Fat absorption in cystic fibrosis mice is impeded by defective lipolysis and post-lipolytic events

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Bijvelds, Marcel J. C., Inez Bronsveld, Rick Havinga, Maarten Sinaasappel, Hugo R. de Jonge, and Henkjan J. Verkade. Fat absorption in cystic fibrosis mice is impeded by defective lipolysis and post-lipolytic events. Am J Physiol Gastrointest Liver Physiol 288: G646–G653, 2005. First published November 4, 2004; doi: 10.1152/ajpgi.00295.2004.—Cystic fibrosis (CF) is frequently associated with progressive loss of exocrine pancreas function, leading to incomplete digestion and absorption of dietary fat. Supplemen ting patients with pancreatic lipase reduces fat excretion, but it does not completely correct fat malabsorption, indicating that additional pathophysiological processes affect lipolysis and/or uptake of lipolytic products. To delineate the role of such (post) lipolytic processes in CF-related fat malabsorption, we assessed fat absorption, lipolysis, and fatty acid uptake in two murine CF models by measuring fecal fat excretion and uptake of oleate- and triolein-derived lipid. Pancreatic and biliary function was investigated by determining lipase secretion and biliary bile salt (BS) secretion, respectively. A marked increase in fecal fat excretion was observed in cftr null mice but not in homozygous ΔF508 mice. Fecal BS loss was enhanced in both CF models, but biliary BS secretion rates were similar. Uptake of free fatty acid was delayed in both CF models, but only in null mice was a specific reduction in lipolytic activity apparent, characterized by strongly reduced triglyceride absorption. Impaired lipolysis was not due to reduced pancreatic lipase secretion. Suppression of gastric acid secretion partially restored lipolytic activity and lipid uptake, indicating that incomplete neutralization of gastric acid impedes fat absorption. We conclude that fat malabsorption in cftr null mice is caused by impairment of lipolysis, which may result from aberrant duodenal pH regulation.

bile flow; cystic fibrosis transmembrane conductance regulator chloride channel; intestinal transport; mouse model; pancreas

Cystic fibrosis (CF) is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR). CFTR is a phosphorylation/ATP-regulated chloride channel involved in exocrine functions in many epithelial tissues. In the exocrine pancreas, loss of CFTR function is associated with progressive fibrosis, which severely compromises pancreatic fluid secretion. Because of a reduced output of lipolytic (co)enzymes and bicarbonate to the duodenum, such pancreatic insufficiency impairs the hydrolysis of lipids, decreasing the efficacy of fat absorption and frequently resulting in steatorrhea.

Oral supplementation of pancreatic lipase reduces fat loss, but some degree of steatorrhea persists in CF patients receiving enzyme therapy (45). Fat malabsorption may persist because (orally administered) lipase is inactivated by acidification of the duodenum, resulting from reduced duodenal and pancreatic bicarbonate secretion (4, 5, 32, 37). In addition, post-lipolytic processing of lipids, e.g., micellar solubilization, transport, and/or chylomicron production, may be impaired (23). CF patients commonly display fecal bile salt (BS) wasting, suggesting that perturbed BS handling may be causally related to fat malabsorption. Low BS secretion may result in a reduced micellar solubilization of long-chain fatty acids and, thus, reduce the efficacy of fat absorption. Duodenal acidification may inactivate BS by protonation, leading to premature uptake or precipitation (3). Apart from these solubilization defects, alterations in composition or function of the intestinal epithelium may impede epithelial transport of fatty acids (11, 35).

Over the last decade, several mouse models have been developed by targeted mutation or disruption of the cftr gene. The phenotype of these murine CF equivalents varies considerably, depending on the site of gene mutation and the genetic background, yet the absence or drastic reduction of intestinal cAMP-dependent chloride secretion is invariably observed. In most strains, this leads to a high incidence of intestinal obstruction syndromes in early life, which are a frequent cause of death. In this respect, the intestinal pathology seems more pronounced in CF mice than in patients (meconium ileus affects ~15% of CF newborns). In contrast, spontaneous lung and pancreatic disease is much less evident in the presently available murine models (10, 17).

