CHAPTER 6

Fat malabsorption in cystic fibrosis patients on enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids


CHAPTER 6

Fat malabsorption in cystic fibrosis patients on enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids

Abstract

Background & Aim: Pancreatic enzyme replacement therapy frequently fails to correct intestinal fat malabsorption completely in cystic fibrosis (CF) patients. The reason behind therapy failure in these patients is unknown. We investigated whether fat malabsorption in CF patients treated with pancreatic enzymes is caused by insufficient lipolysis of triacylglycerols or by defective intestinal uptake of long-chain fatty acids. Methods: In 10 CF patients receiving their habitual pancreatic enzymes, lipolysis was determined by analysis of breath $^{13}$CO$_2$ recovery after oral ingestion of 1,3-distearoyl, 2[1-$^{13}$C]octanoyl glycerol ($^{13}$C-MTG). Intestinal uptake of long-chain fatty acids was determined by analysis of plasma $^{13}$C-linoleic acid concentrations after oral ingestion of $^{13}$C-linoleic acid ($^{13}$C-LA). For 3 days, dietary intake was recorded and feces was collected. Results: Fecal fat excretion ranged from 5.1 to 27.8 g day$^{-1}$ (mean ± SD: 11.1 ± 7.0 g day$^{-1}$) and fat absorption ranged from 79 to 93% (89 ± 5%). After ingestion of $^{13}$C-MTG no relationship was observed between breath $^{13}$CO$_2$ recovery and dietary fat absorption ($r$=0.04). In contrast, a strong relationship was observed between 8-h plasma $^{13}$C-LA concentrations and dietary fat absorption ($r$=0.88, $P<0.001$). Conclusion: Our results suggest that continuing fat malabsorption in CF patients on enzyme replacement therapy is not likely due to insufficient lipolytic enzyme activity, but rather due to either incomplete intraluminal solubilization and/or reduced mucosal uptake of long-chain fatty acids.
Impaired uptake of fats in CF patients

Introduction

In humans, triacylglycerols composed of long-chain fatty acids constitute 92 to 96% of dietary fats [1]. Absorption of these fats comprises two main processes. Firstly, lipolysis, by lipolytic enzymes originating predominantly from the pancreas, leads to hydrolysis of triacylglycerols into fatty acids and 2-monoacylglycerols. And secondly, intestinal uptake involves the formation of mixed micelles composed of bile components and lipolytic products, followed by the desintegration of the mixed micelles in the unstirred water layer, and the translocation of the lipolytic products across the intestinal epithelium [1-4].

Most CF patients have a considerable malabsorption of dietary fats due to pancreatic insufficiency leading to impaired lipolysis [5,6]. The symptoms of pancreatic insufficiency, such as steatorrhea and poor growth, can be alleviated by oral supplementation of pancreatic enzymes. However, despite recent improvements in the pharmacokinetics of the supplements, many patients continue to experience a certain degree of steatorrhea [7-9], with fat absorption reaching 80 to 90% of their dietary fat intake. It has not been elucidated if the remaining fat malabsorption is due to an insufficient dosage of pancreatic enzyme replacement therapy. This possibility is not unlikely because a decreased pancreatic bicarbonate secretion may negatively affect enzyme activity by sustaining a low pH in the duodenum [10,11]. At a low duodenal pH, the release of the enzymes from the (micro)capsules is inhibited and the denaturation of the enzymes is stimulated [11,12]. However, it has been demonstrated that increasing the pancreatic enzyme dosages does not completely correct fat malabsorption [13]. In addition, attempts to increase lipolysis by high-strength pancreatic enzyme supplements has led to the reported association with fibrosing colonopathy [14-16].

An alternative explanation for the continuing fat malabsorption in CF patients on pancreatic enzyme replacement therapy may involve inefficient intestinal uptake of fatty acids [7,17]. Impaired uptake in CF patients can be due to an altered bile composition, decreased bile salt secretion by the liver, bile salt precipitation, a decreased bile salt pool size, and/or bile salt inactivation at low intestinal pH [9,17-20]. Furthermore, small bowel mucosal dysfunction or alterations in the mucus layer contribute to inefficient intestinal uptake of long-chain fatty acids in CF patients [5,21].

