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

Mini Kalivianakis, Deanna M Minich, Charles MA Bijleveld, Wim MC van Aalderen, Frans Stellaard, Marianne Laseur, Roel J V onk, and Henkjan J V erkade

ABSTRACT

Background: Pancreatic enzyme replacement therapy frequently fails to correct intestinal fat malabsorption completely in cystic fibrosis (CF) patients. The reason for this failure is unknown.

Objective: 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.

Design: Lipolysis was determined on the basis of breath $^{13}$CO$_2$ recovery in 10 CF patients receiving pancreatic enzyme replacement therapy after they ingested 1,3-distearoyl,2[$^{1-13}$C]octanoyl glycerol ($[^{13}$C]MTG). Intestinal uptake of long-chain fatty acids was determined by analyzing plasma $[^{13}$C]linoleic acid ($[^{13}$C]LA) concentrations after patients ingested $[^{13}$C]LA. For 3 d, dietary intakes were recorded and feces were collected.

Results: Fecal fat excretion ranged from 5.1 to 27.8 g/d ($\bar{x} \pm SD$: 11.1 $\pm$ 7.0 g/d) and fat absorption ranged from 79% to 93% ($89 \pm 5\%$). There was no relation between breath $^{13}$CO$_2$ recovery and dietary fat absorption ($r = 0.04$) after ingestion of $[^{13}$C]MTG. In contrast, there was a strong relation between breath $^{13}$CO$_2$ recovery and 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 receiving enzyme replacement therapy is not likely due to insufficient lipolytic enzyme activity, but rather to incomplete intraluminal solubilization of long-chain fatty acids, reduced mucosal uptake of long-chain fatty acids, or both. Am J Clin Nutr 1999;69:127–34.

KEY WORDS Breath test, mixed triacylglycerol, $[^{13}$C]linoleic acid, fat malabsorption, fat balance, lipolysis, stable isotopes, cystic fibrosis, recommended dietary allowance, children, long-chain fatty acids

INTRODUCTION

In humans, triacylglycerols composed of long-chain fatty acids constitute 92–96% of dietary fats (1). Absorption of these fats is by 2 main processes. Lipolysis, by lipolytic enzymes originating predominantly in the pancreas, leads to hydrolysis of triacylglycerols into fatty acids and 2-monoacylglycerols. Second, intestinal uptake involves the formation of mixed micelles composed of bile components and lipolytic products, followed by the disintegration of the mixed micelles in the unstirred water layer and the translocation of the lipolytic products across the intestinal epithelium (1–4).

Most cystic fibrosis (CF) patients malabsorb dietary fats because of pancreatic insufficiency, which leads to impaired lipolysis (5, 6). The symptoms of pancreatic insufficiency, such as steatorrhea and poor growth, can be alleviated by oral supplementation with pancreatic enzymes. However, despite recent improvements in the pharmacokinetics of these supplements, many patients continue to experience a certain degree of steatorrhea (7–9), with 10–20% of the dietary fat they consume being malabsorbed. It has not been elucidated whether this malabsorption is due to insufficient pancreatic enzyme replacement therapy. This possibility is likely because 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 enzymes from the microcapsules is inhibited and denaturation of the enzymes is stimulated (11, 12). However, it has been shown that an increase in the dose of pancreatic enzymes does not completely correct fat malabsorption (13). In addition, the use of high-strength pancreatic enzyme supplements to increase lipolysis has been reported to be associated with fibrosing colonopathy (14–16).

An alternative explanation for the continuing fat malabsorption in CF patients receiving pancreatic enzyme replacement therapy may involve inefficient intestinal uptake of fatty acids (7, 17). Impaired uptake in CF patients may be due to altered bile composition, decreased bile salt secretion by the liver, bile salt precipitate-
tion, a decreased bile salt pool size, or bile salt inactivation at a low intestinal pH (9, 17–20). Furthermore, small-bowel mucosal dysfunction or alterations in the mucus layer contributes to inefficient intestinal uptake of long-chain fatty acids in CF patients (5, 21).

