Gastric Function in Children with Cystic Fibrosis: Effect of Diet on Gastric Lipase Levels and Fat Digestion

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ABSTRACT

The effect of diet, usual ($44 \pm 4\%$ energy as fat), high-fat ($49 \pm 4\%$ energy as fat), and moderate-fat ($33 \pm 2\%$ energy as fat), on gastric function (lipase and pepsin activities, pH, emptying rate) and intragastric digestion of fat were assessed in six children with cystic fibrosis. Fasting and postprandial activity of digestive enzymes, gastric pH, and gastric volume measured before, during, and after 120 min of feeding did not differ significantly as a function of fat intake. Postprandial gastric lipase output (units per kilogram of body weight) during usual, moderate-fat, and high-fat diets was close to or higher than (38.8 ± 7.2, 44.9 ± 8.6, and 54.8 ± 5.5 U/kg per 20 min) gastric lipase output of premature infants (22.5 ± 6.4 to 28.3 ± 6.6 U/kg per 20 min) or of healthy adults (5.4 ± 0.4 U/kg per 15 min) fed a high-fat diet. Postprandial pepsin output was higher (4749 ± 797, 6117 ± 925, and 5444 ± 819 U/kg per 20 min) than in premature infants (597 ± 77 to 743 ± 97 U/kg per 20 min) or healthy adults (781 ± 56 U/kg per 15 min). Eighty minutes after feeding gastric lipolysis reached 20 to 36%. This study shows that gastric lipase activity is high in cystic fibrosis patients maintained on diets providing 32% to 49% energy as fat, and that gastric lipase level did not increase over the ranges of dietary fat intake tested. (*Pediatr Res* **55**: 457–465, 2004)

Abbreviations
CF, cystic fibrosis
CFTR, CF transmembrane conductance regulator
PI, pancreatic insufficiency
TG, triglycerides
DG, diglycerides
MG, monoglycerides
PL, phospholipids
PEG, polyethylene glycol
RDA, recommended daily allowance

CF is the most common autosomal recessive disorder in Caucasians and results in pulmonary, gastrointestinal, metabolic and nutritional disturbances (1–3). The basic defect in CF involves faulty regulation of the epithelial cell chloride channel caused by mutations in the CFTR gene (4). Deletion of phenylalanine at amino acid position 508 (ΔF508) in the CFTR protein accounts for 70% of CF gene abnormalities in patients of Northern European ancestry (5). The ΔF508 mutation leads to defective maturation of CFTR and absence of the protein at the apical membrane (6). Greater than 98% of ΔF508 homozygotes have PI.

PI, resulting in fat malabsorption, is found in at least 85% of CF patients (7–9). Lacking pancreatic enzymes, CF patients are dependent on pancreatic enzyme supplements (10–12). However, the degree to which fat is absorbed by these patients varies, and even when pancreatic lipase activity is completely absent, dietary fat is absorbed. Indeed, Ross (13) reported fat absorption of 26 to 81% in a group of children with CF, although most of them did not have detectable pancreatic lipase activity. Lapey *et al.* (14) reported greater than 50% fat absorption in 15 patients with exocrine PI secondary to CF, without any oral enzyme supplementation. Ross and Sammons (15) have therefore postulated the presence of compensatory lipolytic activity in PI. It was shown subsequently that this compensation is provided by gastric lipase, the enzyme that initiates fat digestion in the stomach (16, 17). Because of lower pH in the upper small intestine in this condition (18–20), gastric lipase is able to act not only in the stomach but also in

the duodenum (18–20). Indeed, gastric lipase amounted to 90% of total lipase activity delivered to the ligament of Treitz throughout a 2-h postprandial period in patients with PI secondary to CF (18) or chronic alcoholism (20). Pancreatic enzyme supplements fail frequently to normalize fat absorption in PI patients (10–12). Inactivation in the stomach and suboptimal release in the duodenum because of low pH are the main reasons that only as little as 8% of lipase given in coated supplements reaches the ligament of Treitz (11). Thus, gastric lipase might provide additional therapy for the prevention of steatorrhea in CF because of the characteristics of the enzyme and the specific conditions in the upper small intestine.

