Fat malabsorption in cystic fibrosis patients receiving enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids[1–3]

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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 ± 7.0 g/d) and fat absorption ranged from 79% to 93% (89 ± 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 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 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 precipita-

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 $\Delta F508/\Delta F508$ genotype and patients 1, 4, 8, and 9 had the $\Delta F508$/other genotype. All patients were pancreatic insufficient and therefore took enteric-coated pancreatic enzymes. None of the patients took antacids.

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 $(X - x)$/SD, where X is the patient's measurement and 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

TABLE 1
Comparison of energy intake, ingested lipase enzymes, and fasting plasma concentrations of bile salts in cystic fibrosis patients

Patient and sex	Age	Weight	Energy	Carbohydrates	Fats	Proteins	Lipase enzymes	Plasma bile salts[1]
	y	kg	% of RDA[2]	% of energy	% of energy	% of energy	IU/g fat ingested	µmol/L
1, F	18	55	66	52	33	15	560	13.8
2, F	18	58	104	52	31	17	710	13.5
3, M	16	53	115	48	39	13	1820	20.6
4, M	15	56	113	48	38	15	680	12.8
5, F	9	34	91	52	35	13	440	23.4
6, F	9	26	102	57	30	13	1520	13.5
7, M	8	23	110	50	37	13	830	11.8
8, F	7	27	111	52	35	13	590	18.2
9, F	7	24	92	56	32	12	860	11.6
10, F	7	23	121	54	35	11	460	30.3
$\bar{x} \pm$ 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).

difference per milliliter from the reference standard Pee Dee Belemnite 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.

Percentage of total fat absorption =

$$\frac{\text{fat intake (g/d)} - \text{fecal fat output (g/d)}}{\text{fat intake (g/d)}} \times 100\% \quad (1)$$

Aliquots of freeze-dried feces were extracted according to the method of Bligh and Dyer (35) and were subsequently hydrolyzed and methylated (33). 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 after ingestion of [13C]LA were determined with an enzymatic fluorimetric assay (36). 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 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 $^{13}CO_2$ 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-

TABLE 2
Fecal bile salt concentrations, fecal fat balance, and results of the [^{13}C]linoleic acid ([^{13}C]LA) and 1,3-distearoyl,2[1-^{13}C]octanoyl glycerol ([^{13}C]MTG) tests

					[^{13}C]LA test			[^{13}C]MTG test
Patient	Fecal bile salts[1]	Fat intake	Fecal fat	Total fat absorbed	Cumulative, 6-h breath $^{13}CO_2$	8-h Plasma [^{13}C]LA concentration	Fecal [^{13}C]LA concentration	6-h Breath $^{13}CO_2$
	mmol/kg wet wt	g/d	g/d	%	% of dose	% of dose/L	% of dose	% of dose
1	30.2	54	4.9	91	11.0	1.5	0.6	2.4
2	10.6	85	7.0	92	1.6	1.9	0.2	9.7
3	0.7	124	14.8	88	2.2	0.9	1.8	15.1
4	8.7	133	27.8	79	1.7	0.6	0.6	5.8
5	22.1	92	9.6	90	0.2	1.3	0.0	30.8
6	20.5	66	5.1	92	3.6	2.0	0.7	11.3
7	5.3	85	6.1	93	1.1	1.3	0.2	40.2
8	6.8	93	16.7	82	3.1	0.5	1.3	28.9
9	16.1	64	9.2	86	2.4	0.5	0.3	11.5
10	16.7	88	7.1	92	0.5	1.2	0.2	8.7
$\bar{x} \pm$ SD	13.8 ± 9.0	84 ± 22	11.1 ± 7.0	89 ± 5	2.7 ± 3.1	1.2 ± 0.5	0.6 ± 0.6	16.4 ± 12.5

[1] Normal range: 0.1–1 mmol/kg wet wt.

erence 50th percentile line (z score = 0), indicating that there was 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 profiles. Before the study began, subject 3 was determined to have liver cirrhosis with portal hypertension and was taking ursodeoxycholic acid (750 mg/d); the condition of this patient was stable over the 2 y preceding the study. The plasma bile salt concentration of this subject was not significantly different from 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 all patients, ≈50% of energy intakes were derived from carbohydrates, 35% from fat, and 15% from protein. The dose of pancreatic enzyme supplements was ≈440–1820 IU lipase/g fat ingested (Table 1).