The gold standard for monitoring enzyme replacement therapy is the fat balance. A drawback of the fat balance is that it does not provide insight into the pathophysiology of fat malabsorption. Insight into the adequacy of these separate processes (lipolysis, intestinal uptake) would enable treatment in individual patients by modulating diet therapy, pancreatic enzyme replacement therapy and supplementation of antacids and bile salts. So far, it has not been possible to determine whether fat malabsorption in CF patients is due to impaired lipolysis or due to impaired uptake of long-chain fatty acids. Therapeutic improvements of fat absorption may be of benefit for CF patients, as a positive correlation has been observed between a good nutritional status and long-term survival or well-being of CF patients [22].

The aim of the present study was to determine whether continued fat malabsorption encountered in pediatric CF patients on their habitual pancreatic enzyme replacement therapy results from either insufficient lipolysis or from defective intestinal uptake of long-chain fatty acids in the lumen. We choose to measure lipolysis and uptake by two independent tests in CF...
patients in vivo. Previously, a test to determine lipase activity was described and validated in CF patients, based on oral ingestion of a $^{13}$C-labeled mixed triglyceride ($^{13}$C-MTG, 1,3-distearoyl, 2[1-$^{13}$C]octanoyl glycerol) and excretion of $^{13}$C in breath [23-26]. Inadequate intestinal uptake can be measured by oral ingestion of long-chain fatty acids, e.g. $^{13}$C-labeled linoleic acid ($^{13}$C-LA) [27,28]. The concentration of $^{13}$C-LA in plasma and the expiration of $^{13}$CO$_2$ could then serve as parameters to quantify uptake of $^{13}$C-LA.

### Subjects and Methods

#### Patient characteristics

The study protocol was approved by the Medical Ethics Committee of the University Hospital Groningen, and included informed consent obtained from the parents and the children.

**Patients.** The study group included 10 pediatric CF patients, three male and seven female, ranging in age from 7 to 18 y. The diagnosis of CF had been established by the sweat test and a DNA genotype analysis [29]. The ΔF508/ΔF508 genotype was present in six patients, and the ΔF508/other in four (subjects 1, 4, 8, 9; Table 6.1). All patients were pancreatic insufficient and therefore received enteric coated pancreatic enzymes. None of the patients received antacids.

#### Table 6.1  Comparison of energy intake, ingested lipase enzymes, and fasting plasma concentrations of bile salts in individual CF patients and mean ± SD.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>Energy (% RDA)</th>
<th>Carbohydr. (% energy)</th>
<th>Fats (% energy)</th>
<th>Proteins (% energy)</th>
<th>Lipase (IU/g fat)</th>
<th>Plasma bile (µmol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F</td>
<td>18</td>
<td>55</td>
<td>66</td>
<td>52</td>
<td>33</td>
<td>15</td>
<td>560</td>
<td>13.8</td>
</tr>
<tr>
<td>2 F</td>
<td>18</td>
<td>58</td>
<td>104</td>
<td>52</td>
<td>31</td>
<td>17</td>
<td>710</td>
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</tr>
<tr>
<td>3 M</td>
<td>16</td>
<td>53</td>
<td>115</td>
<td>48</td>
<td>39</td>
<td>13</td>
<td>1820</td>
<td>20.6</td>
</tr>
<tr>
<td>4 M</td>
<td>15</td>
<td>56</td>
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<td>38</td>
<td>15</td>
<td>680</td>
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</tr>
<tr>
<td>5 F</td>
<td>9</td>
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<td>91</td>
<td>52</td>
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<td>13</td>
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</tr>
<tr>
<td>6 F</td>
<td>9</td>
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<td>102</td>
<td>57</td>
<td>30</td>
<td>13</td>
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<td>13.5</td>
</tr>
<tr>
<td>7 M</td>
<td>8</td>
<td>23</td>
<td>110</td>
<td>50</td>
<td>37</td>
<td>13</td>
<td>830</td>
<td>11.8</td>
</tr>
<tr>
<td>8 F</td>
<td>7</td>
<td>27</td>
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<td>52</td>
<td>35</td>
<td>13</td>
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<td>9 F</td>
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<td>7</td>
<td>23</td>
<td>121</td>
<td>54</td>
<td>35</td>
<td>11</td>
<td>460</td>
<td>30.3</td>
</tr>
</tbody>
</table>

103 ± 16  52 ± 3  35 ± 3  14 ± 2  850 ± 460  16.6 ± 5.9

F, female; M, male. Normal range fasting plasma bile salts, 1-10 µmol L$^{-1}$.