Because of a beneficial combination of diet and genetic background, the cftr^tm1CAM null mice and homozygous ΔF508-cftr^tm1EUR mice presented here show a relatively low incidence of intestinal obstruction, obviating the use of specially formulated liquid diets or osmotic laxatives. In addition, these mice do not display an essential fatty acid deficiency or a lipid imbalance (42), as may contribute to the intestinal and pancreatic phenotype of certain CF mouse strains reared on a lipid diet (12). Because of an adequate exocrine pancreas function and an intestinal phenotype that seems less excessive than in other CF mouse models, these particular CF mouse models are well suited to elucidate the role of the intestinal pathophysiology in CF-related fat malabsorption. We have exploited these models to assess the role of lipolysis and post-lipolytic events in fat malabsorption by performing direct comparison of dietary triglyceride and free fatty acid processing.

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MATERIALS AND METHODS

Animals

Homozygous ΔF508 mice (9), cfr null mice (33) (with a FVB:129 and C57BL/6;129 background, respectively), and wild-type (WT) littermate controls were reared in an environmentally controlled facility at the Erasmus MC (Rotterdam, The Netherlands) and had free access to water and a semisynthetic diet with standard amounts of fat (14 ±% of 19 kJ/g diet; Hope Farms, Woerden, The Netherlands). Animals were obtained from CF colonies that are maintained by crossing of heterozygous progeny. This breeding strategy, continued for over 20 generations, has resulted in apparently homogeneous populations. Animals of both sexes were used, but littermates were always matched by gender. Although this setup allows for potential gender-related differences, an effect of gender on any of the reported parameters except, potentially, body mass, was not evident. All experiments were performed on animals 10–20 wk of age. Experimental protocols were approved by the Ethical Committee for Animal Experiments of the Erasmus MC.

Experimental Procedures

Fecal fat and BS excretion. Mice were housed individually for 72 h. During this period, dietary intake was measured and feces were quantitatively collected. Samples were freeze-dried, weighed, and mechanically homogenized. Fats were extracted, hydrolyzed, and methylated (26), and resulting fatty acid methyl esters were analyzed using gas chromatography to calculate fat intake and fecal fat excretion (22). Extracted BS (21) were measured using an enzymatic fluorimetric assay (30).

Collection of biliary and pancreatic secretions. Mice were anesthetized with Hypnorm (fentanyl/fluanisone, 2 g/kg body mass ip; Janssen Pharmaceutica, Beerse, Belgium) and diazepam (10 mg/g body mass mp; Centrafarm, Etten-Leur, The Netherlands). For measuring biliary secretion, the gallbladder was cannulated retrogradely from the duodenum by using polyethylene tubing. For measuring (combined) pancreaticobiliary secretion, the common bile duct of a separate group of animals was cannulated. Biliary and pancreatic secretions were collected in 20-min intervals for a total of 80 min. After collection of two 20-min basal fractions, pancreaticobiliary secretion was stimulated by combined administration (intraperitoneally) of cholecystokinin (CCK-8, 100 pmol/g body mass; Sigma, St. Louis, MO) and secretin (20 fmol/g body mass; BDH, Poole, UK). After 80 min, a final fraction (40–70 % of total) was collected under paraffin, in which pH was measured with a mini electrode (Radiometer, Copenhagen, Denmark) after adjustment of the volume to 90 μl with degassed distilled water. Biliary and pancreaticobiliary flow rates were assessed gravimetrically by assuming that 1 g of secretion corresponds to 1 ml. Lipase activity in pancreatic secretions was measured using a Lipase-PS kit (Trinity Biotech, Wicklow, Ireland). Total BS concentration in bile was determined by an enzymatic fluorimetric assay (30).

Omeprazole treatment. Omeprazole (Sigma) was dissolved in a 1:1 mixture of polyethylene glycol 400 and 200 mmol/l NaHCO₃ to a final concentration of 5 g/l. Animals were administered single daily doses (0.04 mg/g body mass) for 5 consecutive days by intragastric infusion (40). The final dose was administered 16 h before measurement of fatty acid and triglyceride uptake (see below).

