CHAPTER 7

Increased fecal bile salt excretion is independent of the presence of dietary fat malabsorption in two mouse models for cystic fibrosis

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Abstract

Background & Aim: Recent studies from our laboratory suggested that fat malabsorption in cystic fibrosis (CF) patients on pancreatic enzyme replacement therapy is partially due to impaired intestinal uptake of long-chain fatty acids [1], which may be due to bile-related processes. To obtain more insight into the effects of CF on fat absorption and bile formation, we studied two mouse models for CF: mice homozygous for the ΔF508 mutation in the cftr gene (ΔF508/ΔF508), and mice in which the cftr gene is disrupted (cftr -/-). Methods: Fat absorption was studied by means of a 3-day fat balance, after feeding a standard (14 en% fat) or a high-fat (35 en% fat) diet for 2 weeks. Biliary bile salt secretion was determined during 80 min after cannulation of the gallbladder. Fecal bile salts were determined for 3 days. Results: In ΔF508/ΔF508 mice, dietary fat absorption was not significantly different from controls, and above 94% in all groups. However, dietary fat absorption in cftr -/- mice was significantly decreased compared to controls: standard diet: 82.8 ± 3.0% (mean ± SEM) and 93.9 ± 1.3% (P<0.01); high-fat diet: 88.8 ± 1.6% and 95.0 ± 1.4% (P<0.01), respectively. Biliary bile salt secretion rates were similar for the CF mouse models and their respective controls on either diet. The contribution of cholic acid to the biliary bile salt pool was slightly increased in both CF mice at the expense of deoxycholic acid. Primary bile salts were slightly increased, whereas secondary bile salts were slightly decreased in CF mice. Fecal bile salt excretion was increased in ΔF508/ΔF508 and in cftr -/- mice when compared with their respective controls (10 versus 5 µmol g⁻¹ feces, respectively, P<0.01). No significant correlation was observed between fecal excretion of bile salts and of fats. Conclusion: Cftr -/- mice, but not ΔF508/ΔF508 mice, have an impaired dietary fat absorption, which does not result from alterations in bile production. In both CF mouse models, fecal bile salt excretion was increased, but this was not related to increased fecal fat excretion. Bile composition data indicate that the increased fecal loss of bile salts is compensated for by an increased bile salt neosynthesis.
Introduction

Cystic fibrosis (CF), the most common recessive disorder in Caucasian populations, is caused by mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR gene encodes for a phosphorylation-regulated Cl⁻ channel and is expressed in the apical membrane of various epithelial cells [2-4]. Malfunction of this chloride channel in CF patients is associated with obstruction and inflammation of airways, pancreatic ducts, intestine and bile ducts, frequently resulting in intestinal fat malabsorption [5,6]. The most common mutation in the CFTR gene in Caucasian populations is a deletion of a phenylalanine residue at amino acid position 508 of the protein (ΔF508), which is found in 90% of the CF patients in Northern Europe [3,4]. The ΔF508 mutation disrupts the biosynthetic processing of CFTR to its mature glycosylated form [7], so that the protein is retained in the endoplasmic reticulum and subsequently degraded [8]. The protein does not reach the apical plasma membrane, and as a result, affected epithelia lack CFTR in the apical membrane and are deficient in cAMP-stimulated Cl⁻ permeability [8].

In an attempt to further elucidate the pathophysiology of CF, mouse models were developed [9-11]. The initial excitement generated by the emergence of these mouse models was somewhat tempered by the finding that none of the models showed spontaneous airway disease, which is primarily responsible for most of the morbidity and mortality in the human CF population. However, the various CF mouse models are remarkably similar to their human counterparts with respect to intestinal pathophysiology [11-14]. Most importantly, the intestinal tract of the CF mouse models demonstrates the absence or decrease of cAMP-mediated chloride transport which often results in intestinal obstructions, a hallmark of CF.