**Anthropometry.** Anthropometric evaluation consisted of weight, height, midarm circumference, and skinfold thickness measurements at 4 sites (biceps, triceps, subscapula, and suprailiac), done by one pediatrician. The Z-scores of all these anthropometric parameters were calculated based on the reference data for Dutch children described by Gerver and De Bruin [30]. The Z-score is defined as $X - \bar{x} / S$ where $X$ is the patient’s measurement, $\bar{x}$ is the
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A negative value indicates a value under the median reference value.

Pulmonary function. Pulmonary function was assessed by standard spirometric techniques and was characterized by the parameters forced vital capacity, and forced expiratory volume in one second. For each patient, results were expressed as percentage of predicted (control) values for sex and height [31].

Liver function tests. Liver function had been screened during a standard routine control at the time of the study using serum enzyme activities: g-glutamyl transpeptidase, aspartate transaminase, and alanine transaminase.

Diet evaluation. Intake of nutrients was calculated from 3-day consecutive food diaries by a clinical dietitian using The Netherlands Nutrients Table “NEVO” 1993. Intakes were expressed as the recommended dietary allowance (RDA) for weight, age and sex (Table 6.1).

13C-labeled substrates
The mixed triglyceride (1,3-distearoyl, 2[1-13C]octanoyl glycerol; S*OS) was purchased from Euriso-Top (Saint Aubin Cedex, France) and was 99% 13C-enriched. In the original literature, the breath test performed with the use of this molecule has been named the mixed-triglyceride breath test or the 13C-MTG breath test [23,25]. For reasons of consistency, we adhered to this nomenclature. Uniformly labeled 13C-linoleic acid (13C-LA), obtained from Campro Scientific B.V. (Veenendaal, The Netherlands), had an enrichment exceeding 97%. 13C-LA was included into a gelatin capsule coated with an acid-resistant layer consisting of 4.8% cellulose acetate hydrogen phthalate in acetone.

Study protocol
The subjects were instructed to avoid consumption of naturally 13C-enriched foods (e.g. corn or corn products, pineapple, cane sugar) for at least two days prior to the study. The 13C-LA test and the 13C-MTG test were performed on two subsequent days. On day 1, after an overnight fast, the patients received a capsule with 13C-LA (1 mg kg\(^{-1}\) BW), together with their habitual breakfast (bread, butter, ham, cheese, etc.) and pancreatic enzymes. A baseline blood sample (EDTA) was collected before consumption of breakfast, every 2 h for 8 h, and at 24 h. Immediately after sampling, plasma was isolated and stored frozen (-20ºC) until further analysis. Breath samples were collected in duplicate at baseline and every 30 min for 6 h. On day 2, the patients received 13C-MTG (4 mg kg\(^{-1}\) BW) mixed with their habitual breakfast and pancreatic enzymes. Breath samples were collected in duplicate at baseline and every 30 min for 6 h. The fecal fat balance and both breath tests were performed in the same 3-day period. On the day before the 13C-LA test, a feces sample was collected for baseline 13C-measurements. After consumption of the breakfast on the first day, all feces passed was collected for three days (72 h) to determine the presence of fat malabsorption and the amount of 13C-LA excretion into the feces. Collected feces was stored at -20ºC. During this period, intake of nutrients was determined from food diaries also. During the first six hours of both tests, no additional food or liquids were permitted except for non-caloric drinks such as water.
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and tea (without milk and sugar). After 6 hours, patients were allowed to have their habitual lunch, including pancreatic enzymes.

**Analytical techniques**

**Breath sample analysis.** End expiratory breath was collected via a straw into a 10 ml tube (Exetainers; Labco Limited, High Wycombe, United Kingdom), from which aliquots were taken to determine $^{13}$C-enrichment by means of continuous flow isotope ratio mass spectrometry (Finnigan Breath MAT, Finnigan MAT GmbH, Bremen, Germany), conform previous experiments [24]. The $^{13}$C-abundance of breath CO$_2$ was expressed as the difference per mil from the reference standard Pee Dee Belemnite limestone ($\delta^{13}$C$_{PDB}$, ‰).