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

The aim of the present study was to determine whether continued fat malabsorption encountered in pediatric CF patients receiving habitual pancreatic enzyme replacement therapy results from either insufficient lipolysis or defective intestinal uptake of long-chain fatty acids from the lumen. We chose to measure lipolysis and uptake in CF patients with 2 independent tests validated previously. A $^{13}$C-labeled mixed triacylglycerol (1,3-distearoyl,2[1–$^{13}$C]octanoyl glycerol; $^{13}$C-MTG) was ingested and the amount of $^{13}$C excreted in the breath was measured (23–26). Intestinal uptake of long-chain fatty acids was determined by analyzing plasma concentrations of $^{13}$C-linoleic acid ($^{13}$C-LA) after ingestion of $^{13}$C-LA (27, 28). The concentration of $^{13}$C-LA in plasma and the expiration of $^{13}$CO$_2$ thus serve as indexes to quantify the uptake of $^{13}$C-LA.

SUBJECTS AND METHODS

Patient characteristics

Patients

The study protocol was approved by the Medical Ethics Committee of the University Hospital Groningen and informed consent was obtained from the parents and the children. The study group included 10 CF patients, 3 boys and 7 girls, ranging in age from 7 to 18 y. CF had been diagnosed by the sweat test and a DNA genotype analysis (29). Patients 2, 3, 5, 6, 7, and 10 had the F508/DelF508 genotype and patients 1, 4, 8, and 9 had the other genotype. All patients were pancreatic insufficient (lipolysis and intestinal uptake) would enable treatment in individual patients by modulating diet therapy, pancreatic enzyme replacement therapy, and supplementation with antacids and bile salts.

Anthropometry

Weight and height, skinfold thicknesses at 4 sites (biceps, triceps, subscapula, and suprailiac), and midarm circumference were measured by the same pediatrician. The $z$ scores for all anthropometric indexes were calculated based on the reference data for Dutch children described by Gerver and De Bruin (30). The $z$ score is defined as $(X - \bar{x})/SD$, where $X$ is the patient’s measurement and $\bar{x}$ is the median value for age and sex. A negative value indicates a value below the median reference value.

Pulmonary and liver function tests

Pulmonary function was assessed with standard spirometric techniques and was characterized by forced vital capacity and forced expiratory volume in 1 s. For each patient, results were expressed as a percentage of predicted (control) values for sex and height (31). Liver function was assessed at the beginning of the study on the basis of the following serum enzyme activities: γ-glutamyl transferase, aspartate aminotransferase, and alanine aminotransferase.

$^{13}$C-Labeled substrates

$^{[13}$C]MTG was purchased from Euriso-Top (Saint Aubin Cedex, France) and was 99% $^{13}$C-enriched. In the original study, the breath test performed with this molecule was named the mixed-triacylglycerol breath test or the $^{[13}$C]MTG breath test (23, 25). For consistency, we used the same name. $^{[13}$C]LA, obtained from Campro Scientific BV (Veenendaal, Netherlands), had an enrichment > 97%. $^{[13}$C]LA was incorporated 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 foods naturally enriched with $^{13}$C (eg, corn or corn products, pineapple, and cane sugar) for ≥ 2 d before the study began. The $^{[13}$C-LA test and the $^{[13}$C]MTG breath test were performed on 2 subsequent days. On day 1, after an overnight fast, the patients ingested a capsule containing 1 mg $^{[13}$C-LA/kg body wt with their habitual breakfast (eg, bread, butter, ham, and cheese) and pancreatic enzymes. A baseline blood sample was collected into an EDTA-containing tube before breakfast, every 2 h for 8 h after breakfast, and 24 h after breakfast. Immediately after collection, plasma was isolated and stored frozen (–20°C) until analyzed further. Breath samples were collected in duplicate at baseline and every 30 min for 6 h. On day 2, the patients received $^{[13}$C]MTG (4 mg/kg body wt) mixed with their habitual breakfast and pancreatic enzymes. Breath samples were collected in duplicate at baseline and every 30 min for 6 h. Fecal fat balance studies and both breath tests were performed in the same 3-d period. On the day before the $^{[13}$C-LA test, a feces sample was collected for baseline $^{13}$C measurements. After subjects consumed breakfast on the first day, all feces passed were collected for 3 d (72 h) to determine fat malabsorption and the amount of $^{[13}$C-LA excreted into the feces. Collected feces were stored at –20°C. During this period, nutrient intakes were determined from food diaries. Nutrient intakes were calculated from consecutive 3-d food diaries by a clinical dietitian using The Netherlands Nutrients Table NEVO 1993 and intakes were expressed as the recommended dietary allowance for weight, age, and sex (32) (Table 1). During the first 6 h of both tests, no additional foods or liquids were permitted, except for non-energy-containing drinks such as water and tea (without milk or sugar). After 6 h, patients were allowed to have their habitual lunch, including pancreatic enzymes.