Most CF patients display a considerable malabsorption of dietary fats due to pancreatic insufficiency resulting in impaired lipolysis [5,15]. Pancreatic insufficiency can be alleviated by oral supplementation of pancreatic enzymes, however, many patients continue to experience a degree of steatorrhoea, with fat absorption ranging from 80 to 90% of their dietary fat intake [16-18]. Recently, we reported strong indications that fat malabsorption in CF patients on pancreatic enzyme replacement therapy is partially due to impaired intestinal uptake of long-chain fatty acids [1]. Impaired uptake may involve bile-related processes such as altered bile composition, decreased bile salt secretion, or bile salt inactivation at low intestinal pH [18-22]. Furthermore, small bowel mucosal dysfunction or alterations in the mucus layer have been suggested to contribute to inefficient intestinal uptake of long-chain fatty acids in CF patients [5,23]. So far it has been difficult to differentiate between these processes, partially due to the relative inaccessibility of the processes for mechanistic investigations in humans. Yet, a more detailed insight into the processes causing impaired uptake of long-chain fatty acids would allow the development of improved nutritional therapies. This will likely benefit CF patients, because the positive correlation between a good nutritional status and long-term survival or well-being of CF patients is well documented [24].

To obtain more insight into the effects of CF on fat absorption and bile formation we studied two recently generated CF mouse models: (i) Mice homozygous for the ΔF508 mutation in the cftr gene [11]. This mouse model was chosen because the ΔF508 mutation is the most frequently observed mutation in cystic fibrosis patients in Caucasian population; (ii)
Mice with complete inactivation of the cftr gene, cftr -/- “null” mice [14], expected to result in complete inactivation of the cftr gene, the most severe phenotype.

Materials and Methods

Animals
The male and female mice with the ΔF508 mutation (ΔF508/ΔF508) used in this study were generated by Van Doorninck et al. and are described in reference [11]. Mice with a targeted disruption in the cftr gene (cftr<sup>m1cam</sup> knockout mice, cftr -/-), resulting in complete loss of CFTR function, and their controls (cftr +/+) were described by Ratcliff et al. [14]. Experiments involving ΔF508/ΔF508 mice and their controls (N/N) were performed with the strain in 129/FVB genetic background [11], whereas experiments involving cftr -/- and cftr +/+ mice were performed with the strain in 129/C57/Bl6 genetic background [14]. All mice were obtained from the breeding colony at the Erasmus University Rotterdam, The Netherlands. The animals used for the experiments reported here were approximately 2-3 months old, had no obvious signs of disease or discomfort, and an average weight of 29 ± 1 g. The genotype of each individual animal was tested by Southern blotting of tail DNA [4]. For two weeks prior to the experiments, animals were kept on a semi-synthetic diet with standard amounts of fat (14 en% fat; 4.538 kcal kg<sup>-1</sup> food; fatty acid composition: C8-C12, 2.6%; C16:0, 13.5%; C18:0, 4.2%; C18:1n-9, 21.3%; C18:2n-6, 48.4%; C18:3n-3, 1.1%) or an isocaloric high fat diet (35 en% fat; 4.538 kcal kg<sup>-1</sup> food; fatty acid composition: C8-C12, 4.4%; C16:0, 28.5%; C18:0, 3.9%; C18:1n-9, 33.2%; C18:2n-6, 29.3%; C18:3n-3, 0.2%) (Hope Farms BV, Woerden, The Netherlands). The high-fat diet was applied to challenge the absorptive system for fats in the mouse intestine. Mice were housed in an environmentally controlled facility with diurnal light cycling and had free access to chow and tap water. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Erasmus University Rotterdam.

Study protocol
For fat balance measurements, feces was collected and food intake was recorded for 3 days. The gallbladder of mice was cannulated under Hypnorm (fentanyl/fluanisone, 1 mL kg<sup>-1</sup> body weight) and Diazepam anesthesia (10 mg kg<sup>-1</sup> body weight) and bile was collected in 20-minutes fractions for 80 minutes. Bile production was assessed by weight assuming that 1 mL of bile corresponds to 1 g of bile. After bile collection, a large blood sample (0.5-1 mL) was collected by cardiac puncture.