Mean values of whole body CO$_2$ excretion were measured by indirect calorimetry (Oxycon, model ox-4, Dräger, Breda, The Netherlands) at 2 separate periods of 5 minutes during both test days. This sampling method was compared to sampling every 30 min (results not shown). The results indicated that, under the test conditions chosen, the mean values of the CO$_2$ production obtained from 2 randomly chosen periods were within the 95% confidence interval of the mean values obtained when sampling occurred every 30 min.

**Plasma fats.** Plasma fats were extracted, hydrolyzed and methylated according to Lepage and Roy [32]. Resulting fatty acid methyl esters were analyzed both by gas chromatography and by gas chromatography combustion isotope ratio mass spectrometry. Quantification of the resulting fatty acid methyl esters was performed with the use of heptadecanoic acid (C17:0) as an internal standard.

**Fecal fats.** After thawing, feces was weighed and homogenized. Fecal fat was determined according to the method of Van de Kamer et al. [33] and expressed as g fat day$^{-1}$. The percentage of total fat absorption was calculated from the daily dietary fat intake and the daily fecal fat output and expressed as a percentage of the daily fat intake.

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

Aliquots of freeze-dried feces were extracted according to the method of Bligh and Dyer [34], and subsequently hydrolyzed and methylated [32]. Resulting fatty acid methyl esters were analyzed by both gas chromatography and gas chromatography combustion isotope ratio mass spectrometry.

**Plasma and fecal bile salts.** Fasting and postprandial plasma bile salts up to 8 h were determined by an enzymatic fluorimetric assay [35]. Results were expressed as µmol L$^{-1}$ plasma. Fecal bile salts were extracted from an aliquot of dried homogenate of a 24-h feces fraction [36] and fluorimetrically measured [35].

**Gas liquid chromatography.** Fatty acid methyl esters were separated and quantified by gas liquid chromatography on a Hewlett Packard gas chromatograph Model 5880 equipped with a CP-SIL 88 capillary column (Chrompack; 50 m x 0.32 mm) and an FID detector [37,38]. The gas chromatograph oven was programmed from an initial temperature of 150°C to 240°C in 2 temperature steps (150°C held 5 min; 150-200°C, ramp 3°C min$^{-1}$, held 1 min; 200-240°C, ramp 20°C min$^{-1}$, held 10 min). Adequate separation of linoleic acid could be
achieved in this way. Quantification of the fatty acid methyl esters was done by adding heptadecanoic acid (C17:0) as internal standard.

Gas chromatography combustion isotope ratio mass spectrometry. $^{13}$C-enrichment of the palmitic acid methyl esters was determined on a gas chromatography combustion isotope ratio mass spectrometer (Delta S/GC Finnigan MAT, Bremen, Germany) [39]. Separation of the methyl esters was achieved on a CP-SIL 88 capillary column (Chrompack; 50 m x 0.32 mm). The gas chromatograph oven was programmed from an initial temperature of 80°C to 225°C in 3 temperature steps (80°C held 1 min; 80-150°C, ramp 30°C min$^{-1}$; 150-190°C, ramp 5°C min$^{-1}$; 190-225°C, ramp 10°C min$^{-1}$, held 5 min). Adequate separation of linoleic acid could be achieved in this way.

Statistics
The experimental data are reported as means ± SD. Corresponding to the literature [40-42], relationships between the percentage of total fat absorption and either plasma $^{13}$C-LA concentrations or breath $^{13}$CO$_2$ expiration were considered exponential. All other correlations were assumed to be linear. Correlations between variables were calculated with the least square method and are expressed as Pearson’s coefficient of variation $r$. Differences between means were considered statistically significant at the level of $P<0.05$.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fecal bile salts (mmol/kg wet weight)</th>
<th>Fat intake (g day$^{-1}$)</th>
<th>Fecal fat (g day$^{-1}$)</th>
<th>Total fat absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.2</td>
<td>54</td>
<td>4.9</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>10.6</td>
<td>85</td>
<td>7.0</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>124</td>
<td>14.8</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>8.7</td>
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<td>27.8</td>
<td>79</td>
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</tr>
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<td>6</td>
<td>20.5</td>
<td>66</td>
<td>5.1</td>
<td>92</td>
</tr>
<tr>
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<td>85</td>
<td>6.1</td>
<td>93</td>
</tr>
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</tr>
<tr>
<td>10</td>
<td>16.7</td>
<td>88</td>
<td>7.1</td>
<td>92</td>
</tr>
</tbody>
</table>