Analytic techniques

Breath sample analysis

End expiratory breath was collected with a straw into a 10-mL tube (Exetainers; Labco Limited, High Wycombe, United Kingdom) from which aliquots were taken to determine $^{13}$C enrichment with a continuous-flow isotope ratio mass spectrometer (Finnigan Breath MAT; Finnigan MAT GmbH, Bremen, Germany) in conformance with previous experiments (24). The abundance of $^{13}$C in breath carbon dioxide was expressed as the
IMPAIRED UPTAKE OF FATS IN CF PATIENTS

Comparison of energy intake, ingested lipase enzymes, and fasting plasma concentrations of bile salts in cystic fibrosis patients

<table>
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<tr>
<th>Patient and sex</th>
<th>Age</th>
<th>Weight y (kg)</th>
<th>% of RDA1</th>
<th>% of energy</th>
<th>% of energy</th>
<th>% of energy</th>
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<td>11</td>
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X ± SD — — 103 ± 16 52 ± 3 35 ± 3 14 ± 2 850 ± 460 16.6 ± 5.9

1 Normal range: 1–10 µmol/L.
2 Recommended dietary allowance (32).

The difference per milliliter from the reference standard Pee Dee Belemnit limestone (%).

Mean whole-body carbon dioxide was determined by indirect calorimetry (Oxycon, model ox-4; Dräger, Breda, Netherlands) in 2 separate 5-min time periods during both test days. This method was compared with a method in which carbon dioxide was sampled every 30 min (results not shown). The results indicated that mean carbon dioxide production values obtained during 2 randomly chosen periods were within the 95% CIs of mean values obtained when sampling occurred every 30 min.

Plasma fats

Plasma fats were extracted, hydrolyzed, and methylated according to the method of LePage and Roy (33). Resulting fatty acid methyl esters were analyzed both by gas chromatography and by gas chromatography–combustion isotope ratio mass spectrometry. The resulting fatty acid methyl esters were quantified by using heptadecanoic acid (17:0) as an internal standard.

Fecal fats

After being thawed, feces samples were weighed and homogenized. Fecal fat was determined according to the method of Van de Kamer et al (34) and expressed as g fat/d. Total fat absorption (%) was calculated from the daily dietary fat intake and the daily fecal fat output and expressed as percentage of the daily fat intake (g/d). Results were expressed as µmol/L plasma. Fecal bile salts were extracted from a sample of dried homogenate from a 24-h feces fraction (37) and fluorimetrically measured (36).

Gas-liquid chromatography

Fatty acid methyl esters were separated and quantified with a gas-liquid chromatograph (model 5880; Hewlett-Packard, Palo Alto, CA) equipped with a CP-SIL 88 capillary column (50 m × 0.32 mm; Chrompack, Middelburg, Netherlands) and a flame ionization detector (38, 39). The gas chromatograph oven was programmed to hold an initial temperature of 150°C for 5 min, to then increase by 3°C/min to 200°C and hold for 1 min, and to then increase by 20°C/min to 240°C and hold for 10 min. Adequate separation of LA was thus achieved. The fatty acid methyl esters were quantified by adding heptadecanoic acid (17:0) as an internal standard.