Because a positive correlation has been reported between good nutritional status and long-term survival and well-being in CF patients (21), numerous published recommendations for the nutritional management of children with CF encourage an energy-dense diet, especially a high-fat intake (22, 23), to counteract energy losses from malabsorption and increased energy expenditure.

The fat content of the diet affects the level of digestive lipases (24–28). Several animal studies have shown that doubling the usual fat intake leads to a 95% increase in lingual lipase levels in tongue tissue in the rat (24) and a 70% increase in gastric lipase levels in gastric mucosa of rabbits or pigs within 1 to 2 wk (27). In healthy adult human subjects a 50 to 70% increase in gastric lipase content occurs within 2 wk after change of dietary fat from 25 to 50% (28).

In light of all these studies, it might be important to explore the possibility of increasing the endogenous levels of gastric lipase in CF patients. Thus, this study aimed to assess whether fat intake modulates gastric lipase level in children with CF, *i.e.* whether a high-fat diet (approximately 50% energy as fat) can induce an increase in lipase level compared with a moderate-fat diet (approximately 30% energy as fat). In parallel, intragastric fat digestion was also studied.

METHODS

Subjects. Six children aged 2.5 to 14 y old [6.4 ± 1.6 y (mean ± SD), three girls and three boys] were enrolled in the study after informed consent was given by the parents. The experimental protocol was approved by the institutional review boards of Johns Hopkins Medical Institution (Baltimore, MD,

U.S.A.) and Georgetown University Medical Center (Washington, DC, U.S.A.). The diagnosis of CF was based on clinical evaluation. PI was documented by abnormal quantitative fecal fat in all patients. All children had evidence of pulmonary disease, and their Taussig clinical score ranged from 50 to 95 (29). Individual clinical data on the patients are presented in Table 1. Growth characteristics and anthropometric data were in the ranges of data previously published for CF patients (30). Four children were ΔF508 homozygotes, one ΔF508/unknown, and one G542X/2814 delA genotype. All patients had gastrostomy feeding tubes, and all but patient 2 were receiving enteral feeds during the period of the study. Patients were studied as outpatients. All patients were treated with pancreatic enzyme supplements: three with Pancrease (Ortho McNeil Pharmaceuticals, Raritan, NJ, U.S.A.), two with Creon (Solvay Pharmaceuticals, Marietta, GA, U.S.A.), and one with Ultrase (Scandi Pharmaceuticals, Birmingham, AL, U.S.A.). No medication that could interfere with gastrointestinal function and no enzyme supplements were used on the days of the study. Each child served as his or her own control.

CFTR mutation detection. Mutation detection was performed using reverse dot-blot technology and reagents and methods supplied by the manufacturers (Perkin-Elmer, Norwalk, CT, U.S.A.). One patient had her second mutation (2814delA) identified by Genzyme (Framingham, MA, U.S.A.).

Diets and test meals. All patients were regularly followed by a dietitian [A.K.] and advised to eat an energy-dense diet. The usual diet and nutritional habits of each subject were determined using a 3-d diet record. Dietary analyses were done using the Nutritionist III software (Oregon, U.S.A.).

The patients were studied at three different periods: first during their usual diet, then 2 wk after consuming a moderate-fat diet (approximately 30% of calories derived from fat), and finally 2 wk after consuming a high-fat diet (approximately 50% of calories derived from fat). The diets were given in random order. The experimental diets (moderate and high-fat diets) were calculated in accordance with the regular energy intake of the subjects (2122 ± 210 kcal/d). The diets during the experimental periods were evaluated by a 3-d dietary survey.