Mean ± SD 13.8 ± 9.0 84 ± 22 11.1 ± 7.0 89 ± 5

Normal range fecal bile salt: 0.1-1 mmol kg$^{-1}$ fecal wet weight.

Results

Patient characteristics
Z-scores for all anthropometric parameters in CF patients were low to normal. For all parameters, the 95% confidence interval does include the reference 50th centile line (Z-score 0), indicating that there is no significant difference between our study group and the healthy reference population. Most patients had some degree of lung disease. Subjects 1 and 6-10 had
normal liver biochemistry. Previously, subject 3 was diagnosed as having liver cirrhosis with portal hypertension. This patient receives ursodeoxycholic acid (750 mg day\(^{-1}\)) and the condition of this patient has been stable for the past few years. The bile salt concentration in plasma of this subject is in the same range as that of the other patients (Table 6.1). Analysis of 3-day dietary food records is shown in Table 6.1. Energy intake of 7 patients exceeded the recommended dietary allowance. In all patients approximately 50% of the energy was derived from carbohydrates, 35% from fat, and 15% from protein. Patients took pancreatic enzyme supplements in a dosage of approximately 440 - 1820 IU lipase per gram fat ingested (Table 6.1).

<table>
<thead>
<tr>
<th>Table 6.3</th>
<th>Results of the (^{13})C-LA test, and (^{13})C-MTG test in 10 individual CF patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Breath 6-h cum (^{13})CO(_2) (% dose)</td>
</tr>
<tr>
<td></td>
<td>Plasma (^{13})C-LA at 8 h (% dose L(^{-1}))</td>
</tr>
<tr>
<td>1</td>
<td>11.0</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
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<tr>
<td>3</td>
<td>2.2</td>
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<tr>
<td>4</td>
<td>1.7</td>
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<td>5</td>
<td>0.2</td>
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<tr>
<td>6</td>
<td>3.6</td>
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<tr>
<td>7</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>3.1</td>
</tr>
<tr>
<td>9</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.7 ± 3.1</td>
</tr>
</tbody>
</table>

LA, linoleic acid; MTG, mixed triglyceride. Normal range fecal bile salt: 0.1-1 mmol kg\(^{-1}\) fecal wet weight.

**Fat balance**

In the studied CF patients, dietary intake of fat over the 3-day period ranged from 54 to 130 g day\(^{-1}\), and the excretion of fat in feces ranged from 4.9 to 27.8 g day\(^{-1}\) (Table 6.2). The percentage of total fat absorption ranged from 79 to 93% (Table 6.2). Under physiological conditions, healthy individuals excrete approximately 4-6 g day\(^{-1}\) of fat via the feces [43], which generally means that over 96% of the dietary fats entering the intestinal lumen is absorbed [43]. These observations were confirmed by experiments performed in our own laboratory with dietary records and feces of healthy human adults (n=13, fecal fat excretion: 3.0 ± 0.9 g day\(^{-1}\), total fat absorption: 97 ± 2%, data not shown). Despite standard pancreatic enzyme replacement therapy, fecal fat excretion in 8 out of 10 patients was higher than 6 g fat per day, and the percentage of total fat absorption was below 96% in all patients studied. According to the prevailing reference values [43], all patients but 2 have fat malabsorption.

In studies in infants between 0 and 6 months, Fomon et al. [44] found that fecal fat excretion per kg body weight correlated with fat intake per kg body weight. In our study we observed a similar curvilinear correlation (r=0.71, P<0.05) despite a considerably lower intake.
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of fat per kg BW compared to infants [44]. However, when we compared fat intake per kg body weight with percentage of total fat absorption, no correlation was observed ($r=0.06$), indicating that fat malabsorption in our study was not due to high fat intake. In addition, no correlation was observed between the percentage of total fat absorption and the amount of pancreatic enzymes ingested ($r=0.12$).