Gas chromatography–combustion isotope ratio mass spectrometry

13C Enrichment of the palmitic acid methyl esters was determined with a gas chromatograph–combustion isotope ratio mass spectrometer (Delta S/GC; Finnigan MAT) (40). Methyl esters were separated on a CP-SIL 88 capillary column (50 m × 0.32 mm; Chrompack). The gas chromatograph oven was programmed to rise from an initial temperature of 80°C to 225°C in 3 steps: 80°C for 1 min, then an increase of 30°C/min to 150°C, then an increase of 5°C/min to 190°C, and then an increase of 10°C/min to 225°C, which was held for 5 min. Adequate separation of LA was thus achieved.

Statistical analysis

The experimental data are reported as means ± SDs. In agreement with other studies (41–43), relations between the percentage of total fat absorption and either plasma [13C]LA concentrations or breath 13CO2 expiration were exponential. All other correlations were assumed to be linear. Correlations between variables were calculated with the least-squares method and are expressed as Pearson’s coefficient of variation (r). Differences between means were considered statistically significant at a P value <0.05. The analyses were performed by using SPSS for WINDOWS (SPSS Inc, Chicago).

RESULTS

Patient characteristics

The z scores for all anthropometric indexes in CF patients were low to normal. For all indexes, the 95% CIs include the ref-
Fat balance

In the CF patients studied, dietary fat intakes over the 3-d period ranged from 54 to 133 g/d and the excretion of fat in feces ranged from 4.9 to 27.8 g/d (Table 2). The percentage of total fat absorption ranged from 79% to 93%. Under physiologic conditions, healthy individuals excrete $\approx 4-6$ g fat/d via the feces (44), which generally means that $>96\%$ of the dietary fats entering the intestinal lumen are being absorbed. These observations were confirmed by experiments performed in our laboratory with dietary food records are also shown in Table 1. The energy intake of those of the other patients (Table 1). Data derived from the 3-d dietary food records are also shown in Table 1. The energy intake of 7 patients exceeded the recommended dietary allowance. 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

Baseline $^{13}$C abundance in breath before consumption of $[^{13}C]$MTG was $-23.2 \pm 2.6\%$ (range: $-25.5\%$ to $-17.1\%$). After ingestion of $[^{13}C]$MTG, different time-course patterns were observed for the expiration of $^{13}$C over the 6-h study period (Figure 1). When expressed as a proportion of administered $^{13}$C, the expiration rate reached a mean maximum value of 4.9 $\pm$ 3.1%/h between 3 and 6 h after administration of the label (range: 0.7–10.7%). Over the 6-h study period, the cumulative expiration of $^{13}$C was 16.4 $\pm$ 12.5% of the dose, ranging from 2.4% to 40.2% (Figure 1, Table 2). If defective lipolysis was 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 relation 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 ($r = 0.04$).

$[^{13}C]$LA test

Baseline $[^{13}C]$LA abundance in plasma before consumption of the $[^{13}C]$LA label was $-29.1 \pm 2.2\%$ (range: $-32.6\%$ to $-25.5\%$). The $[^{13}C]$LA concentration in plasma samples, expressed as a percentage of the dose per liter plasma, increased steeply after $\approx 6$ h (Figure 2). Peak $[^{13}C]$LA concentrations in plasma after administration occurred between 8 and 24 h; 24 h after the label was ingested, $[^{13}C]$LA enrichment in plasma had not yet returned to the baseline $^{13}$C abundance value. Plasma $[^{13}C]$LA concentrations varied from 0.5% to 2.0% of the dose administered (Table 2).

