile acid glycocholate equivalent/g stool/24 hours (data not shown). Three of four patients with the highest faecal bile acid losses had normal or near normal fat excretions of 4.5, 6.9, and 7.1 g/24 hours.

SeHCAT RETENTION

The mean percentage seven day retention of SeHCAT in patients with liver disease was 30.0 (8.3)% and comparable with that in control subjects (36.8 (5.9)%; *p*=NS). In contrast a mean percentage seven day retention in patients without liver disease of 19.9 (3.8)% was lower (*p*<0.05) than in control subjects and patients with liver disease although the second did not reach statistical significance (Table II). Seven day SeHCAT retention was abnormal in eight (38%) of 21 cystic fibrosis patients only one of whom had liver disease. SeHCAT retention in the remaining five patients with liver disease was similar to, or greater than, the value in control subjects (Fig 2).

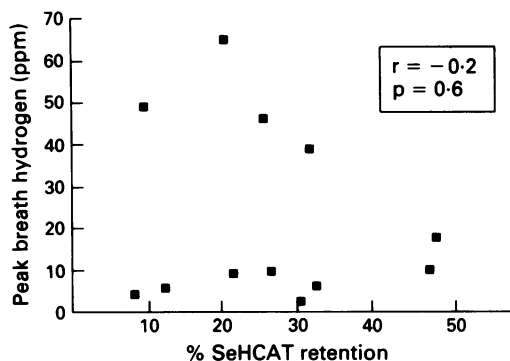


Figure 4: Correlation between breath hydrogen excretion and % SeHCAT retention in 12 patients with cystic fibrosis.

BREATH HYDROGEN EXCRETION

Sixteen (48.5%) of 33 cystic fibrosis patients had an increased fasting breath hydrogen (three of five patients with liver disease and 13 of 28 patients without liver disease; $p=NS$) (Table III) with eight patients having appreciably raised (>75 ppm) values (range 75–780 ppm). The hydrogen breath test was positive in 20 of 33 (66%) patients and no difference was seen between the patients with and without liver disease (Table III). Fasting breath hydrogen in control subjects was less than 12 ppm (range 0–11 ppm) and did not increase after sucrose given orally.

RELATION BETWEEN BREATH HYDROGEN EXCRETION, FAECAL FAT AND FAECAL BILE ACID LOSSES, AND SeHCAT RETENTION

There was no significant difference in faecal fat excretion in patients with a positive and negative hydrogen breath test ($59.3 (8.6) \times 10^{-3}$ v $47.1 (18.9) \times 10^{-3}$ g fat/g stool/24 hours respectively ($p=NS$)). Similarly there was no significant difference in faecal bile acid excretion in patients with a positive and negative hydrogen breath test ($167.1 (37)$ v $125.4 (22.9)$ mg bile acid/g stool/24 hours respectively; $p=NS$; Fig 3). There was no

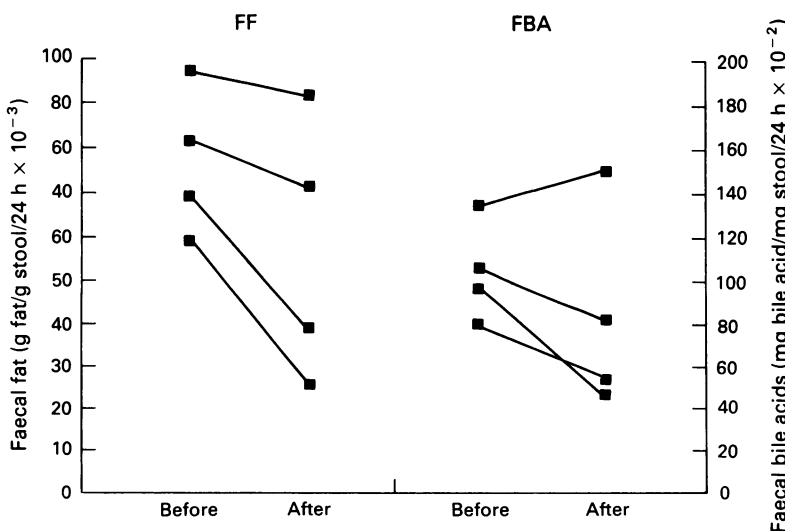


Figure 5: Faecal fat (FF) and faecal bile acid (FBA) excretion in four patients before and after seven days treatment with oral metronidazole. Breath hydrogen excretion had returned to normal in all four patients.

correlation between peak, after sucrose, breath hydrogen excretion and percentage SeHCAT retention in the 12 patients in whom both studies were performed ($r=-0.02$, $p=NS$; Fig 4).