Analytical techniques
Lipids. Rat chow and feces were freeze-dried and mechanically homogenized. Aliquots of chow and feces were extracted, hydrolyzed and methylated [25]. Resulting fatty acid methyl esters were analyzed by gas chromatography to calculate fat intake and fecal fat excretion, as detailed below. Percentage of total fat absorption was calculated from the daily fat intake and the daily fecal fat excretion and expressed as a percentage of the daily fat intake.
Fat (mal)absorption in CF mice

Percentage of total fat absorption = \( \frac{\text{Fat intake (g day}^{-1}) - \text{Fecal fat output (g day}^{-1})}{\text{Fat intake (g day}^{-1})} \times 100\% \)

**Bile salts.** Total bile salt concentrations in bile and plasma were determined by an enzymatic fluorimetric assay [26]. Individual bile salts in bile were analyzed by gas chromatography after extraction with commercially available Sep-Pak-C\(_{18}\) cartridges (Waters Associates, Milford, MA, USA) [27]. Total fecal bile salt concentrations were extracted from an aliquot of freeze-dried homogenate [28] and fluorimetrically measured [26].

**Gas liquid chromatography.** Fatty acid methyl esters were separated and quantified by gas liquid chromatography on a Hewlett Packard gas chromatograph Model 6890 equipped with a capillary column (Hewlett Packard - Ultra 1, crosslinked methyl silicone gum; 50 m x 0.2 mm) and an FID detector. The gas chromatograph oven was programmed from an initial temperature of 160°C to 290°C in 2 temperature steps (160°C held 2 min; 160-240°C, ramp 2°C min\(^{-1}\), held 1 min; 240-290°C, ramp 10°C min\(^{-1}\), held 10 min). Quantification of the fatty acid methyl esters was performed by adding heptadecanoic acid (C17:0) as internal standard.

Bile salts were separated and quantified with a CP-SIL 19CB capillary column (25 m x 0.25 mm; Chrompack, Middelburg, The Netherlands). The gas chromatograph oven was programmed from an initial temperature of 240°C to 280°C in 1 temperature step (240°C held 4 min; ramp 10°C min\(^{-1}\); held 26 min). Quantification of bile salts was performed by adding 5β-cholestane-3βol (coprostanol) as internal standard.

**Calculations and statistics**
The experimental data are reported as means ± SEM. Differences between sample means of CF mice and their controls were analyzed by the two-tailed Student’s t-test for unpaired data or one-way ANOVA followed by post-hoc analysis (Student-Newman-Keuls). Differences between means were considered statistically significant at the level of \( P < 0.05 \). Analysis was performed with SPSS for Windows software (SPSS, Chicago, IL, USA).

**Results**

**Fecal fat balance**
Nutritional data of ΔF508/ΔF508 mice, \( cftr^{-/-} \) mice and their respective controls on standard chow and high fat chow are shown in Table 7.1. No differences were observed between ΔF508/ΔF508 and their controls with respect to fat intake, fecal fat excretion, net fat uptake or percentage of dietary fat absorption on either standard or high-fat diet. In \( cftr^{-/-} \) mice on standard diet, fecal fat excretion was significantly increased when compared with controls (82 ± 12 vs. 31 ± 4 µmol day\(^{-1}\), respectively, \( P < 0.01 \)). Accordingly, percentage of dietary fat absorption was significantly decreased from 93.9 ± 1.3% in \( cftr^{+/+} \) mice to 82.8 ± 3.0% in \( cftr^{-/-} \) mice (\( P < 0.01 \)). Similar results were obtained when \( cftr \) mice were fed the high-fat diet, although net fat uptake was approximately three-fold higher when compared with the standard diet: fecal fat excretion was significantly increased and percentage of dietary fat absorption...
was significantly reduced in \textit{cftr} -/- mice when compared with their control counterparts (Table 7.1).