Partitioning of orogastrically administered free fatty acid and triglyceride. Mice, fasted overnight, were anesthetized with isoflurane and administered Triton WR1339 (12.5 mg/0.1 ml saline iv) to block lipoprotein lipase-dependent lipolysis (38). Without delay, a blood sample (75 μl) was collected by puncture of the orbital plexus using heparin-coated microhemocrit tubes, and an intragastric dose of glycerol tri-[9,10(n)-3H]-oleate (triolein) and [1-14C]oleate (200 kBq each) in 0.1 ml of olive oil was administered. Further blood samples were collected hourly. After 4 h, a final blood sample was collected, and the liver, stomach and intestinal tract were excised. Lipids were extracted from plasma (1) and separated by thin-layer chromatography (eluents: hexane/diethyl ether/acetic acid 80:20:1; Kieselgel 60 plate; Merck, Darmstadt, Germany). Autoradiography showed that radioactive label on the thin-layer plates was contained to the triglyceride fraction (not shown). Silicate scrapings of triglyceride spots were dispersed in 1 ml of water, and 10 ml of scintillation cocktail were added. The contents of the small intestine were collected by flushing with 5 ml of 0.5 mmol/l taurocholate-150 mmol/l NaCl. Collected tissue was weighed and then digested in Soluene (3 ml/g tissue, 30 h at 50°C; Packard Chemicals, Meriden, CT). The digest was sampled gravimetrically (30–50 mg), and these samples were dispersed in 10 ml of Hionic scintillation cocktail (Packard Chemicals). Radioactivity in all samples was assessed via liquid scintillation analysis by using a dual-label dpm program (Tri-Carb 2500TR; Packard Instruments, Meriden, CT). Plasma triglyceride concentration was determined using a reagent kit (Sigma) (28).

Statistical Analysis

Data are presented as means ± SE. The statistical significance of differences between means was assessed using Student’s t-test (two sided). For assessing differences in mean body mass, a paired analysis was preferred, because this parameter showed considerably more variability between litters than within a litter (i.e., between littermates). All other significance levels reported refer to an unpaired t-test. For comparison of pH values, the Mann-Whitney U-test was applied.

RESULTS

Fecal Fat Balance

Nutritional data for homozygous ΔF508 mice, cfr null mice, and their respective controls are shown in Table 1. No differences were observed between homozygous ΔF508 mice and their controls with respect to fat intake, fecal fat excretion, net fat uptake (i.e., the quantity of fat ingested but not excreted), or percentage of dietary fat absorption. In cfr null mice, fecal fat excretion was significantly increased compared with controls, resulting in a lower efficacy of fat absorption. Analysis of the

Table 1. Nutritional data for homozygous ΔF508 mice, cfr null mice, and WT littermates

<table>
<thead>
<tr>
<th>Category</th>
<th>Body Mass, g</th>
<th>Food Intake, g/day</th>
<th>Fat Intake, μmol/day</th>
<th>Fecal Fat, μmol/day</th>
<th>Net Fat Uptake, μmol/day</th>
<th>Fat Absorption, % intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>30.0 ± 1.9</td>
<td>4.0 ± 0.4</td>
<td>626 ± 49</td>
<td>24 ± 2</td>
<td>602 ± 47</td>
<td>96.1 ± 0.2</td>
</tr>
<tr>
<td>Δ/Δ</td>
<td>27.2 ± 1.1</td>
<td>3.6 ± 0.3</td>
<td>580 ± 50</td>
<td>29 ± 2</td>
<td>550 ± 50</td>
<td>94.8 ± 0.5</td>
</tr>
<tr>
<td>+/−</td>
<td>29.0 ± 1.5</td>
<td>4.8 ± 0.3</td>
<td>690 ± 37</td>
<td>41 ± 2</td>
<td>649 ± 35</td>
<td>94.0 ± 0.3</td>
</tr>
<tr>
<td>−/−</td>
<td>25.9 ± 1.5*</td>
<td>4.4 ± 0.3</td>
<td>647 ± 60</td>
<td>67 ± 11*</td>
<td>580 ± 53</td>
<td>89.7 ± 1.2**</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5. ΔF508 mice and cfr null mice (−/−), and their wild-type (WT) littermates were all males, 11–14 wk of age, of FVB; 129 and C57BL/6;129 genetic background, respectively. A symbol indicates a significant difference compared to controls; *P < 0.05; **P < 0.01.

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fatty acid composition of the feces did not reveal major qualitative differences in fat excretion between CF mice and controls (Fig. 1). In CF mice as well as in control mice, the feces were slightly enriched in saturated fatty acids (16:0, 18:0) compared with the levels found in the chow, whereas the relative concentration of unsaturated fatty acids (18:1, 18:2, 22:6) was somewhat lower than in the chow, suggesting that unsaturated fatty acids are absorbed more efficiently than saturated species.

