In Vivo Bile Acid Uptake from Terminal Ileum in Cystic Fibrosis

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ABSTRACT. The cause(s) of excessive fecal bile acid loss in cystic fibrosis (CF) has not yet been fully determined, but *in vitro* studies have suggested that a primary mucosal defect in ileal bile acid uptake may be of importance. To examine this mechanism *in vivo*, the terminal ileal uptakes of taurocholate and glycocholate were determined using a marker-perfusion technique in three CF infants and four controls. Normal and CF ileal conditions were simulated by varying the taurocholate:glycocholate concentration ratio (normal 1:1, CF 1:4) and pH (normal 7.8, CF 6.0) of the perfusate. The mean bile acid uptake under normal perfusate conditions was not significantly different in CF subjects (taurocholate $0.124 \mu\text{mol/min/cm ileum} \pm \text{SD } 0.127$, glycocholate $0.117 \mu\text{mol/min/cm ileum} \pm 0.114$), and controls (0.142 ± 0.164 and 0.115 ± 0.120 respectively). Similarly, under CF conditions, bile acid uptake values in CF subjects and controls were similar. The results are not consistent with deranged ileal bile acid reabsorption being a major cause of fecal bile acid loss in CF. (*Pediatr Res* 23: 323–328, 1988)

Abbreviations

CF, cystic fibrosis
G/T ratio, glycine/taurine conjugation ratio
BSP, bromosulphophthalein
HPTLC, high performance thin layer chromatography

Excessive fecal bile acid loss has been well documented in patients with CF (1–3). The loss may predispose to depletion of total bile acid pools (3) and possibly to impaired nutrient absorption. In addition, because of man's limited ability to biosynthesize taurine (4), excessive bile acid loss may deplete taurine-conjugated bile acid pools and result in elevation of the G/T ratio of bile acids. Elevated G/T ratio has been noted frequently in CF (5–7), and may contribute to fat malabsorption (8).

The cause(s) of excessive fecal bile acid loss in CF have not yet been clearly identified. Proposed mechanisms (2) include excessive intraluminal binding of bile acids to food residues, precipitation of bile acids in the acidic duodenal contents of most CF subjects, interference with bile acid absorption by unhydrolyzed triglycerides and/or phospholipids, and defective bile acid uptake in the terminal ileum. Fondacaro *et al.* (9) have recently proposed on the basis of *in vitro* measurements of taurocholate uptake that bile acid malabsorption in CF results from a primary mucosal defect in active ileal bile acid transport mechanisms. The finding has not yet been confirmed *in vivo*,

nor has the ileal transport of other bile acids been examined in CF.

Our study was designed to examine the uptake of taurocholate and glycocholate *in vivo* in the ileum of CF infants with ileostomies after meconium ileus.

METHODS

Subjects. *CF Subjects.* Three CF infants (mean age 1.5 months, range 1–2) who had developed the neonatal complication of meconium ileus and required a temporary ileostomy of either the Bishop-Koop (two infants) or double-barrel (one infant) type (Fig. 1) were studied. Ileostomies in these children all gave access to a 10- to 20-cm segment of intact terminal ileum that had been sited within 25 cm of the ileocecal valve before surgery. The diagnosis of CF was based on elevated chloride concentrations ($>60 \text{ mmol/liter}$) in at least 100 mg of sweat collected after pilocarpine iontophoresis (10). All CF subjects had pancreatic insufficiency and were taking Pancrease R (Johnson & Johnson, Skillman, NJ: 1 capsule/90 ml of feed). All were feeding normally on a medium chain triglyceride-based formula.

Controls. Four non-CF infants (mean age 3.2 months, range 2.5–4) were studied as controls. Each infant had a temporary or permanent Bishop-Koop (one infant), double-barrel (one infant), or terminal (two infants) ileostomy (Fig. 1) that gave access to a terminal ileal segment 10–20 cm in length as described above. All controls were feeding normally on standard infant formulae at the time of study.

Control subject 1 was a prematurely born infant who developed necrotizing enterocolitis requiring total colectomy and terminal ileostomy in the neonatal period. The infant, aged 3 months postterm, had bronchopulmonary dysplasia and required additional oxygen at the time of study but was otherwise healthy. Subject 2, a full-term infant, required a double-barrel ileostomy because of necrotizing enterocolitis that developed after exchange transfusion in the 1st wk of life. No ileum was resected and the distal ileal stump was 2 cm in length. Subject 3 had Down's syndrome and a cyanotic congenital heart lesion requiring oxygen supplements. He developed necrotizing enterocolitis requiring a terminal ileostomy in the neonatal period but was active and feeding normally at the time of study. Subject 4 had long-segment Hirschsprung's disease requiring an ileostomy. The distal ileal loop measured 1 cm in length. The ileum had been biopsied and was not involved in the disease process.