**Bile salt concentrations**

\textit{Plasma}. Plasma total bile salt concentrations of \(\Delta F508/\Delta F508\) mice, \(\text{cftr} -/-\) mice and their respective controls on standard and high-fat chow are shown in Table 7.2. In CF patients, elevated plasma total bile salt concentrations are often an indication of liver disease or cholestasis [29]. No significant differences in plasma bile salt concentrations were observed between CF mice and their controls indicating that hepatic secretion of bile salts was not likely to be inhibited in either group.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.1.png}
\caption{Bile salt output during 20-minute fractions of bile collection after gallbladder cannulation for 80 minutes in \(\Delta F508/\Delta F508\) mice (129/FVB genetic background) (■) and their controls (□), and in \textit{cftr} -/- mice (129/C57/Bi6 genetic background) (●) and their controls (○) on a standard diet (14 en\% fat) and a high fat diet (35 en\% fat).}
\end{figure}

\textit{Bile}. Total biliary bile salt secretion in the 80-min period of bile collection was not significantly different between the CF mouse models and their respective controls on either diet (Table 7.2). In the \(\Delta F508/\Delta F508\) mice and their controls biliary bile salt secretion appeared to be slightly increased when they were fed the high-fat diet, however, the difference
was not significant. Bile salt secretion during the 80 minutes of bile collection decreased in all mice, indicating that the bile salt pool was depleting (Figure 7.1). In the 20-min period immediately after cannulation of the gallbladder, biliary bile salt profiles show that cholic acid and β-muricholic acid are the most predominant bile salts present in bile, together accounting for approximately 90% of the biliary bile salts (Table 7.2). The percentual contribution of cholic acid to the bile salt pool in cftr -/- mice on standard diet was significantly increased when compared with their controls (P<0.01). The percentual contribution of deoxycholic acid to the bile salt pool was decreased in ΔF508/ΔF508 mice on standard and high-fat diet, and in cftr -/- mice on a high-fat diet, compared with their controls (P<0.01). This tendency could also be observed in the other groups although the differences did not reach significance (P=0.07 and P=0.08). No differences were observed between CF mice and their controls with respect to concentrations of chenodeoxycholic acid, ursodeoxycholic acid, and β-muricholic acid on either diet.

Figure 7.2 Fecal bile salt excretion in ΔF508/ΔF508 mice (129/FVB genetic background), cftr -/- mice (129/C57/Bl6 genetic background) and their respective controls (N/N and cftr +/-) on a standard diet (14 en% fat) and a high-fat diet (35 en% fat).

Figure 7.3 Correlation between fecal fat excretion and fecal bile salt excretion in ΔF508/ΔF508 mice (129/FVB genetic background) (Ã), cftr -/- mice (129/C57/Bl6 genetic background) (~) and their respective controls (N/N (Ã) and cftr +/- (~)) on a standard diet (14 en% fat) and a high-fat diet (35 en% fat).
### Table 7.1 Nutritional data of ΔF508/ΔF508 mice (129/FVB genetic background), cftr −/− mice (129/C57Bl/6 genetic background) and their respective controls (N/N and cftr +/+ ) on standard chow (14 en% fat) and high-fat diet (35 en% fat) (mean ± SEM).

<table>
<thead>
<tr>
<th>Category</th>
<th>Diet</th>
<th>n</th>
<th>Food intake (g day⁻¹)</th>
<th>Fat intake (µmol day⁻¹)</th>
<th>Fecal fat (µmol day⁻¹)</th>
<th>Net fat uptake (µmol day⁻¹)</th>
<th>Fat absorption (% intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/N</td>
<td>Standard</td>
<td>6 (6M)</td>
<td>3.3 ± 0.4</td>
<td>534 ± 60</td>
<td>22 ± 2</td>
<td>513 ± 59</td>
<td>95.8 ± 0.4</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>Standard</td>
<td>5 (4M/1F)</td>
<td>3.5 ± 0.4</td>
<td>565 ± 73</td>
<td>29 ± 3</td>
<td>536 ± 72</td>
<td>94.8 ± 0.7</td>
</tr>
<tr>
<td>N/N</td>
<td>High fat</td>
<td>6 (3M/3F)</td>
<td>4.6 ± 0.5</td>
<td>1990 ± 215</td>
<td>94 ± 31</td>
<td>1896 ± 202</td>
<td>95.5 ± 1.2</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>High fat</td>
<td>5 (4M/1F)</td>
<td>4.6 ± 0.3</td>
<td>2024 ± 126</td>
<td>123 ± 27</td>
<td>1900 ± 115</td>
<td>94.0 ± 1.3</td>
</tr>
</tbody>
</table>