If defective intestinal uptake of long-chain fatty acids was 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. Relations between 8-h plasma $[^{13}C]$LA concentrations and either fecal fat excretion or the percentage of

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**TABLE 2**

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<th>Patient</th>
<th>Fecal bile salts $^{\text{1}}$</th>
<th>Fat intake</th>
<th>Fecal fat</th>
<th>Total fat absorbed</th>
<th>Cumulative, % of dose</th>
<th>8-h Plasma $[^{13}C]$LA concentration</th>
<th>Fecal $[^{13}C]$LA concentration</th>
<th>6-h Breath $^{13}$CO$_2$</th>
<th>6-h Breath $^{13}$CO$_2$</th>
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$^{\text{1}}$Normal range: 0.1–1 mmol/kg wet wt.

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The plasma bile salt concentration of this subject was not significantly different from that of the other patients (Table 1). Data derived from the 3-d dietary food records are also shown in Table 1. The energy intake of 7 patients exceeded the recommended dietary allowance. In all patients, $\approx 50\%$ of energy intakes were derived from carbohydrates, 35% from fat, and 15% from protein. The dose of pancreatic enzyme supplements was $\approx 440$–1820 IU lipase/g fat ingested (Table 1).

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The concentration of this subject was not significantly different from that of the other patients (Table 1). Data derived from the 3-d dietary food records are also shown in Table 1. The energy intake of 7 patients exceeded the recommended dietary allowance. In all patients, $\approx 50\%$ of energy intakes were derived from carbohydrates, 35% from fat, and 15% from protein. The dose of pancreatic enzyme supplements was $\approx 440$–1820 IU lipase/g fat ingested (Table 1).
total fat absorption are shown in Figure 3. A strong, negative
relation was observed between fecal fat excretion and 8-h plasma
\([^{13}\text{C}]\text{LA}\) concentrations \((r = -0.75, P < 0.01)\) and, correspond-
ingly, a strong, positive relation was observed between the per-
centage of total fat absorption and 8-h plasma \([^{13}\text{C}]\text{LA}\) concen-
trations \((r = 0.88, P < 0.001)\).

Because a breath test would be more convenient for patients
than a test requiring blood sampling, we investigated whether
similar information on intestinal uptake of long-chain fatty acids
could be derived from measurements of breath \(^{13}\text{CO}_2\) after
\([^{13}\text{C}]\text{LA}\) ingestion. Baseline \(^{13}\text{C}\) abundance in breath before
ingestion of \([^{13}\text{C}]\text{LA}\) was \(-24.3 \pm 2.3\‰\) (range: \(-27.2\‰\) to
\(-20.9\‰\)). In most subjects, the expiration rate of \(^{13}\text{C}\) was low
during the first hours, then increased rapidly and reached its
maximum value 6 h after administration of the label (Figure 4).
In most subjects, no decay was observed during the study. This
time-course pattern was similar to the pattern obtained for
\([^{13}\text{C}]\text{LA}\) concentrations in plasma, except for subject 1, whose
\(^{13}\text{CO}_2\) expiration rate had already increased 90 min after inges-
tion of the label. The 6-h cumulative \(^{13}\text{CO}_2\) expiration value was
2.7 ± 3.1\% of the dose (Table 2). Cumulative \(^{13}\text{CO}_2\) expiration
over the 6 h is plotted in Figure 4. In contrast with the significant
relation between fat absorption or fecal fat excretion and plasma

![Figure 1: Expiration rate of \(^{13}\text{C}\) and cumulative expiration of \(^{13}\text{CO}_2\) in breath over the 6-h study period in 10 cystic fibrosis patients after ingestion of 4 mg 1,3-distearoyl,2[1-\(^{13}\text{C}\)]octanoyl glycerol (\([^{13}\text{C}]\text{MTG}\)/kg body wt at time 0. Each symbol represents one patient.]

![Figure 2: Appearance of \([^{13}\text{C}]\text{linoleic acid (}[^{13}\text{C}]\text{LA}) in plasma over 24 h in 10 cystic fibrosis patients after a single oral dose of 1 mg\([^{13}\text{C}]\text{LA/kg body wt at time 0. Each symbol represents one patient.]