**Biliary BS Secretion**

Fecal BS excretion rates were significantly increased in both homozygous ΔF508 mice and cftr null mice compared with their respective controls (Fig. 2). Enhanced fecal BS loss may lead to depletion of the BS pool and, by reducing biliary BS secretion, may affect solubilization of dietary fat. To assess whether biliary BS output is indeed lowered in CF mice, we measured biliary BS secretion, by cannulation of the gallbladder, directly after acute interruption of the enterohepatic circulation. In homozygous ΔF508 mice, both bile flow and the rate of biliary BS secretion were similar to controls (4.3 ± 0.3 vs. 4.3 ± 0.4 μl·min⁻¹·100 g body mass⁻¹, P = 0.97, and 143 ± 10 vs. 139 ± 16 nmol·min⁻¹·100 g body mass⁻¹, P = 0.86, respectively). Cftr null mice also showed similar bile flow rates compared with controls (5.4 ± 0.5 vs. 5.1 ± 0.5 μl/min, P = 0.70, respectively) but tended to secrete more BS in bile, although the apparent difference between means was not statistically significant (294 ± 58 vs. 163 ± 33 nmol·min⁻¹·100 g body mass⁻¹, P = 0.11).

**Partitioning of Orogastrically Administered Free Fatty Acid and Triglyceride**

To investigate the relative role of lipolytic and post-lipolytic processes in CF-related fat malabsorption, we gave mice an intragastric dose of [³H]triolein and [¹⁴C]oleate and assessed the metabolic fate of these labels by measuring radioactivity in plasma, liver, stomach, small intestine (content and wall separately), and colon. After 4 h, the small intestinal wall of cftr null mice contained significantly less of both labels (³H and ¹⁴C) than WT tissue (Fig. 3B). In line with enhanced fecal fat excretion, a large quantity of nonabsorbed label was found in the distal intestine of null mice (Fig. 3B). Plasma radioactivity and triglyceride content (Fig. 4B) also were lowered in null mice, indicating that both triglycerides and free fatty acids are malabsorbed. In homozygous ΔF508 mice, the apparent difference in label content of the intestinal wall was much less pronounced, and these mice did not show enhanced accumulation of label in the colon (Fig. 3A). Still, in homozygous ΔF508 mice, as in null mice, plasma radioactivity and triglyceride concentration were decreased (Fig. 4A). In WT mice and in CF mice, the plasma ³H/¹⁴C ratio was lower than in the administered bolus (1:1 ratio), indicating that uptake of triolein-derived fatty acid continuously lagged behind uptake of the corresponding free fatty acid (Fig. 4). In all mice, this ratio increased during the course of the experiment. Cftr null mice, but not homozygous ΔF508 mice, showed a further reduction in the plasma ³H/¹⁴C ratio compared with WT mice, indicating a specific defect in the uptake of triolein-derived lipid relative to free fatty acid (Fig. 4B).

Recently, it was shown that the duodenum of cftr null mice is abnormally acidic and that this can be corrected by omeprazole-mediated inhibition of the gastric parietal cell H⁺-K⁺-ATPase (24). To test the effect of duodenal pH correction on fat absorption, we likewise applied omeprazole to suppress gastric acid secretion. The amount of ³H label retained in the stomach 4 h after lipid dosage was similar to that in untreated mice, suggesting that gastric emptying is not affected by omeprazole treatment. In contrast, omeprazole seemed to reduce deposition of nonabsorbed ³H-labeled lipid in the colon (35 ± 6% in untreated null mice vs. 17 ± 9% in omeprazole-treated animals, P = 0.12) to a level approaching that found in omeprazole-treated WT littermates (9 ± 4%, P = 0.47; Fig. 5A). These data suggest that an increase in the duodenal pH reduces fecal fat loss. In accordance with reduced fat loss, omeprazole stimulated accumulation of lipids in plasma of null mice compared with untreated animals (Fig. 5C). Omeprazole...
also stimulated the rate of lipid uptake in control mice (Fig. 5C). At 3–4 h after lipid dosage, omeprazole significantly enhanced plasma $^3$H accumulation, increasing the plasma $^3$H/$^14$C ratio in null and WT mice, suggesting that gastric acid suppression particularly enhanced lipolytic activity (Fig. 5B).