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 ≈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 records and feces of 13 healthy human adults: fecal fat excretion, 3.0 ± 0.9 g/d; total fat absorption, 97 ± 2% (data not shown). Despite standard pancreatic enzyme replacement therapy, fecal fat excretion in 8 of 10 patients was >6 g/d and the percentage of total fat absorption was <96% in all patients studied. On the basis of prevailing reference values (44), fat was malabsorbed by all but 2 patients.

In studies of infants between 0 and 6 mo of age, Fomon et al (45) found that fecal fat excretion per kilogram body weight correlated with fat intake per kilogram body weight. In our study, we observed a curvilinear correlation ($r = 0.71$, $P < 0.05$) similar to that for the infants in Fomon et al's study, despite the fact that our subjects had a considerably lower intake of fat per kilogram body weight. However, when we compared fat intake per kilogram body weight with the 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

Baseline ^{13}C abundance in breath before consumption of [^{13}C]MTG was −23.2 ± 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 ± 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 ± 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 ± 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 ≈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
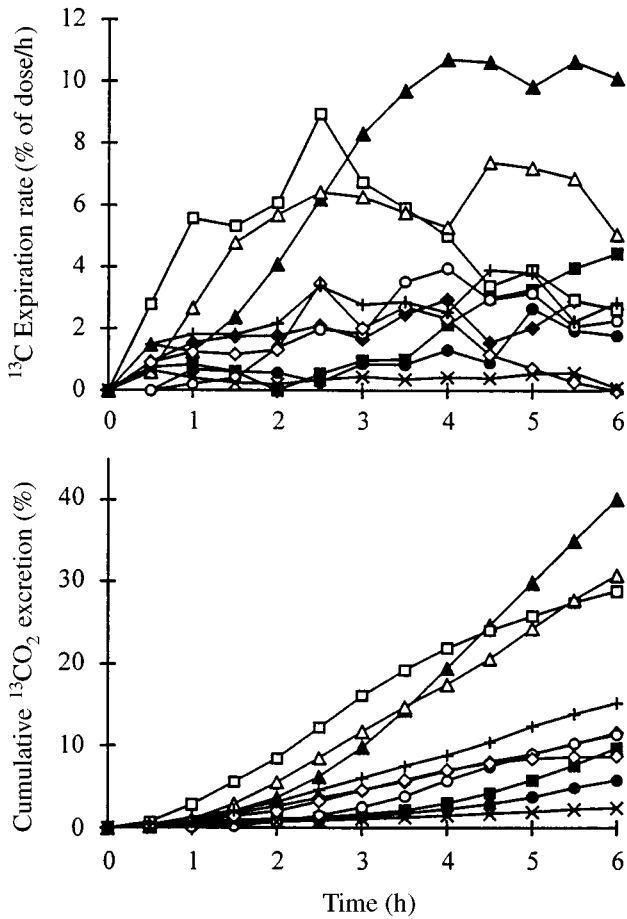
FIGURE 1. Expiration rate of ^{13}C and cumulative expiration of $^{13}CO_2$ in breath over the 6-h study period in 10 cystic fibrosis patients after ingestion of 4 mg 1,3-distearoyl,2[1-^{13}C]octanoyl glycerol ([^{13}C]MTG)/kg body wt at time 0. Each symbol represents one patient.

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

centage of total fat absorption and 8-h plasma [^{13}C]LA concentrations ($r = 0.88$, $P < 0.001$).