The two subjects who required oxygen supplements were normoxemic at the time of study. The remaining terminal ileum was macroscopically and microscopically normal in all controls. Studies were performed in all children only when they were fully recovered from the effects of surgery (at least 3 wk postsurgery). All were gaining weight normally and none had excessive fluid loss through the ileostomy.

Marker-perfusion technique. The marker-perfusion technique

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Fig. 1. Configuration of ileostomies used in marker-perfusion techniques.

was adapted from the methods of Torres-Pinedo *et al.* (11) and Krag and Phillips (12).

Tube Characteristics and Placement. For subjects with a Bishop-Koop or terminal ileostomy a triple-lumen tube was constructed by introducing an orifice (port B) at 10 cm from the end (port A) of a 10-gauge double-lumen gastric tube (Porges, Paris, France). An 8-gauge infant feeding tube with its orifice (port C) 10–20 cm from the end of the gastric tube was then attached as shown in Figure 2a. The tube was inserted into the subject's ileostomy with port A proximal and port C distal and as close as possible to the ileostomy. In subjects with a double-barrel ileostomy, the distal ileal loop was perfused. This loop was not exposed to intestinal contents from above, obviating the need for a mixing segment. A double-lumen tube was therefore created by attaching an infant feeding tube to the gastric tube with its orifice (port A) 10–20 cm from the end (port C) of the gastric tube (Figure 2b). The tube was placed in the ileostomy so that port C was as close as possible to the ileocecal valve; proximal reflux of perfusion solution was prevented by gently inflating the tip of a 10-gauge Foley catheter proximal to port A. For both tubes, the introduction into the ileostomy of a 20-cm test segment (Fig. 2) was attempted. In three subjects (two CF, one control) the test segment length was reduced because of difficult access into, or reduced length of, the available ileal segment. The mixing segment of the triple-lumen tube was a constant 10 cm. Despite careful securing to the subject's ileostomy bag, the tubes sometimes became displaced. Results of perfusion studies were discarded for periods during which malposition of the tube had occurred. Subjects tolerated the procedures well, requiring no

Fig. 2. Tube systems used in marker-perfusion techniques. Infusions were administered through port A and samples collected through ports B and C. *a*, triple-lumen tube used with Bishop-Koop and terminal ileostomies. *b*, double-lumen tube used with double-barrel ileostomy.

sedation, and were fed and cared for normally throughout each study.

Perfusate Composition. Glycocholate, taurocholate (sodium salts; Sigma Chemical Co., St. Louis, MO) and electrolytes were added to create solutions of differing G/T ratio and pH and electrolyte composition (Table 1). Two solutions were made up to approximate the conditions found in the ileum of CF subjects (CF pH, CF G/T ratio: pH of approximately 6, G/T ratio of 4:1) and in the ileum of normal subjects (normal pH, normal G/T ratio: pH 7.8, G/T ratio 1:1). A further two solutions were composed of low pH with normal G/T ratio (CF pH, normal G/T ratio) and normal pH with elevated G/T ratio (normal pH, CF G/T ratio). BSP 0.01% w/v was added to each solution as a marker. A potential disadvantage of BSP as a marker is that it may bind to proteins, causing an uneven distribution of the marker within intestinal contents (13). The protein content of 15 intestinal fluid samples collected from port B in CF subjects was minimal (mean 2 g/liter, range 0–4).

Perfusion Method and Sample Collection. Each perfusate was infused via port A at 1.5 ml/min for 2 h using a constant rate infusion pump (IVAC model 700). Solutions were infused in random order over 2×2 h periods on 2 successive days, or over 4×2 h periods on a single day. For each solution an equilibration period of 20 min was allowed. Samples were then collected from port C by continuous siphonage over five or six 20-min periods. The expected transit time of ileal contents over the perfused segment is about 5 min (15). Staggering the sampling so that the proximal sample corresponds to the midpoint of each distal intestinal fluid collection period considerably increases the reproducibility of results of perfusion studies (15). A spot sample was therefore collected from port B by suction 5 min after the commencement of each port C collection period. The initial 2–3 ml of aspirate (corresponding to the dead space of the tube in use) was discarded before each sample of approximately 2 ml was collected from port B.