The two test meals were similar to the experimental diets and contained a similar amount of protein. The moderate-fat

Table 1. *Anthropometric and clinical data of the study CF children*

Patients	1	2	3	4	5	6
Age (y)	2.4	4.5	5.0	5.5	7.0	14.0
Height (cm)*	89.8 (25–50)	96.5 (3)	104.4 (10–25)	115.7 (75)	109.4 (<3)	154.4 (10–25)
Weight (kg)*	12.9 (25–50)	15.6 (10)	16.8 (25–50)	17.7 (25)	17.2 (<3)	42.0 (5–10)
Gender (M/F)	M	M	M	F	F	F
Taussig score	95	85	71	50	45	68
AC (cm)	16.7	17.0	15.6	16.0	16.3	24.0
TSF (mm)	7.2	5.5	3.5	3.0	5.5	13.0
MAMA (mm^2)	1 658	1 856	1 672	1 803	1 689	3 153
AFA (mm^2)	560	444	797	234	427	1 427
Age at diagnosis	7mo	4mo	1wk	5mo	4mo	13mo
CF genotype	ΔF508/ukn	ΔF508/ΔF508	ΔF508/ΔF508	ΔF508/ΔF508	ΔF508/ΔF508	G542X/2814delA

* Number in parentheses indicate percentiles for each patient.

Abbreviations: AC, arm circumference; TSF, triceps skin fold; MAMA, mid-arm muscle area; AFA, arm fat area; ukn, unknown.

test meal consisted of 240 mL of vanilla Ensure formula containing 8.8 g protein, 34.4 g carbohydrate, and 8.8 g fat and provided 252 kcal, 14% protein, 54.5% carbohydrate, and 31.4% fat (Mead Johnson Nutritionals, Evansville, IL, U.S.A.). The high-fat test meal was composed of 138 mL Ensure, 4 teaspoons of protein powder (Promod, Ross Products Division, Abbott Laboratories, Columbus, OH, U.S.A.), and 11 mL of corn oil and water (final volume 240 mL) and provided 267 kcal, 16% protein, 30% carbohydrate, and 54% fat. As the size of the fat droplets affects lipase activity (31, 32), the high-fat test meal was mixed in a blender for 10 min. The Ensure formula fat droplet size was 0.52 ± 0.03 μm, and the high-fat test meal fat droplet size was 0.77 ± 0.12 μm; the specific surface area of the emulsions was 17.4 ± 1.1 and 11.8 ± 2.4 m^2/g of lipid, respectively.

Collection of gastric contents. At the completion of 2 wk on each diet the patients were studied at 0800–0900 h after a 9-h fast. Fasting gastric aspirates were collected at 10-min intervals for a period of 30 min through a gastrostomy tube (Bard International Products, Billerica, MA, U.S.A.). Then, a standardized liquid test meal containing PEG-4000, a nonabsorbable marker (50 mg PEG/test meal), was administered directly into the stomach through the gastrostomy tube for a period of 10 min. The high-fat test meal was used after the usual and the high-fat diet periods, and the moderate-fat test meal after the moderate-fat diet period. Postprandial gastric specimens (6 to 7 mL) were sampled at 20-min intervals, timed from the time when half of the meal volume was fed, for an overall sampling period of 80 to 120 min depending on the patient. Gastric samples were withdrawn by a syringe after careful mixing by repeated manual aspiration–reinstillation to ensure that representative samples were obtained. Immediately after collection of each sample, the appearance was recorded, the pH was measured using a portable pH meter (model 840008 CMS; Spree Scientific, Houston, TX, U.S.A.), and the sample was neutralized to pH 4.5–5.0, when necessary, to prevent the inactivation of gastric lipase. Neutralization did not interfere with pepsin activity because this enzyme is stable when stored at pH 1 to 5. A cocktail of inhibitors (33, 34) (10% vol/vol) was added to the sample aliquot taken for neutral lipid analysis to prevent lipolysis during storage. Under these conditions, approximately 85 to 90% of gastric lipase activity is inhibited (34).