cftr +/+

<table>
<thead>
<tr>
<th>Category</th>
<th>Diet</th>
<th>n</th>
<th>Food intake (g day⁻¹)</th>
<th>Fat intake (µmol day⁻¹)</th>
<th>Fecal fat (µmol day⁻¹)</th>
<th>Net fat uptake (µmol day⁻¹)</th>
<th>Fat absorption (% intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>6 (5M/1F)</td>
<td>3.5 ± 0.3</td>
<td>562 ± 56</td>
<td>31 ± 4</td>
<td>531 ± 58</td>
<td>93.9 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>cftr −/−</td>
<td>Standard</td>
<td>6 (1M/5F)</td>
<td>3.0 ± 0.1</td>
<td>488 ± 22</td>
<td>82 ± 12*</td>
<td>406 ± 30</td>
<td>82.8 ± 3.0*</td>
</tr>
<tr>
<td>cftr +/+</td>
<td>High fat</td>
<td>6 (2M/4F)</td>
<td>3.5 ± 0.4</td>
<td>1507 ± 185</td>
<td>76 ± 23</td>
<td>1430 ± 178</td>
<td>95.0 ± 1.4</td>
</tr>
<tr>
<td>cftr −/-</td>
<td>High fat</td>
<td>5 (2M/3F)</td>
<td>3.1 ± 0.4</td>
<td>1371 ± 171</td>
<td>151 ± 25*</td>
<td>1221 ± 162</td>
<td>88.8 ± 1.6*</td>
</tr>
</tbody>
</table>

M, male; F, female. A symbol indicates a significant difference from controls; # P<0.05, * P<0.01.

### Table 7.2 Plasma bile salt concentration, biliary bile salt output during the total 80-min period and bile salt composition during the first 20 minutes of bile cannulation in ΔF508/ΔF508 mice (129/FVB genetic background), cftr −/− mice (129/C57Bl/6 genetic background) and their respective controls (N/N and cftr +/+ ) on standard chow (14 en% fat) and high-fat diet (35 en% fat) (mean ± SEM).

<table>
<thead>
<tr>
<th>Category</th>
<th>Diet</th>
<th>Plasma bile salts (µM)</th>
<th>Biliary bile salts (µmol/100 g BW)</th>
<th>C (%)</th>
<th>BMC (%)</th>
<th>CDC (%)</th>
<th>DC (%)</th>
<th>UDC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/N</td>
<td>Standard</td>
<td>25.4 ± 5.5</td>
<td>8.2 ± 0.7</td>
<td>61.0 ± 2.8</td>
<td>29.4 ± 2.8</td>
<td>2.2 ± 0.5</td>
<td>5.0 ± 0.9</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>Standard</td>
<td>16.2 ± 2.3</td>
<td>11.7 ± 2.2</td>
<td>70.7 ± 3.7</td>
<td>25.1 ± 3.4</td>
<td>1.5 ± 0.1</td>
<td>0.1 ± 0.1*</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>N/N</td>
<td>High fat</td>
<td>16.6 ± 1.3</td>
<td>18.7 ± 1.7</td>
<td>61.8 ± 2.4</td>
<td>18.2 ± 1.4</td>
<td>4.8 ± 0.4</td>
<td>7.6 ± 1.4</td>
<td>7.6 ± 1.4</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>High fat</td>
<td>12.4 ± 1.3</td>
<td>20.1 ± 4.5</td>
<td>70.6 ± 2.6</td>
<td>18.6 ± 2.3</td>
<td>4.9 ± 0.7</td>
<td>1.5 ± 0.3*</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td>cftr +/+</td>
<td>Standard</td>
<td>14.3 ± 3.0</td>
<td>11.2 ± 4.9</td>
<td>46.5 ± 3.8</td>
<td>42.6 ± 3.2</td>
<td>3.4 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>cftr −/−</td>
<td>Standard</td>
<td>19.9 ± 4.1</td>
<td>14.2 ± 2.6</td>
<td>61.3 ± 1.7*</td>
<td>33.8 ± 1.4*</td>
<td>2.0 ± 0.2*</td>
<td>0.6 ± 0.4</td>
<td>2.3 ± 0.2*</td>
</tr>
<tr>
<td>cftr +/+</td>
<td>High fat</td>
<td>13.1 ± 1.6</td>
<td>11.3 ± 2.0</td>
<td>53.5 ± 6.1</td>
<td>33.1 ± 5.8</td>
<td>4.5 ± 0.9</td>
<td>3.8 ± 0.7</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>cftr −/-</td>
<td>High fat</td>
<td>18.5 ± 3.2</td>
<td>15.3 ± 0.9</td>
<td>63.8 ± 5.9</td>
<td>27.3 ± 6.4</td>
<td>4.9 ± 0.6</td>
<td>0.9 ± 0.3*</td>
<td>3.1 ± 0.7</td>
</tr>
</tbody>
</table>