Sample analysis. Intestinal fluid samples were frozen immediately after collection and, together with an aliquot of perfusate,

Table 1. Composition of perfusates used in marker-perfusion studies

	G/T ratio*			
	Normal	CF	Normal	CF
	pH and electrolyte composition†			
	CF	CF	Normal	Normal
Bile acids (mmol/liter)				
Taurocholate	5	2	5	2
Glycocholate	5	8	5	8
pH	6	6	7.8	7.8
Electrolytes (mmol/liter)				
Sodium	130	130	125	125
Potassium	6	6	8	8
Chloride	86	86	120	120
Magnesium	1.75	1.75	1.75	1.75
Calcium	1.0	1.0	1.1	1.1
Bicarbonate	0.5	0.5	13	13
Glucose (mmol/liter)	4.0	4.0	4.0	4.0
Osmolarity (mosm/kg)	255	255	275	275

* The ratio of glycine:taurine conjugation of bile acids.

† Composition of 'CF' perfusate was based on analysis of ileal fluid contents from the first CF subject studied, while composition of normal perfusate was based on normal literature values (14).

were stored at -20°C until assayed. Sample pH was measured with a glass pH meter.

Bile Acids. Bile acids were measured in intestinal fluid samples by HPTLC as previously described (16).

BSP. BSP was measured by a method based on that of Oppe and Gibbs (17), except that acidification of the blank solution with 7.5 mmol/liter acetic acid was necessary because many samples of higher pH exhibited some color change that remained if the usual deionized water was used. Some samples, particularly those from CF subjects, were excessively turbid in neutral or acid solution and became less turbid with alkalization. The turbidity, which resulted in high absorbance at 580 nm in blank (acid) solutions and therefore interfered with BSP determination, could be removed by prewashing 1 ml of sample with 5 ml water-saturated spectrophotometric grade ether (Merck, Darmstadt, West Germany). The mean BSP recovered from 11 perfusate solutions assayed by this alternative technique was $98.1\% \pm \text{SD } 6.7$ and correlation between BSP values obtained from nonturbid intestinal fluid solutions with and without ether prewashing was good ($r = 0.992$, $n = 8$). BSP assays of intestinal fluid samples derived from a given perfusate in each individual subject were all performed by the same technique.

Bile acid uptake calculation. Net bile acid uptake was calculated by a method adapted from that described by Fordtran *et al.* (18):

For subjects with a Bishop-Koop or terminal ileostomy:

$$\text{net bile acid uptake} = \frac{V_B \cdot [X]_B - V_C \cdot [X]_C}{L} - S_B$$

($\mu\text{mol/min/cm ileum}$)

For subjects with a double-barrel ileostomy:

$$\text{net bile acid uptake} = \frac{V_A \cdot [X]_A - V_C \cdot [X]_C}{L}$$

($\mu\text{mol/min/cm ileum}$)

where $[X]_A$ = bile acid concentration of perfusate (mmol/liter); $[X]_B$ = bile acid concentration at port B (mmol/liter); $[X]_C$ = bile acid concentration at port C (mmol/liter); S_B = rate of sample collection at port B (ml/min); L = length of test segment (cm); V_A = infusion rate at port A = 1.5 ml/min; V_B = flow rate at port B (ml/min) = $V_A [BSP]_A / [BSP]_B$; $[BSP]_A$ = BSP concentration of perfusate = 100 mg/liter; $[BSP]_B$ = BSP concentration at port B (mg/liter); V_C = flow rate at port C (ml/min):

for Bishop-Koop or terminal ileostomy

$$V_C = V_B \cdot \frac{[BSP]_B}{[BSP]_C} - S_B$$

for double-barrel ileostomy

$$V_C = V_A \cdot \frac{[BSP]_A}{[BSP]_C}$$

$[BSP]_C$ = BSP concentration of port C (mg/liter).

Binding of bile acids to ileal mucosa has not, to our knowledge, been examined in man. In rats and hamsters, bile acid binding is less than 10% of uptake (19). Passive binding of bile acids was ignored in our study. The calculations assume constancy of the parameters described by Poiseuille's law, *i.e.* constancy between ports A and C of viscosity of intestinal fluid, and of pressure within, and radius and length of, the ileal segment. The net change in the values of these parameters over a number of time periods could be expected to be minimal. The model is only useful, however, when a mean of uptake values from a number of periods is taken.