**Exocrine Pancreas Function**

The low lipolytic capacity that was seen in $cftr$ null mice may be caused by pancreatic disease reducing secretion of lipolytic enzymes and bicarbonate. To test this assumption, we assessed exocrine pancreas function in null mice and control.
littermates. Neither the volume collected by cannulation of the common bile duct nor the lipase activity in this secretion differed between these groups (Fig. 6). Combined secretin/CCK stimulation increased fluid and lipase output in both groups to a similar extent. The pH of the secretions collected under paraffin ranged between 7.7 and 8.1 and did not reveal significant differences in pancreatic bicarbonate production between null mice and controls. In accordance, chloride levels, which typically are complementary to bicarbonate in pancreatic secretions, also were similar in null mice and controls (73.7/11006 3.3 vs. 79.3/11006 5.7 mmol/l, respectively, \( P < 0.44 \)).

Thus, with respect to bicarbonate and lipase secretion rates, dysfunction of the exocrine pancreas is not indicated in this strain of cftr null mice.

**DISCUSSION**

Although pancreatic lipase deficiency is a major cause of fat malabsorption in CF patients, it is thought that additional factors contribute to aberrant fat processing. However, because CFTR activity has a pervasive effect on gastrointestinal physiology through control of ion and fluid balance of pancreatic, hepatobiliary, and intestinal secretions, it has been difficult to pinpoint exactly these modulators of fat absorption. Because of the invariably mild pancreatic and hepatobiliary pathology of CF mice, we reasoned that these murine models would allow us to focus on the role of intestinal CFTR in lipid processing. Indeed, our data indicate that fat malabsorption does occur in cftr null mice, although they display apparently adequate pancreatic lipase and bicarbonate secretion and biliary BS secretion. Our data show that fat malabsorption is associated with impaired lipolysis and uptake of fatty acids and strongly suggest a critical role of duodenal bicarbonate production in processing of dietary lipid.

The cftr null mice excreted 10% of the fat contained in a comparatively low-fat diet (7.3 mass%), whereas WT and homozygous \( \Delta F508 \) mice excreted only 5% of ingested fat. Null mice showed enhanced excretion of all long-chain fatty acids analyzed. This overt fat wasting coincided with defective digestion of triglycerides, as indicated by a lowered plasma \( {\text{H}}/{\text{14C}} \) ratio, suggesting that incomplete lipolysis restricts the amount of fatty acids and monoglycerides available for uptake, leading to high fat excretion.

Whereas our data indicate that impaired lipolysis contributes significantly to fat malabsorption in null mice, the role of impaired post-lipolytic processing is less evident. In homozygous \( \Delta F508 \) mice, plasma triglyceride accumulation and free fatty acid uptake measured over a 4-h period after olive oil dosage were reduced to an extent similar to that in null mice, but this did not enhance fecal fat excretion. Importantly, homozygous \( \Delta F508 \) mice did not display a reduction in triglyceride-derived lipid uptake (i.e., the plasma \( {\text{H}}/{\text{14C}} \) ratio was not lowered), indicating that lipolytic activity had not

Fig. 5. Effect of omeprazole treatment on fat absorption in cftr null mice and WT littermates. A: label content of stomach and colon of omeprazole-treated animals was assessed 4 h after Triton WR1339 administration and intragastric dosage of [\( ^{3} \)H]triolein and [\( ^{14} \)C]oleate in 0.1 ml of olive oil. The \( {\text{H}}/{\text{14C}} \) ratio (B) and total triglyceride levels (C) in plasma are shown in comparison with untreated animals with the same cftr genotype (see Fig. 4). *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \); \( n = 4–6 \).

Fig. 6. Pancreaticobiliary secretion in cftr null mice and WT littermates. Fluid was collected in 20-min fractions by cannulation of the common bile duct before (basal) and after administration of secretin and cholecystokinin (CCK) (\( n = 3 \)).
decreased and that delayed lipid uptake must be ascribed to impaired processing of free fatty acid and lipolytic products. A similar delay in uptake of lipids without a concomitant effect on fecal fat excretion was found for mdr2 null mice (showing defective biliary phospholipid secretion) (41) and mice deficient in the pancreatic triglyceride lipase (PTL) (19). In the latter study it was shown that in PTL null mice, uptake of $^3$H-labeled lipids shifts to more distal sections of the small intestine but that there is no reduction in the total amount of label associated with the intestinal wall or the amount finally absorbed. Correspondingly, the amount of label recovered from the entire small intestinal wall was not significantly reduced in homozygous ΔF508 mice compared with WT mice (in contrast to null mice). Thus our data are compatible with a redistribution of lipid uptake along the longitudinal axis of the small intestine of homozygous ΔF508 mice. Indeed, taking into account the amount of label still contained in the stomach and in the small intestinal lumen, as well as the low level found in the colon, there was no indication for enhanced fat loss in homozygous ΔF508 mice, even when a high fat dosage (i.e., 0.1 ml of olive oil, equivalent to ~20% of the daily ingested lipid dose) was given acutely. In contrast, in null mice, olive oil dosage further increased fractional fat excretion, because ~30% of the administered label accumulated in the colon. These observations strongly indicate that in homozygous ΔF508 mice, a reduced rate of lipid uptake, caused by inefficient transfer to the epithelial surface, cellular uptake, or chylomicron assembly, is compensated for by surplus absorption capacity along the small intestine.