All specimens were collected on ice, and then kept in dry ice until transfer to the laboratory. All specimens were stored at $-70°$C until assay. Under these storage conditions, lipase activity is stable for months (35, 36).

Measurement of gastric content volume. We used the double-sampling marker dilution method of George (37), using PEG-4000 as a marker, to measure gastric volume to quantify total gastric lipase activity level, and to compare gastric emptying rate between both test meals. The meals used were fully homogenized liquid test meals that have been shown to lead to simultaneous emptying of the aqueous and lipid phases (38, 39). Thus, under these conditions, PEG-4000, similar to phenol red (35), is a marker of the aqueous phase and can be used to monitor the emptying of all the meal components (32). A small amount of concentrated PEG marker was added (1 mL, con-

taining 50 mg of PEG) at each collection time after a sample was collected just before addition of a new dose of marker. Volumes present in the stomach (secretion plus emulsion) were calculated by the following equation:

$$V_1 = V_2 \frac{(C_2 - C_3)}{(C_3 - C_1)} \tag{1}$$

where V_1 is the volume to be determined; C_1 the initial concentration of marker in V_1; V_2 the volume of concentrated marker added to V_1; C_2 the concentration of added marker; C_3 the final concentration of the marker; the mass of marker in V_1 is given by $V_1 \times C_1$; and the mass of marker in V_2 is given by $V_2 \times C_2$.

Sampling was taken into account when determining the final volume. The amount of fat present in the stomach contents was calculated by multiplying the gastric volume by the fat concentration at each time. Rates of fat emptying were thus calculated knowing the initial quantity of fat ingested (8.8 or 16 g) and the total amount of fat present in the stomach at each time.

Quantification of gastric lipase activity. Gastric lipase activity in the aspirates was quantified using a stable emulsion of tri[^{3}H]olein as previously described (28, 40). The [^{3}H]oleic acid produced was quantified after separation by liquid-liquid partition (41). One lipase unit is expressed as the release of 1 μmol of FFA per minute (international unit). Gastric lipase output (concentration times volume) is expressed as units per kilogram of body weight.

Because it was previously reported that PEG in the range of 1–5 mg/mL inhibits pancreatic lipase activity (42), we have tested the effect of PEG-4000 on gastric lipase and pepsin using human gastric juice. The results showed no effect of PEG when present at the above concentrations (data not shown).

Quantification of pepsin activity. Pepsin activity was measured in 20–40 μL of gastric aspirates (43, 44) as previously described (28, 40). One pepsin unit is defined as the amount of enzyme required to produce 0.1 μmol of tyrosine-containing peptides at 37°C in 10 min at pH 1.8 from a 2% Hb solution. Pepsin output (concentration times volume) is expressed as units per kilogram of body weight.

Lipid analysis. Lipids in test meals and gastric aspirates were homogenized and extracted in chloroform–methanol (2:1, vol/vol), containing 0.01% BHT according to Folch *et al.* (45). To ensure complete protonation of fatty acids, the organic solvent phases were partitioned with 20% (vol/vol) 0.15 M aqueous NaCl containing 2% glacial acetic acid (vol/vol, pH 3.0) as previously described (33).

Neutral lipid classes (triacylglycerol, diacylglycerol, monoacylglycerol, FFA) were separated by two-stage, one-dimensional thin-layer chromatography according to Bitman and Wood (46) and stained in 10% copper sulfate–8% phosphoric acid (wt/vol) solution. Lipid was quantified by densitometry with a Shimadzu dual-wavelength densitometer (deuterium lamp, 340 nm; Shimadzu Scientific Instruments, Columbia, MD, U.S.A.). The densitometric areas were fitted by computer to a linear regression model, and the coefficient of determination, r^2, was in the range of 0.90–0.98. A standard

sample (5–20 μg) was included in every experimental plate to correct for deviations from the standard calibration curve. Values were converted into moles using average molecular masses calculated according to the fatty acid composition of the test meals (TG = 872; DG = 612; MG = 352; FFA = 278).