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 $^{13}CO_2$ after [^{13}C]LA ingestion. Baseline ^{13}C abundance in breath before ingestion of [^{13}C]LA was -24.3 ± 2.3‰ (range: -27.2‰ to -20.9‰). In most subjects, the expiration rate of ^{13}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}C]LA concentrations in plasma, except for subject 1, whose $^{13}CO_2$ expiration rate had already increased 90 min after ingestion of the label. The 6-h cumulative $^{13}CO_2$ expiration value was 2.7 ± 3.1% of the dose (Table 2). Cumulative $^{13}CO_2$ 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

total fat absorption are shown in **Figure 3**. A strong, negative relation was observed between fecal fat excretion and 8-h plasma [^{13}C]LA concentrations ($r = -0.75$, $P < 0.01$) and, correspondingly, a strong, positive relation was observed between the per-
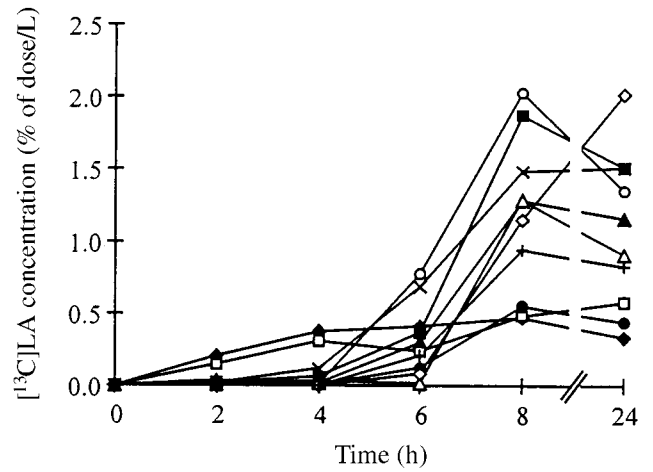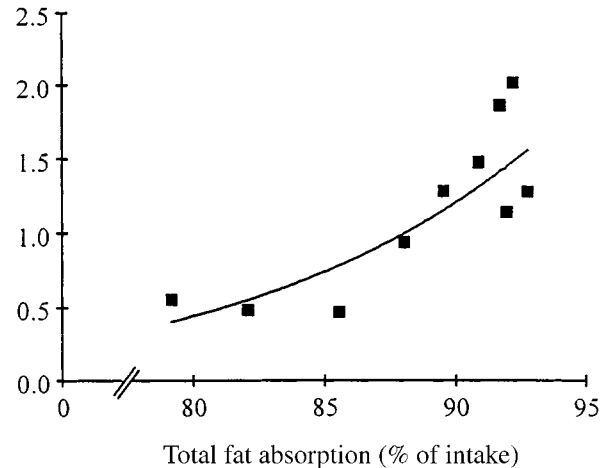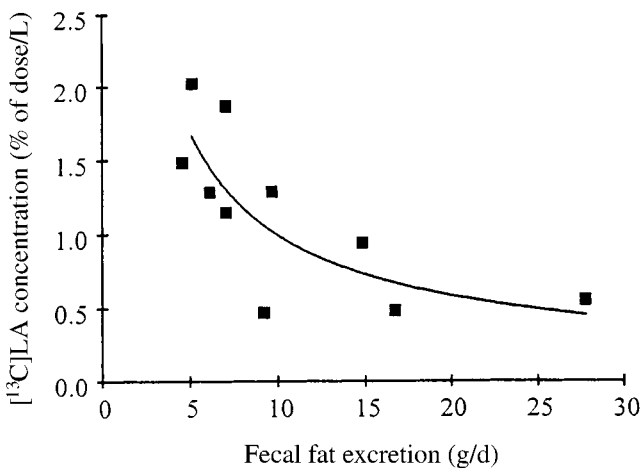