![Figure 3: Relation between 8-h plasma \([^{13}\text{C}]\text{linoleic acid ([^{13}\text{C}]\text{LA}) concentrations and daily fecal fat excretion (r = -0.75, P < 0.01) and total fat absorption (r = 0.88, P < 0.001) in 10 cystic fibrosis patients after a single oral dose of 1 mg \([^{13}\text{C}]\text{LA/kg body wt at time 0.]

![Figure 4: Appearance of \([^{13}\text{C}]\text{linoleic acid ([^{13}\text{C}]\text{LA}) in plasma over 24 h in 10 cystic fibrosis patients after a single oral dose of 1 mg\([^{13}\text{C}]\text{LA/kg body wt at time 0. Each symbol represents one patient.]

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after meal ingestion. Fecal total bile salt concentrations in most CF patients were higher than normal reference values (range: 0.7–30.2 mmol/kg fecal wet wt; \( x = 13.8 \) mmol/kg fecal wet wt), indicating that the patients had bile salt malabsorption (Table 2). Bile salt malabsorption may have resulted 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 the 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 fat absorption to values obtained in control subjects. Our results from the 3-d fat balance study confirmed that the pediatric CF patients receiving enzyme replacement therapy had mild-to-moderate fat malabsorption (percentage of total fat absorption: 79–93%), 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 was due to deficient lipolysis of triacylglycerols or to impaired intestinal uptake of fatty acids.

We used 2 fat substrates with different physical and chemical properties: \([13\text{C}]\text{MTG}\) and \([13\text{C}]\text{LA}\). The principle of the \([13\text{C}]\text{MTG}\) breath test is based on lipolysis-dependent \(^{13}\text{CO}_2\) expiration. Efficient absorption of the \(^{13}\text{C}\) label from the mixed triacylglycerol is limited primarily by lipolysis (23), and the \([13\text{C}]\text{MTG}\) test therefore distinguishes pancreatic insufficiency from deficient intestinal uptake of long-chain fatty acids. After \([13\text{C}]\text{MTG}\) ingestion, no relation was observed between \(^{13}\text{CO}_2\) recovery in breath and the percentage of total fat absorption, indicating that fat malabsorption in the CF patients was probably not related to defective lipolysis. The recovery of expired \(^{13}\text{CO}_2\) in the present study was similar to that obtained in other studies, indicating that the CF patients were sufficiently supplemented with pancreatic enzymes. In healthy adults, the 6-h cumulative percentage of expired \(^{13}\text{C}\) after ingestion of \([13\text{C}]\text{MTG}\) 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 \(^{13}\text{CO}_2\) in CF patients receiving regular amounts of pancreatic enzymes varied between 0% and 45% (23, 25). In neither of these studies was total fat absorption related to the percentage of \(^{13}\text{C}\) recovered in the breath.

Efficient absorption of \([13\text{C}]\text{LA}\), a long-chain nonesterified fatty acid, differs predominantly from \([13\text{C}]\text{MTG}\) in its rate-limiting factors for adequate intestinal uptake (27). Minich et al (28) showed in a rat model for fat malabsorption (permanently interrupted enterohepatic circulation) that measuring plasma \([13\text{C}]\text{LA}\) concentrations is a valuable method for assessing the intestinal uptake of long-chain fatty acids. The \([13\text{C}]\text{LA}\) test therefore distinguishes pancreatic insufficiency from deficient intestinal uptake of long-chain fatty acids (28). After \([13\text{C}]\text{LA}\) ingestion, a strong relation was observed between \(^{13}\text{CO}_2\) expiration and either fecal fat excretion (\(r = 0.23\)) or the percentage of total fat absorption (\(r = 0.13\)). In addition, there was no correlation between plasma \([13\text{C}]\text{LA}\) concentrations and cumulative breath \(^{13}\text{CO}_2\) excretion (\(r = 0.32\)), indicating that the multitude of metabolic processes limits the utility of breath samples for determining the intestinal uptake of long-chain fatty acids (46). The results indicate that plasma sampling cannot be easily replaced by breath sampling for determining the intestinal uptake of long-chain fatty acids.