The extent of gastric TG hydrolysis was calculated as the percent of TG disappearance from total glycerides present (TG + DG + MG; %TG total) and from the percentage of TG originally present in the test meals (%TG at time 0), *i.e.* TG disappearance (%) = %TG time 0 − %TG total, as previously described (32, 34, 40).

PEG determination. PEG concentration in gastric contents was determined according to the method of Hyden (47) as modified by Ulyatt (48).

Statistical analysis. Data are given as mean ± SEM of six or four subjects. Data were tested by two-way ANOVA for paired values using the statistical package (StatView 512+) for the Macintosh. Significance level was set at $p < 0.05$ for any variable tested (49).

RESULTS

This study determined whether gastric function assessed as pH, gastric emptying, digestive enzyme activity, and intragastric lipolysis is modulated by the amount of fat in the diets given to children with CF.

Composition of regular and experimental diets. Nutrient intake data are shown in Table 2. Energy provided by fat ranged between 35 and 60% of the total energy supply in the usual diet, and the fat consumption was 65 to 148 g of fat per day (3.5 to 8.8 g fat/kg). During the moderate-fat period, fat ranged between 27.9 and 37.9% of the total energy, and daily intake of fat was 33 to 82 g (2 to 4 g/kg per day). During the high-fat diet period 43 to 62% of energy was provided by fat with a daily fat intake of 83 to 148 g/d, representing 3.5 to 8.8 g/kg per day. Caloric intake was lower in the moderate-fat diet; however, protein intake was relatively close in the three diets (70–76 g/d).

Gastric pH throughout digestion. Gastric pH during the usual, high-fat, and moderate-fat diet periods is shown in Figure 1. During the usual diet, the pH values varied between 1.5 and 3.0 in fasting conditions, increased to 4.5–5.9 immediately after a meal was ingested, and then gradually decreased postprandially to 2.0–4.6 at 1 h and to 1.6–3.1 at 2 h. During moderate-fat and high-fat diets, pH values varied between 1.73 and 2.03 and 1.87 and 2.05 at baseline, respectively, then increased to 5.4–5.5 after the test meals, and gradually de-
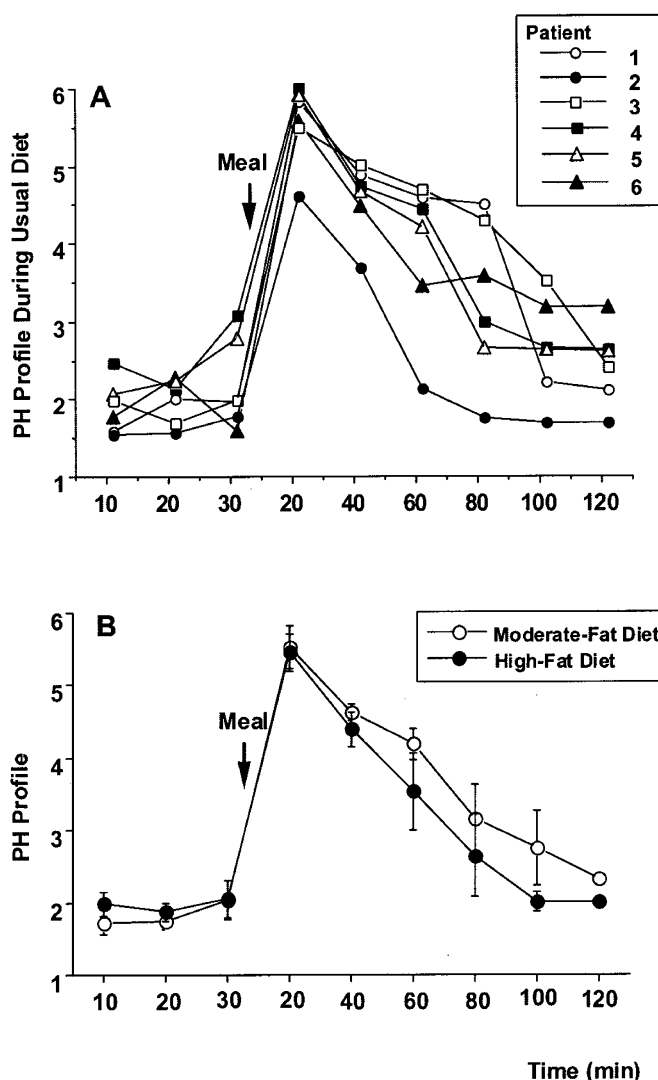
Figure 1. pH of gastric aspirates in children with CF during usual diet (*A*; individual data) and during a moderate-fat (○) or a high-fat (●) diet (*B*; data are mean ± SEM of four subjects). The pH of gastric aspirates was measured at 10-min intervals for 30 min during fasting conditions and at 20-min intervals for 120 min after gastric feeding of a test meal of 267 kcal containing 16% protein, 30% carbohydrate, 54% fat for the regular and the high-fat diet periods, and of a test meal of 252 kcal containing 14% protein, 54.5% carbohydrate, 31.4% fat for the moderate-fat diet period.