*Significantly different from controls: * P<0.05, * P<0.01. C, cholic acid; BMC, β-muricholic acid; CDC, ursodeoxycholic acid; DC, deoxycholic acid; UDC, ursodeoxycholic acid.
Fat (mal)absorption in CF mice

Feces. Fecal total bile salts were significantly increased in ΔF508/ΔF508 mice and cftr -/- mice when compared with their respective controls on a standard diet (ΔF508/ΔF508 mice: 9.1 ± 0.4 versus 5.9 ± 0.6 µmol per g dry weight of feces, respectively, P<0.01; cftr -/- mice: 10.8 ± 1.0 versus 5.4 ± 0.3 µmol per g dry weight of feces, respectively, P<0.01, Figure 7.2). A significant increase was also obtained for ΔF508/ΔF508 mice on a high-fat diet (10.8 ± 1.0 versus controls 5.4 ± 0.3 µmol per g dry weight P<0.001). There was no significant correlation between fecal fat excretion and fecal bile salt excretion in the group as a whole (r=0.27, P=0.07; Figure 7.3), nor when the mice were stratified according to their genotype.

Discussion

Recently, we reported strong indications suggesting that fat malabsorption in CF patients on pancreatic enzyme replacement therapy is partially due to impaired intestinal uptake of long-chain fatty acids [1], which may involve bile-related processes. The aim of the present study was to investigate in more detail fat absorption and bile formation in two recently generated CF mouse models: ΔF508/ΔF508 mice (129/FVB genetic background) and cftr -/- mice (129/C57/Bl6 genetic background) [11,14].

The current data show that cftr -/- mice exhibit fat malabsorption, in contrast to ΔF508/ΔF508 mice. No differences were observed with respect to biliary bile salt output. Hence, this is not likely the cause of the fat malabsorption. The observed fat malabsorption is not likely due to differences in the composition of the bile salt pool either, since these differences were observed in both ΔF508/ΔF508 mice and cftr -/- mice, whereas only cftr -/- mice exhibit fat malabsorption. It has been speculated that liver disease develops in patients with CF as a consequence of the plugging of intrahepatic bile ducts [30-32]. The lack of CFTR in the apical membrane of bile duct cells may lead to abnormalities in biliary drainage with chronic cholestasis [30]. Cholestasis would result in increased amounts of bile salts in plasma and decreased amounts of bile in the intestine, and absorption of dietary fats would be impaired [29,33]. Our results show that intestinal bile salts were similar in all mice, indicating that cholestasis is probably not a cause of fat malabsorption in cftr -/- mice. Plasma bile salts were similar in all mice.