Finally, we investigated the excretion of \([13\text{C}]\text{LA}\) in feces. The apparent absorption of the \(^{13}\text{C}\) label was determined from the difference between the amount of \([13\text{C}]\text{LA}\) administered and that excreted in feces. \([13\text{C}]\text{LA}\) excretion in feces over the 3-d period was low and varied between 0.0% and 1.8% of the dose (Table 2). No metabolites of \([13\text{C}]\text{LA}\) were observed in the feces and there was no significant correlation observed between \([13\text{C}]\text{LA}\) excretion and total fat in feces (\(r = 0.22\), \(P = 0.54\)).

**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 higher than those of healthy control subjects and ranged from 11.6 to 30.3 \(\mu\text{mol/L (} x = 17.2 \mu\text{mol/L)}\) (Table 1). There was no significant change in total plasma bile salts (data not shown) from 11.6 to 30.3 \(x\) was higher than those of healthy control subjects and ranged from 8 to 28 mmol/L. Fasting plasma total bile salt concentrations in CF patients were higher than normal reference values (range: 11.6–30.3 mmol/L) (Table 1). There was no significant correlation observed between plasma total bile salt concentrations and cumulative breath \(^{13}\text{CO}_2\) excretion (\(r = 0.00\)).
cipitated in an acid milieu (17) and duodenal bile salt concentrations may fall below the critical micellar concentration, thereby exacerbating fat malabsorption. Precipitated bile salts also appear to be 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 the impaired uptake of long-chain fatty acids in CF patients, possibly because of impaired chylomicron assembly and secretion (47). 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 finding of increased fecal bile salt losses agrees with the findings of several other studies (48–50); these increased losses may lead to a smaller bile salt pool in CF patients. Watkins et al (18) showed that bile acid pool size nearly doubled in a group of CF patients with normal fecal bile salt losses after treatment with pancreatic enzymes. Although the present findings suggest that the pathophysiology of continued fat malabsorption despite pancreatic enzyme treatment in CF is related to insufficient long-chain fatty acid uptake, the data do not clearly indicate the individual process responsible for the impaired uptake.

The [13C]LA bolus was administered in an acid-resistant coated capsule, preventing the capsule from opening in a low-pH environment (gastric or intestinal). In patients with a low intestinal pH, eg, due to inadequate bicarbonate secretion (10, 11), [13C]LA bioavailability is hypothesized to be impaired, resulting in decreased [13C]LA incorporation into plasma LA. Because a low intestinal pH affects the uptake of long-chain fatty acids, we reasoned that the acid-resistant capsule probably enhanced the effect of the [13C]LA test in correctly diagnosing solubilization disorders. The release of the substrate may be delayed in some patients, which can explain the differences in plasma appearance rates of [13C]LA curves between the patients. In addition, delayed time courses for the onset of 13CO2 in breath have been observed in other studies, one explanation being delayed gastric emptying (24, 51, 52).

The study was designed so that the patients served as their own control subjects. Thus, for each individual patient, we calculated the percentage of total fat absorption and related these results to the results of the [13C]MTG breath test and the [13C]LA test. We reasoned that this design was appropriate because neither an optimal positive control group (pancreatic-sufficient CF patients with known impaired intestinal uptake) nor an optimal negative control group (pancreatic-sufficient CF patients with no intestinal uptake disorders) existed or was available. The present approach allowed us to relate the results of total fat absorption to the results of lipolysis and intestinal uptake in individual patients.

In conclusion, the fat balance data indicated that, despite enzyme replacement therapy, the pediatric CF patients in the present study excreted too much fat in the feces and, correspondingly, absorbed too little fat. Results of the [13C]MTG and [13C]LA tests indicated that persistent fat malabsorption in these CF patients was not likely due to insufficient enzyme replacement therapy, but rather to either incomplete intraluminal solubilization or to reduced mucosal uptake of long-chain fatty acids. Indirect indications exist that increased bile salt losses 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.

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