Statistical analysis. The unpaired Wilcoxon rank sum test (20) was used to compare bile acid uptake volume in CF and control subjects as the uptake values were of non-Gaussian distribution.

All subjects participated in the study with the informed consent of their parents. The study was approved by the Adelaide Children's Hospital Research Ethics Committee.

RESULTS

The first 20-min sampling period yielded erratic results in subjects with a Bishop-Koop or terminal ileostomy which suggested that equilibration was incomplete. Uptake values for subsequent periods, however, and for all periods in subjects with a double-barrel ileostomy, were more consistent. In subjects with a double-barrel ileostomy, the lack of proximal intestinal secretions entering the test segment probably shortened the equilibration time. Uptake values from the first 20-min period in subjects with Bishop-Koop or terminal ileostomy were excluded from further analysis, effectively increasing the equilibration time to 40 min.

Bile acid uptake values for each individual were variable between periods but showed no particular trend. Bile acid uptake values from the CF subject with a double-barrel ileostomy were similar to those of CF subjects with a Bishop-Koop ileostomy. Data from all periods were therefore combined and are presented in Figure 3 and Table 2. Under all conditions of G/T ratio and pH and electrolyte composition, uptake of both taurocholate and glycocholate appeared to be similar in CF and control subjects.

Perfusion methods similar to those used in the current study (11, 15, 21) have, when used for the measurement of electrolyte and water uptake, resulted in large SD of uptake values similar to those noted in Table 2. Wide variations in results, even within an individual between periods, appear inevitable when using a model that assumes the steady state of the conditions described by Poiseuille's law, in the human intestine, where such steady state conditions cannot be consistently obtained. The similarity between the variability obtained in our study and that obtained elsewhere in normal individuals (11, 12, 15) suggests the variations did not arise from alteration to the ileum by surgery in subjects in our study.

Before the current perfusion studies, taurocholate and glycocholate levels were measured in intestinal fluid discharged from the ileostomies of two CF and two control subjects. The ileostomy fluid mean taurocholate concentration in the CF subjects was 0.50 mmol/liter whereas that of glycocholate was 3.5 mmol/liter (mean G/T ratio 7/1); both bile acids were below lower limits of detection of the HPTLC method (about 0.01 mmol/liter) in ileostomy fluid from each control.

Fig. 3. Bile acid uptake from terminal ileum. Bile acid uptake values from all collection periods at equilibrium are combined for each perfusate. ●, CF; ○, controls. * Perfusates were composed as described in Table 1.

During perfusion of normal perfusate, the pH values of 10 ileal contents samples collected from each of ports B (mean pH 8.11) and C (mean pH 8.38) in one CF subject was similar to that of the perfusate (pH 7.8). Similarly, during perfusion of CF perfusate, the pH values of four samples collected from each of ports B (mean pH 6.09) and C (mean pH 6.20) in one control subject were similar to that of the perfusate (pH 6.0). The concentration of bile acids at port B was similar to that of the perfusate for each perfusate in both CF and control subjects.

DISCUSSION

Herein we demonstrate no obvious decrease in ileal bile acid uptake rates in CF subjects compared with controls. The controls were slightly older than the CF subjects, a factor that may be relevant considering the rapid development of intestinal function in infancy. Whereas the exact ontogeny of bile acid uptake is not known in man, the uptake increases with age in the rat (19) and dog (22), suggesting that age-corrected values in CF subjects

Table 2. Bile acid uptake from terminal ileum in CF and control subjects

Perfusate composition†		Bile acid uptake ($\mu\text{mol}/\text{min}/\text{cm}$ ileum, mean $\pm$ SD)			
G/T ratio‡	pH and electrolytes	n‡	CF subjects	n*	Controls
Taurocholate					
Normal	CF	12	0.231 ± 0.166	14	0.054 ± 0.058
CF	CF	8	0.058 ± 0.127	14	0.049 ± 0.080
Normal	Normal	13	0.124 ± 0.127	13	0.142 ± 0.164
CF	Normal	11	0.010 ± 0.017	12	0.016 ± 0.062
Glycocholate					
Normal	CF	12	0.200 ± 0.174	14	0.034 ± 0.038
CF	CF	8	0.194 ± 0.303	14	0.081 ± 0.075
Normal	Normal	13	0.117 ± 0.114	13	0.115 ± 0.120
CF	Normal	11	0.103 ± 0.045	12	0.089 ± 0.081

* The ratio of glycine:taurine conjugation of bile acids.

† As described in Table 1.