Apparently, the fat malabsorption in the cftr -/- mice can not be explained by processes regarding bile formation in the liver. Other mechanisms that may contribute to inefficient fat absorption are intestinal mucosal dysfunction or alterations in the mucus layer. This would also be in concordance with the intestinal histologic abnormalities observed in ΔF508/ΔF508 mice and in cftr -/- mice [11,14]. Finally, the difference of fat malabsorption may be due to different pancreatic functioning. However, CF mice do not show major histological abnormalities in the pancreas or pancreatic duct and the secretion of amylase is not impaired [34]. This lack of pancreatic disease in CF mice is most plausibly due to a lower level of CFTR expression and a relatively higher contribution of alternative Ca-activated chloride channels as compared with human pancreas [35,36]. CF-related abnormalities in the intestine rather than hepatobiliary or pancreatic disturbances are therefore the most probable cause of fat malabsorption in cftr -/- mice.
The observation that fat malabsorption is present in \( cftr^{-/-} \) mice but absent in \( \Delta F508/\Delta F508 \) mice, may be due to the fact that the “Rotterdam” \( \Delta F508/\Delta F508 \) mice exhibit residual \( cftr \) activity [11,37], whereas in \( cftr^{-/-} \) mice, \( cftr \) function is completely abolished [14]. Low apical \( cftr \) activity in \( \Delta F508/\Delta F508 \) mice has been observed at physiological temperatures in the gallbladder and in the ileum [11,37]. The level of residual \( cftr \) activity could differ in various tissues and small variations in apical activity levels could have profound effects on pathology. A careful analysis of \( cftr \) processing kinetics between distinct tissues is necessary to confirm this hypothesis. Moreover, it can not be excluded that the different genetic backgrounds (129/FVB and 129/C57/Bl6 for \( \Delta F508/\Delta F508 \) and \( cftr^{-/-} \) mice, respectively) are in part responsible for the different phenotypes of the two CF mouse models. Additionally, studies were performed with both male and female mice (Table 7.1), which may also influence the results. However, it has not been reported before in the literature that fat absorption, bile salt pool size or bile composition differ for males and females.

In the present study, bile salt secretion during an 80-min period decreased by approximately 50%, showing that most of the bile salt pool was collected. Total biliary bile salt output during this period was similar for CF mice and their respective controls, suggesting that bile salt pools of CF and control mice were similar. Previous data on the bile salt pool size in CF patients are conflicting; both a normal and a decreased bile salt pool size have been reported in CF patients exhibiting fat malabsorption [19,38]. Our results indicate that bile salt secretion rates and bile salt pool size in the two mouse models for CF are not affected.

The observation of a relative increase in the proportion of cholic acid at the expense of deoxycholic acid is a well-known phenomenon in CF [38,39]. This result is consistent with the finding of increased fecal bile salts in CF mice. Interruption of the enterohepatic circulation by fecal bile salt loss is normally accompanied by an increase in bile salt synthesis in order to maintain bile salt output [40]. The capacity to increase synthesis is estimated to equal three to four times the pool size [41]. Apparently, the increased fecal loss of bile salts can be compensated for by increased hepatic bile salt neosynthesis.

Both CF mouse models exhibited increased excretion of bile salts in the feces when compared with their respective controls. Fecal bile salt loss is well recognized in patients with CF and has been attributed to various intraluminal factors: 1. unhydrolyzed triacylglycerols and phospholipids, 2. precipitation of bile salts in acidic duodenal content, 3. adsorption of bile salts to non-absorbed dietary residues, 4. modification of bile salts by intestinal microflora and 5. defects in the ileal uptake of bile salts [42-44]. In the CF mouse models, we did not find a significant correlation between fecal bile salt secretion and fecal fat excretion, indicating that unhydrolyzed triacylglycerols and phospholipids do not contribute to the increased fecal bile salt loss. The other factors can not be excluded. Since intestinal histologic abnormalities have been observed in both \( \Delta F508/\Delta F508 \) and in \( cftr^{-/-} \) mice [11,14], we speculate that the most logical explanation for bile salt malabsorption in these two CF mouse models would be defects in the ileal uptake of bile salts due to intestinal mucosal dysfunction or alterations in the mucus layer [45].

In conclusion, in this study we have shown that \( cftr^{-/-} \) mice, but not \( \Delta F508/\Delta F508 \) mice, have an impaired dietary fat absorption, which is not likely due to either decreased bile salt pool size or altered bile composition. In both CF mouse models, fecal bile salt excretion
was increased, which was not secondary to increased fecal fat excretion. Bile composition data indicate that the increased fecal loss of bile salts is compensated for by an increased bile salt neosynthesis.

References