EFFECT OF ANTIMICROBIAL TREATMENT

In four patients with positive hydrogen breath tests treated with oral metronidazole (400 mg three times daily) for seven days, fasting and post sucrose breath hydrogen excretion decreased to normal values of less than 12 ppm, which was associated with a reduction in faecal fat excretion in all four patients from $77.5 (8.4) \times 10^{-3}$ to $57.7 (15)$ g fat/g stool/24 hours $\times 10^{-3}$ ($p=NS$). Faecal bile acid excretion decreased in three of four patients from $105.2 (13.1)$ mg to $84.5 (23.3)$ mg bile acid/g stool/24 hours $\times 10^{-2}$ ($p=NS$; Fig 5).

Discussion

Several studies have shown that faecal bile acid excretion is appreciably raised in patients with cystic fibrosis^{2-4,6} and is comparable with that seen in subjects who have had ileal resection.² The suggestion that excess faecal bile acid losses in cystic fibrosis is related to the presence of intraluminal unhydrolysed triglyceride,^{2,4} is based on: (1) a close correlation between faecal bile acid and faecal fat excretion^{2,4}; (2) the finding that improvements in fat digestion with pancreatic enzyme supplementation was associated with a concomitant reduction in faecal bile acid losses^{2,4,6,12}; and (3) substitution of dietary fat with medium chain triglycerides resulted in a decrease in both faecal fat and faecal bile acid excretion.^{4,6} Not all studies support a direct link between fat malabsorption and faecal bile acid losses. Thus a correlation between faecal fat and faecal bile acid excretion has not been seen in all studies.^{3,5,6} Administration of sodium bicarbonate while reducing faecal fat excretion was not associated with a reduction in faecal bile acid losses and in vitro studies have failed to show an inhibitory effect of unhydrolysed triglycerides on ileal bile acid absorption.⁷

This study confirms previous findings that faecal bile acid losses are increased appreciably in patients with cystic fibrosis. Several findings are inconsistent with the theory that an inhibitory effect of unhydrolysed fat on small intestinal bile acid absorption is a major factor responsible for faecal bile acid losses including: (1) the absence of a correlation between faecal fat excretion and faecal bile acid loss; (2) the normal faecal bile acid losses in patients with liver disease compared with the excessive losses in those without liver disease despite similar faecal fat excretion; and (3) the finding that patients with near normal fat excretion continue to lose large amounts of bile acids. Thus factors other than the presence of intraluminal unhydrolysed triglycerides must be contributing to faecal bile acid loss in this group of patients.

In vitro studies have shown impairment of bile acid absorption in the ileal mucosa of patients with cystic fibrosis.^{9,10} A defect in ileal transport mechanisms is also supported by in vivo findings suggesting a selective malabsorption of cholic acid, a primary bile acid specifically absorbed by

an active transport mechanism in the terminal ileum.²⁰ Using a marker perfusion technique, however, the uptake of taurocholate and glycocholate was measured in three infants with cystic fibrosis and was noted to be similar to control subjects.²¹

The results of this study are consistent with a defect in the ileal transport of bile acids in patients with cystic fibrosis with over a third of such patients retaining significantly less radio-labelled bile acids over a seven day period compared with control subjects. Of particular interest were the findings that the defect was present predominantly in patients without liver disease and that bile acid absorption in patients with liver disease was comparable with control subjects. This finding also provides an explanation for the fact that faecal bile acid excretion in patients with liver disease is comparable with control subjects.

Intestinal motility disturbances with prolonged intestinal transit times are well described in patients with cystic fibrosis¹¹ and may predispose to the development of small bowel bacterial overgrowth, bacterial dehydroxylation and deconjugation of bile acids, decreased bile salt solubility, diminished intestinal bile acid absorption, and excessive faecal bile acid loss.^{17 22 23} Forty per cent of cystic fibrosis patients in this series had evidence of bacterial overgrowth based on a fasting breath hydrogen excretion of greater than 75 ppm after prolonged fasting or a positive hydrogen breath test.^{18 19} The reduction in post sucrose breath hydrogen excretion, faecal fat, and faecal bile acids after antibiotic treatment would suggest that small intestinal bacterial overgrowth contributes to malabsorption in these patients. It is unlikely, however, that bacterial overgrowth is a major factor determining the malabsorption of fat and bile acids as there was no significant difference in faecal fat and faecal bile acid excretion in patients with a positive or negative hydrogen breath test and no correlation was seen between breath hydrogen excretion and SeHCAT retention.

The results of this study show that: (1) excessive faecal bile acid losses in cystic fibrosis occurs almost exclusively in patients without liver disease of whom almost 50% have reduced retention of SeHCAT consistent with the presence of a terminal ileal bile acid active transport defect; (2) faecal bile acid loss is

unrelated to the presence of intraluminal fat; and (3) bacterial overgrowth in these patients plays a minor part in excessive faecal bile acid excretion.

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