**$^{13}$C-MTG test**

The baseline $^{13}$C-abundance in breath prior to consumption of the $^{13}$C-MTG label was $-23.2 \pm 2.6\%$ (range $-25.5$ to $-17.1\%$). After ingestion of the $^{13}$C-MTG label, different time-course patterns were observed for the excretion of $^{13}$C-label in breath over the 6-h study period (Figure 6.1A). When expressed as a proportion of administered $^{13}$C, the excretion rate reached a mean maximum value of $4.9 \pm 3.1\%$ per hour between 3 and 6 h after administration of the label (range 0.7 to 10.7%). Over the 6-h study period the cumulative excretion of $^{13}$C in breath was $16.4 \pm 12.5\%$ of that administered, ranging between 2.4 and 40.2% (Figure 6.1B, Table 6.3). If defective lipolysis would be responsible for the continuing fat malabsorption in CF patients, then a low percentage of fat absorption would be expected to correlate with low expiration of $^{13}$CO$_2$ after $^{13}$C-MTG ingestion. However, no significant relationship was observed between 6-h cumulative $^{13}$CO$_2$ expiration and either daily fecal fat excretion ($r=-0.02$) or the percentage of total fat absorption ($r=0.04$).

![Figure 6.1](image)

**Figure 6.1** Time courses for the excretion of $^{13}$C in breath over the 6-h study period following oral ingestion of $^{13}$C-MTG (4 mg per kg body weight) at time 0 in 10 CF patients. Each symbol represents a patient. Figure (A) represents the excretion rate, whereas figure (B) represents the cumulative $^{13}$CO$_2$ excretion.

**$^{13}$C-LA test**

The baseline $^{13}$C-LA abundance in plasma prior to consumption of the $^{13}$C-LA label was $-29.1 \pm 2.2\%$ (range $-32.6$ to $-25.5\%$). $^{13}$C-LA concentration in plasma samples, expressed as percentage of the dose per liter plasma, increased steeply after approximately 6 h (Figure 6.2). Peak values of $^{13}$C-LA concentrations in plasma after administration occurred between 8 and 24 h. At 24 h after ingestion of the label, the enrichment of $^{13}$C-LA in plasma had not yet returned to the level of baseline $^{13}$C-abundance. Plasma 8-h $^{13}$C-LA concentrations varied from 0.5 to 2.0% dose L$^{-1}$ plasma (Table 6.3).
If defective intestinal uptake of long-chain fatty acids would be responsible for the continuing fat malabsorption in CF patients, then a low percentage of fat absorption would be expected to correlate with low concentrations of \(^{13}\)C-LA in plasma after \(^{13}\)C-LA ingestion. Figure 6.3 shows the relationship between the 8-h plasma \(^{13}\)C-LA concentrations and either fecal fat excretion or the percentage of total fat absorption. A strong, negative relationship was observed between fecal fat excretion and 8-h plasma \(^{13}\)C-LA concentrations (Figure 6.3A; \(r=-0.75, P<0.01\)) and, correspondingly, a strong, positive relationship was observed between the percentage of total fat absorption and 8-h plasma \(^{13}\)C-LA concentrations (Figure 6.3B; \(r=0.88, P<0.001\)).
first hours, then increased rapidly and reached a possible maximum value at 6 h after administration of the label (Figure 6.4A). In most subjects no decay was observed during the time course of the study. This time course pattern was very similar to the pattern obtained for $^{13}$C-LA concentrations in plasma, except for subject 1, whose $^{13}$C excretion rate in breath already increased after 90 min. The 6-h cumulative $^{13}$CO$_2$ expiration (Table 6.3) amounted to 2.7 ± 3.1% dose. In Figure 6.4B the 6-h cumulative $^{13}$CO$_2$ expiration for all patients is plotted. In contrast to plasma values, no significant relationship between 6-h cumulative $^{13}$CO$_2$ expiration and either fecal fat excretion ($r=0.00$) or the percentage of total fat absorption ($r=-0.13$) was observed. In addition, there was no correlation between plasma $^{13}$C-LA concentrations and cumulative breath $^{13}$CO$_2$ expiration ($r=0.32$), indicating that the multitude of metabolic processes limits the utility of breath samples to measure uptake of long-chain fatty acids [45]. The results indicate that for the measuring intestinal uptake of long-chain fatty acids, plasma sampling cannot be easily replaced by breath sampling.