Because both murine CF models showed enhanced fecal loss of BS, we speculated that depletion of the biliary BS pool may occur, limiting the capacity for solubilization and impeding lipid uptake. In rats, it was shown that bile duct ligation impairs solubilization of lipids and enhances fecal lipid excretion (7, 29). However, no reduction in biliary BS secretion rate was observed in CF mice, suggesting that high BS losses are compensated for by enhanced de novo synthesis [as occurs in CF patients (39)]. Still, qualitative changes in biliary BS composition, intraluminal inactivation, or premature uptake of BS may potentially affect solubilization of lipids. Because saturated fatty acids are less soluble in the intraluminal environment than unsaturated species (31), impediment of lipid solubilization primarily affects the uptake of the former, leading to a corresponding shift in fecal fatty acid concentrations (29). Yet, the fecal fatty acid profiles of null mice and controls were very similar and showed only a slight enrichment of saturated fatty acids (16:0, 18:0) and depletion of unsaturated species (18:1, 18:2) compared with diet composition, suggesting that intraluminal solubilization of lipids is not markedly impaired by decreased BS activity.

Pancreatic insufficiency is a major cause of low lipolytic capacity and fat malabsorption in CF patients. Although such overt pancreatic disease has never been observed in CF mice, more subtle changes in pancreatic fluid output and composition have been reported (13) that may affect the activity of lipolytic enzymes. To test this possibility, we assessed exocrine pancreas function in cftr null mice. Lipase activity in pancreatic secretions was not reduced compared with WT mice, and the pH of the secreted fluid also appeared normal, i.e., around pH 8, indicating that pancreatic lipase and bicarbonate secretion are not severely compromised by CFTR inactivation in our mouse model. The pancreatic phenotype of our null mouse (cftr<sup>tm1UNC</sup>, mixed C57BL/6;129 background) seems relatively mild compared with the cftr<sup>tm1HSC</sup> null mouse (C57BL/6), which shows dilatation of the pancreatic ducts and may secrete an abnormally acidic pancreatic fluid (10, 13). However, such clear signs of pancreatic disease are not always observed in cftr<sup>tm1UNC</sup> null mice (44), suggesting that environmental factors (e.g., diet composition, nutritional status, exposure to pathogens) play a role in its development. The relatively mild gastrointestinal phenotype in our model is further underlined by the observation that the cftr<sup>tm1CAM</sup> mice used in this study were reared on a solid diet, whereas cftr<sup>tm1UNC</sup> mice usually are fed a liquid diet to prevent high mortality from intestinal obstructions. Interestingly, a mild intestinal phenotype (defined as prolonged survival on solid food) observed in a subset of cftr<sup>tm1HSC</sup> null mice has been associated with a capacity for calcium-activated intestinal chloride secretion (34). Possibly, a calcium-dependent chloride conductance also secures adequate pancreatic secretion (15). Therefore, we conclude that the exocrine pancreas function in this cftr<sup>tm1CAM</sup> strain, possibly due to a compensatory chloride conductance, is not severely compromised and that impaired lipolysis is not caused by pancreatic insufficiency.