FIGURE 3. Relation between 8-h plasma [^{13}C]linoleic acid ([^{13}C]LA) concentrations and daily fecal fat excretion ($r = -0.75$, $P < 0.01$) and total fat absorption ($r = 0.88$, $P < 0.001$) in 10 cystic fibrosis patients after a single oral dose of 1 mg [^{13}C]LA/kg body wt at time 0.
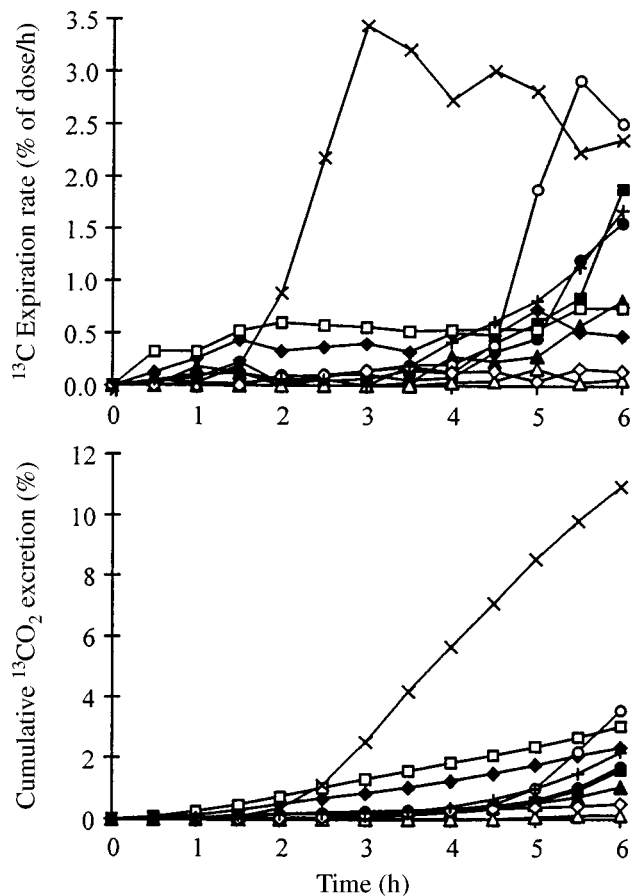
FIGURE 4. Expiration rate of ^{13}C and cumulative ^{13}CO$_2$ expiration over the 6-h study period in 10 cystic fibrosis patients after ingestion of 1 mg [^{13}C]linoleic acid ([^{13}C]LA)/kg body wt at time 0.

concentrations of [^{13}C]LA, there was no significant relation between 6-h cumulative ^{13}CO$_2$ expiration and either fecal fat excretion ($r = 0.00$) or the percentage of total fat absorption ($r = -0.13$). 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 for determining the 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}C]LA in feces. The apparent absorption of the ^{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-d period was low and varied between 0.0% and 1.8% of the dose (Table 2). No metabolites of [^{13}C]LA were observed in the feces and there was no significant correlation observed between [^{13}C]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 μmol/L ($\bar{x}$: 17.2 μmol/L) (Table 1). There was no significant change in total plasma bile salts (data not shown)

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; $\bar{x}$: 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}C]MTG and [^{13}C]LA. The principle of the [^{13}C]MTG breath test is based on lipolysis-dependent ^{13}CO$_2$ expiration. Efficient absorption of the ^{13}C label from the mixed triacylglycerol 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 relation was observed between ^{13}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}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}C 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 was total fat absorption related to the percentage of ^{13}C recovered in the breath.

Efficient absorption of [^{13}C]LA, a long-chain nonesterified fatty acid, differs predominantly from [^{13}C]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}C]LA concentrations is a valuable method for assessing 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 [^{13}C]LA ingestion, a strong relation was observed between 8-h plasma [^{13}C]LA concentrations and total fat absorption, indicating that the observed fat malabsorption in CF patients was 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 pre-

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 [^{13}C]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), [^{13}C]LA bioavailability is hypothesized to be impaired, resulting in decreased [^{13}C]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 [^{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 plasma appearance rates of [^{13}C]LA curves between the patients. In addition, delayed time courses for the onset of $^{13}CO_2$ 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 [^{13}C]MTG breath test and the [^{13}C]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 [^{13}C]MTG and [^{13}C]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.