Finally, we investigated the excretion of $^{13}$C-LA in feces. The apparent absorption of $^{13}$C-label was determined from the difference between the amount of $^{13}$C-LA administered and that excreted in feces. $^{13}$C-LA excretion in feces over the 3-day period was very low and varied between 0.0 and 1.8% of the dose administered (Table 6.3). No metabolites of $^{13}$C-LA were observed in the feces. There was no significant correlation observed between the excretion of $^{13}$C-LA and of total fat in feces ($r=0.22$, $P=0.54$).

Figure 6.4  Time courses for the excretion of $^{13}$C in breath over the 6-h study period following oral ingestion of $^{13}$C-LA (1 mg kg$^{-1}$ body weight) at time 0 in 10 CF patients. Figure (A) represents the excretion rate, whereas figure (B) represents the cumulative $^{13}$CO$_2$ excretion.

Total bile salt concentrations in plasma and feces

Total bile salt concentrations were determined in plasma and feces. Fasting plasma total bile salt concentrations in CF patients were high when compared with normal healthy control values and ranged from 11.6 to 30.3 µmol L$^{-1}$ (mean 17.2 µmol L$^{-1}$) (Table 6.1). Following a meal there was no significant change in total plasma bile salts (data not shown). Fecal total bile salt concentrations in most CF patients were elevated (range 0.7-30.2; mean 13.8 mmol per kg fecal wet weight) when compared with healthy control values, indicating that they had bile salt malabsorption (Table 6.2). Bile salt malabsorption could result in a decreased amount of bile salts available for the formation of mixed micelles, leading to fat malabsorption. However, no
significant correlation was found between percentage of dietary fat absorption and fecal bile salt concentrations ($r=0.26$).

**Discussion**

In CF patients, pancreatic enzyme replacement therapy frequently does not correct disordered fat absorption to values obtained in controls. Our results of the 3-day fat balance confirm the presence of mild to moderate fat malabsorption (percentage of total fat absorption: 79-93%) in a group of pediatric CF patients on enzyme replacement therapy despite good clinical conditions. The aim of the present study was to elucidate whether fat malabsorption in CF patients receiving habitual pancreatic enzyme replacement therapy is due to deficient lipolysis of triacylglycerols or due to impaired intestinal uptake of fatty acids.

We applied two fat substrates with different physical and chemical properties, i.e. $^{13}$C-MTG and $^{13}$C-LA. The principle of the $^{13}$C-MTG breath test is based on lipolysis-dependent $^{13}$CO$_2$ excretion via the breath. Efficient absorption of the $^{13}$C label from the mixed triglyceride is limited primarily by lipolysis [23], and the $^{13}$C-MTG test therefore distinguishes pancreatic insufficiency from deficient intestinal uptake of long-chain fatty acids. After $^{13}$C-MTG ingestion, no relationship was observed between recovery of $^{13}$CO$_2$ in breath and percentage of total fat absorption, indicating that fat malabsorption in CF patients on their habitual enzyme replacement therapy is probably not related to defective lipolysis. The recovery of expired $^{13}$CO$_2$ obtained in the present study was similar to those obtained in other studies, indicating sufficient supplementation of pancreatic enzymes to the CF patients in this study. In healthy adults the 6-h cumulative percentage of $^{13}$C expired via the breath after ingestion of $^{13}$C-MTG varied between 23 and 52% of the dose in one study [23] and between 3 and 48% in another study [24]. The recovery of expired $^{13}$CO$_2$ in CF patients receiving regular amounts of pancreatic enzymes varied between 0 and 45% [23,25]. In neither of these studies total fat absorption was related to the percentage of $^{13}$C recovered in the breath.