Collectively, these results indicate that lipid processing in cftr null mice is not impaired by changes in pancreatic or biliary secretion. However, the activity of pancreatic enzymes and BS, and, therefore, partitioning of lipids in mixed micelles and transfer across the unstirred layer, critically depends on the luminal pH. In humans, pancreatic and duodenal bicanonicsecretions are CFTR-dependent processes, vital for neutralization of gastric acid entering the proximal intestine (4, 5, 32, 37). Clinical trials show a beneficial effect of gastric acid suppression (by omeprazole therapy) on fat absorption in CF patients receiving oral lipase supplementation, indicating that orally administered lipase is inactivated by gastric acid (18). Duodenal acidification may also cause inactivation of endogenous lipase and impede micellar solubilization of lipids. The cftr<sup>tm1UNC</sup> null mice show a moderately lower duodenal pH than control mice (a difference of 0.3 units in fasted mice), at least in part attributable to a lack of duodenal bicarbonate secretion (6). Recently, it was reported that suppression of gastric acid production by omeprazole increased the duodenal pH of these null mice up to the levels found in (untreated) controls (24). Our results show that although the amount of lipid entering the small intestine from the stomach over a 4-h period was not affected, omeprazole treatment reduced the deposition of label in the colon of null mice and increased the rate of triglyceride accumulation in plasma. Furthermore, gastric acid suppression increased the plasma $^{3}$H/$^{14}$C ratio at 3–4 h after olive oil dosage, suggesting that enhanced lipid uptake mainly results from an increase in lipolytic activity.

Surprisingly, omeprazole also increased lipolytic activity in WT mice, suggesting that the prevailing pH in the duodenal lumen limits lipolytic activity. However, even when mice are fed a high-fat diet, lipid absorption reaches 95% efficiency (Verkade HJ, unpublished observations), indicating that, under physiological conditions, lipolysis does not limit fat absorption. A relatively low initial (1 h) lipolytic activity was observed in both omeprazole-treated controls and null mice. Paradoxically, this may also be an effect of gastric acid
suppression, because the absence of acid release in the upper intestine may retard pancreatic fluid secretion (16).

Although omeprazole markedly increased the efficiency of fat uptake, lipolytic capacity in null mice did not reach the levels found in untreated controls, indicating that factors other than incomplete buffering of gastric acid reduce lipolytic activity, specifically in cftr null mice. The nature of these additional factors is speculative but may also be related to defective duodenal pH regulation. CFTR dysfunction, by abolishing cAMP-mediated inhibition, leads to increased activity of the intestinal Na+/H+ exchanger (NHE3) (14, 43), whereas CFTR-dependent bicarbonate secretion is reduced (20, 25, 36). Consequently, in the CF intestine, loss of reciprocal regulation of NHE3 activity and bicarbonate secretion may lead to excessive H+ secretion, further contributing to the pH imbalance. In this context, it is of interest that, in contrast to null mice, the rate of lipolysis and quantitative fat absorption were not affected in homozygous ΔF508 mice. This suggests that these mice have retained some capacity for neutralization of gastric acid. Unlike homozygous ΔF508 patients, who show no (27) or marginal (2, 8) rescue of functional CFTR protein, considerable ΔF508-CFTR-mediated intestinal chloride secretion is apparent in mutant mice (15–20% of WT level; de Jonge HR, unpublished observations). Accordingly, murine ΔF508-CFTR may mediate enough residual duodenal bicarbonate secretion to secure a level of gastric acid buffering that permits hydrolysis of all triglyceride. In addition, because the homozygous ΔF508 mice and null mice presented here have separate genetic backgrounds, secondary genetic factors that modulate intestinal ion transport may play a role in producing these contrasting phenotypes. Delayed processing of lipolytic products and free fatty acids still may be related to defective solubilization, possibly due to more subtle changes in luminal pH, or may be related to disruption of other post-lipolytic processes.

In conclusion, cftr null mice exhibit fat malabsorption, impaired lipolysis, and uptake of long-chain fatty acids despite normal pancreatic lipase secretion and biliary BS delivery. Processing of free fatty acid and lipolytic products is perturbed in both null mice and homozygous ΔF508 mice. Because homozygous ΔF508 mice do not show increased fecal fat loss, post-lipolytic processing affects the kinetics of fat uptake but may be less critical for quantitative fat absorption. Suppression of gastric acid secretion partly restored lipolytic capacity and reduced deposition of fat in the colon, indicating that neutralization of gastric acid is imperative for efficient fat absorption. In view of the absence of gross biliary or pancreatic pathology, a critical role is suggested for duodenal bicarbonate secretion in luminal pH regulation and fat absorption. We conclude that insufficient duodenal bicarbonate secretion leading to lipase inactivation, with or without defective solubilization, is the principle cause of fat malabsorption in cftr null mice.

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