REFERENCES

1. Carey MC, Hernell O. Digestion and absorption of fat. Semin Gastrointest Dis 1992;3:189–208.
2. Tso P. Intestinal lipid absorption. In: Johnson LR, ed. Physiology of the gastrointestinal tract. 3rd ed. New York: Raven Press, 1994:1867–907.
3. Staggers JE, Hernell O, Stafford RJ, Carey MC. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. Biochemistry 1990;29:2028–40.
4. Hernell O, Staggers JE, Carey MC. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry 1990;29:2041–56.
5. Pencharz PB, Durie PR. Nutritional management of cystic fibrosis. Annu Rev Nutr 1993;13:111–36.
6. Shalon LB, Adelson JW. Cystic fibrosis. Gastrointestinal complications and gene therapy. In: Lebenthal E, ed. The pediatric clinics of North America. Pediatric gastroenterology I. Philadelphia: WB Saunders Company, 1996:157–96.
7. Zentler-Munro PL, Fine DR, Batten JC, Northfield TC. Effect of cimetidine on enzyme inactivation, bile acid precipitation, and lipid solubilisation in pancreatic steatorrhoea due to cystic fibrosis. Gut 1985;26:892–901.
8. Regan PT, Malagelada J-R, DiMagno EP, Go VLW. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: correction by treatment. Gastroenterology 1979;77:285–9.
9. Carroccio A, Pardo F, Montalto G, et al. Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy. A long-term study in cystic fibrosis. Dig Dis Sci 1992;37:1441–6.
10. Abrams CK, Hamosh M, Hubbard VS, Dutta SK, Hamosh P. Lingual lipase in cystic fibrosis. Quantitation of enzyme activity in the upper small intestine of patients with exocrine pancreatic insufficiency. J Clin Invest 1984;73:374–82.
11. Weber AM, Roy CC. Intraduodenal events in cystic fibrosis. J Pediatr Gastroenterol Nutr 1984;3(suppl):S113–9.
12. Robinson PJ, Smith AL, Sly PD. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. Dig Dis Sci 1990;35:1299–304.
13. Robinson P, Sly P. High dose pancreatic enzymes in cystic fibrosis. Arch Dis Child 1990;65:311–2.
14. Smyth R, Van Velzen D, Smyth A, Lloyd D, Heaf D. Strictures of ascending colon in cystic fibrosis and high strength pancreatic enzymes. Lancet 1994;343:85–6.
15. MacSweeney E, Oades PJ, Buchdahl RM, Phelan M, Bush A. Relationship of thickening of colon wall to pancreatic enzyme treatment in cystic fibrosis. Lancet 1995;345:752–6.
16. FitzSimmons SC, Burkhart GA, Borowitz D, et al. High-dose pancreatic-enzyme supplements and fibrosing colonopathy in children with cystic fibrosis. N Engl J Med 1997;336:1283–9.
17. Zentler-Munro PL, FitzPatrick WJF, Batten JC, Northfield TC. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhoea due to cystic fibrosis. Gut 1984;25:500–7.
18. Watkins JB, Tercyak AM, Szczepanik P, Klein PD. Bile salt kinetics

in cystic fibrosis: influence of pancreatic enzyme replacement. Gastroenterology 1977;73:1023–8.

19. Roy CC, Weber EA, Morin CL, et al. Abnormal biliary lipid composition in cystic fibrosis. Effect of pancreatic enzymes. N Engl J Med 1977;297:1301–5.

20. Belli DC, Levy E, Darling P, Leroy C, Lepage G. Taurine improves the absorption of a fat meal in patients with cystic fibrosis. Pediatrics 1987;80:517–23.

21. Eggermont E, De Boeck K. Small intestinal abnormalities in patients with cystic fibrosis. Eur J Pediatr 1991;150:824–8.

22. Corey M, McLaughlin FJ, Williams M, Levison H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. J Clin Epidemiol 1988;41:583–91.

23. Vantrappen GR, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. Gastroenterology 1989;96:1126–34.

24. Kalivianakis M, Verkade HJ, Stellaard F, Van der Werf M, Elzinga H, Vonk RJ. The ^{13}C-mixed triglyceride breath test in healthy adults: determinants of the $^{13}CO_2$ response. Eur J Clin Invest 1997;27:434–42.

25. Amarri S, Harding M, Coward WA, Evans TJ, Weaver LT. ^{13}Carbon mixed triglyceride breath test and pancreatic enzyme supplementation in cystic fibrosis. Arch Dis Child 1997;76:349–51.

26. Swart GR, Baartman EA, Wattimena JL, Rietveld T, Overbeek SE, Van den Berg JW. Evaluation studies of the ^{13}C-mixed triglyceride breath test in healthy controls and adult cystic fibrosis patients with exocrine pancreatic insufficiency. Digestion 1997;58:415–20.