Efficient absorption of $^{13}$C-LA, a long-chain unesterified fatty acid, differs predominantly from $^{13}$C-MTG in its dependence on adequate intestinal uptake [27]. Minich et al. [28] showed in a rat model for fat malabsorption (permanently interrupted enterohepatic circulation) that measuring plasma $^{13}$C-LA concentrations is a valuable method to assess the intestinal uptake of long-chain fatty acids and correlates with fat absorption. The $^{13}$C-LA test therefore distinguishes deficient intestinal uptake of long-chain fatty acids from pancreatic insufficiency [28]. After ingestion of $^{13}$C-LA, a strong relationship was observed between 8-h plasma $^{13}$C-LA concentrations and total fat absorption, indicating that the observed fat malabsorption in CF patients on their habitual enzyme replacement therapy is due to defective intestinal uptake of long-chain fatty acids.

Impaired intestinal uptake of long-chain fatty acids may result from several processes. In the absence of adequate bicarbonate secretion, gastric acid entering the duodenum may lower intestinal pH until well into the jejunum [11]. Bile salts are readily precipitated in an acid milieu [17], and duodenal bile salt concentration may fall below the critical micellar concentration, thereby exacerbating fat malabsorption. Precipitated bile salts also appear to be
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lost from the enterohepatic circulation in greater quantities, thus reducing the total bile salt pool and decreasing the fraction of bile salts conjugated with glycine [20]. Intracellular events may also contribute to impaired uptake of long-chain fatty acids in CF patients, e.g. due to absent fatty acid binding proteins or impaired chylomicron assembly and secretion [46]. Viscous, thick intestinal mucus, with altered physical properties, may have a deleterious effect on the thickness of the intestinal unstirred water layer, limiting translocation of long-chain fatty acids over the intestinal epithelium [5,21]. Our data on increased fecal bile salt losses are in agreement with several other studies [47-49] and could be in agreement with a diminished bile salt pool in CF patients. Watkins et al. [18] showed that bile acid pool size was nearly doubled upon treatment with pancreatic enzymes in a group of CF patients with normal fecal bile salt losses. Although the present data suggest that the problem is related to insufficient long-chain fatty acid uptake, they do not allow a clear identification of the individual process responsible for impaired uptake.

The $^{13}$C-LA bolus was administered in an acid-resistant coated capsule, preventing the capsule from being opened at a low pH environment (gastric or intestinal). In patients with a low intestinal pH, e.g. due to inadequate bicarbonate secretion [10,11], the bioavailability of $^{13}$C-LA was hypothesized to be impaired, resulting in a decreased amount of $^{13}$C-LA incorporated into plasma linoleic acid. Since low intestinal pH affects uptake of long-chain fatty acids, we reasoned that the acid-resistant capsule probably enhances the effect of the $^{13}$C-LA test in correctly diagnosing solubilization disorders. The release of the substrate may be delayed in some patients, which can explain the differences in timing for the onset of the individual $^{13}$C-LA curves. In addition, delayed time courses for the onset of $^{13}$CO$_2$ in breath have been observed before and may be explained by, e.g., delayed gastric emptying [24,50,51].

The study was designed such that the patients served as their own controls. Thus, in each individual patient we calculated the percentage of total fat absorption and related these results to the measurements of the $^{13}$C-MTG breath test and the $^{13}$C-LA test. We reasoned that these controls would be the most appropriate, given the fact that neither the optimal positive control group (pancreatic sufficient CF patients with known impaired intestinal uptake) nor the optimal negative control group (pancreatic sufficient CF patients without intestinal uptake disorder) exists or is available. The present approach allowed us to relate the results of total fat absorption to the results of lipolysis and intestinal uptake in the individual patient.

In conclusion, fat balance data indicate that, despite enzyme replacement therapy, pediatric CF patients have increased fecal fat excretion and, correspondingly, decreased percentage of fat absorption. The results of the $^{13}$C-MTG test and $^{13}$C-LA test indicate that continuing fat malabsorption is not likely due to insufficient enzyme replacement therapy, but rather due to either incomplete intraluminal solubilization and/or reduced mucosal uptake of long-chain fatty acids. Indirect indications exist that an increased bile salt loss leading to a diminished bile salt pool may contribute to this problem. Therapeutic attempts to normalize fat absorption in pediatric CF patients need to include a strategy to improve intestinal uptake of long-chain fatty acids.
References

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