‡ Number of 20 min-perfusion periods. None of the differences were statistically significant.

would not be lower, although they could be higher, than those in controls. While intensive mucosal function tests could not be undertaken in controls, the normal macroscopic and microscopic mucosal appearance, normal growth, and normal intestinal fluid output suggest that mucosal function was normal. Our results therefore indicate that ileal bile acid uptake is not impaired in CF.

The finding contrasts sharply with that of Fondacaro *et al.* (9) who demonstrated that taurocholate uptake in ileum from CF subjects in an *in vitro* model was about 30% that in control ileum. They proposed that the decreased uptake may be a manifestation of a primary defect in CF. The mean age of their subjects was, however, 16.7 yr, and it is possible that the defect they demonstrated was secondary to age-related mucosal damage. De Rooij *et al.* (23) also drew similar conclusions from *in vitro* studies of ileal mucosal taurocholate uptake in two CF subjects, in whom bile acid uptake was low but not abnormal compared with controls. Balistreri *et al.* (24), on the other hand, have shown a blunted postprandial rise in serum glycocholate in CF which could be corrected by pancreatic enzyme replacement. They concluded from this indirect measure of ileal function that ileal active transport of bile acids was normal in CF. Further, Roller and Kern (25) made similar conclusions using a $[1-^{14}\text{C}]$ cholyglycine breath test in adolescent CF subjects, although subjects in this study had virtually normal fecal bile acid loss.

The concentrations of bile acids used in perfusates in the current study were more than their respective K_m [0.6 mmol/liter for taurocholate in man (12) and 0.18 mmol/liter for glycocholate in the rat (26)]. These conditions would normally favor passive rather than active bile acid absorption. However, predominately passive absorption of glycocholate would be expected to result in a linear increase in uptake values with increasing intraluminal bile acid concentration. No such relationship was found herein. Ileal passive absorption of both glycocholate and taurocholate appears to be negligible (12); thus the relatively decreased uptake of taurocholate in CF and control subjects for perfusates of CF G/T ratio most likely represents competitive inhibition of taurocholate uptake by the higher glycocholate concentration rather than resulting from passive absorption. This conclusion is consistent with the similarity of uptake values for taurocholate (at normal G/T ratio) and for glycocholate; uptake values of both bile acids are similar in normal man (12). Thus the bile acid absorption demonstrated was almost certainly active for both taurocholate and glycocholate. Although active bile acid transport is absent in the newborn dog it develops rapidly in the neonatal period, reaching adult levels by 5 wk of age (22), and

by 3 wk of age in the rat (19). The apparent predominance of active uptake in the human subjects studied is consistent with similarly rapid development of the transport system in man. The bile acid concentrations were similar to those used *in vitro* by Fondacaro *et al.* (9) (0.1, 1.0, and 10.0 mmol/liter) and to those of the ileostomy fluid of CF subjects in our study before perfusion. The concentrations used thus appear to have been appropriate for the pathophysiology of CF.

Taurocholate and glycocholate uptake during perfusion of a solution of physiologic G/T ratio, pH, and electrolyte composition was similar in both CF and control subjects (Table 2) but the values for taurocholate were lower than those reported by Krag and Phillips (12) in normal adults (0.4 $\mu\text{mol}/\text{min}/\text{cm}$ ileum when corrected for perfusate bile acid concentration). Several factors may contribute to the difference. The infusion of both bile acids together will have resulted in competitive inhibition (12) and the ileal uptake mechanisms may not have been fully mature in subjects in the current study. Also, Krag and Phillips (12) used a perfusate infusion rate of 10 ml/min whereas that used herein (1.5 ml/min) is similar to the normal ileal flow rate of about 0.67 ml/min (15); infusion rates above 7 ml/min may cause physiologic changes in intraintestinal pressure and volume (27).

The cause of excessive fecal bile acid loss in CF remains unexplained. Absorption of bile acids other than cholic acid conjugates was not assessed herein. Cholic acid conjugates do, however, appear to be especially susceptible to malabsorption in CF, both in relation to, and independent of, pancreatic insufficiency (28). The current study showed persistent excretion of bile acids in CF subjects compared with controls in the presence of similar bile acid uptake values, suggesting an increased bile acid load from the proximal ileum in the CF subjects. The study did not examine the effect on bile acid absorption of intraluminal factors such as unhydrolyzed triglycerides and unhydrolyzed phospholipids, precipitation of bile acids in the more acidic CF duodenum, and adsorption of bile acids to food residues. These factors remain possible explanations for excessive fecal bile acid loss in CF (2).

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