27. Watkins JB, Klein PD, Schoeller DA, Kirschner BS, Park R, Perman JA. Diagnosis and differentiation of fat malabsorption in children using ^{13}C-labeled lipids: trioctanoin, triolein and palmitic acid breath tests. Gastroenterology 1982;82:911–7.

28. Minich DM, Kalivianakis M, Havinga R, et al. A novel ^{13}C-linoleic acid absorption test detects lipid malabsorption due to impaired solubilization in rats. Gastroenterology 1997;112(suppl):A894 (abstr).

29. Kerem B-S, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245:1073–80.

30. Gerver WJM, De Bruin R. Paediatric morphometrics: a reference manual. Utrecht, Netherlands: Bunge, 1996.

31. Zapletal A, Samánek M, Paul T. Lung function in children and adolescents. Methods, reference values. Basel, Switzerland: Karger, 1987.

32. Anonymous. NEVO tabel. Nederlands Voedingsstoffenbestand. (NEVO table. Dutch nutrient database.) Den Haag, Netherlands: Voorlichtingsbureau voor de Voeding, 1993 (in Dutch).

33. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986;27:114–20.

34. Van de Kamer JH, Ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in feces. J Biol Chem 1949;177:347–55.

35. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.

36. Murphy GM, Billing BH, Baron DN. A fluorimetric and enzymatic method for the estimation of serum total bile acids. J Clin Pathol 1970;23:594–8.

37. Kalek H-D, Stellaard F, Kruis W, Paumgartner G. Detection of increased bile acid excretion by determination of bile acid content in single stool samples. Clin Chim Acta 1984;140:85–90.

38. Eder K. Gas chromatography analysis of fatty acid methyl esters. J Chromatogr B 1995;671:113–31.

39. Gutnikov G. Fatty acid profiles of lipid samples. J Chromatogr B 1995;671:71–89.

40. Guo Z, Nielsen S, Burguera B, Jensen MD. Free fatty acid turnover measured using ultralow doses of [U-^{13}C]palmitate. J Lipid Res 1997;38:1888–95.

41. Newcomer AD, Hofmann AF, DiMagno EP, Thomas PJ, Carlson GL. Triolein breath test: a sensitive and specific test for fat malabsorption. Gastroenterology 1979;76:6–13.

42. Einarsson K, Björkhem I, Eklöf R, Blomstrand R. ^{14}C-triolein breath test as a rapid and convenient screening test for fat malabsorption. Scand J Gastroenterol 1983;18:9–12.

43. Mills PR, Horton PW, Watkinson G. The value of the ^{14}C breath test in the assessment of fat absorption. Scand J Gastroenterol 1979;14:913–21.

44. Carey MC, Small DM, Bliss CM. Lipid digestion and absorption. Annu Rev Physiol 1983;45:651–77.

45. Fomon SJ, Ziegler EE, Thomas LN, Jensen RL, Filer LJ Jr. Excretion of fat by normal full-term infants fed various milks and formulas. Am J Clin Nutr 1970;23:1299–313.

46. Pedersen NT, Jorgensen BB, Rannem T. The [^{14}C]-triolein breath test is not valid as a test of fat absorption. Scand J Clin Lab Invest 1991;51:699–703.

47. Minich DM, Vonk RJ, Verkade HJ. Intestinal absorption of essential fatty acids under physiological and essential fatty acid-deficient conditions. J Lipid Res 1997;38:1709–21.

48. O'Brien S, Mulcahy H, Fenlon H, et al. Intestinal bile acid malabsorption in cystic fibrosis. Gut 1993;34:1137–41.

49. Weber AM, Roy CC, Morin CL, Lasalle R. Malabsorption of bile acid in children with cystic fibrosis. N Engl J Med 1973;289:1001–5.

50. Weber AM, Roy CC, Chartrand L. Relationship between bile acid malabsorption and pancreatic insufficiency in cystic fibrosis. Gut 1976;17:295–9.

51. Duncan A, Cameron A, Stewart MJ, Russell RI. Limitations of the triolein breath test. Clin Chim Acta 1992;205:51–64.

52. Jones PJH, Pencharz PB, Clandinin MT. Absorption of ^{13}C-labeled stearic, oleic, and linoleic acids in humans: application to breath tests. J Lab Clin Med 1985;105:647–52.