creased to 3.54–4.19 after 1 h and to 2.02–2.33 after 2 h. The pH values appear to be slightly lower during the high-fat diet compared with the moderate-fat diet starting at 60 min after feeding the test meal, but the differences were not statistically significant.

Table 2. *Diet composition and fat intake*

| Components	Usual diet*	High-fat diet†	Moderate-fat diet†
Protein (kcal%)	14.1 ± 0.9 (11.1–17.5)	14.0 ± 1.3 (12.0–17.8)	18.0 ± 1.2 (15.1–20.4)
Carbohydrate (kcal%)	43.2 ± 2.9 (29.7–49.0)	38.2 ± 3.7 (27.4–43.6)	50.2 ± 3.2 (42.3–58.1)
Fat (kcal%)	43.8 ± 3.6 (35.3–60.0)	48.8 ± 4.5 (43.2–62.2)	32.7 ± 2.1 (27.9–37.9)
Energy (kcal/d)	2 122 ± 210 (1 653–3 068)	2 179 ± 316 (1 740–3 068)	1 561 ± 275 (1 076–2 315)
Fat (g/d)	104 ± 14 (65–148)	116 ± 13 (83–148)	57 ± 11 (33–82)
Fat (g · kg^{-1} · d^{-1})	5.6 ± 0.9 (3.5–8.8)	6.1 ± 1.1 (3.5–8.8)	2.9 ± 0.4 (2.0–4.0)

Data are mean ± SEM and ranges (parentheses) from six (*) or four (†) CF children.

Gastric volume and fat emptying rate. Gastric emptying during the moderate-fat or high-fat diets had a similar biphasic pattern. Emptying rate was rapid during the first 40 min after feeding and then decreased thereafter (Fig. 2A). The time required for the volume in the stomach to decrease by half was 34 and 36 min with the moderate-fat and the high-fat diets, respectively. Gastric emptying of fat was similar throughout 120 min of digestion, although the emptying rate tended to be faster (not significantly) with the high-fat test meal (Fig. 2B). During moderate-fat and high-fat diets 59.7 and 74.8% of fat had left the stomach 1 h after feeding and 93.9 and 95.2% after 2 h, respectively. Emptying rates of the test meal were similar during the usual diet to those seen with the high-fat diet (data not shown).

Gastric lipase and pepsin activity throughout digestion. Basal and after feeding activities (units per milliliter) and output of gastric lipase (expressed as units per kilogram of body weight) were similar during the moderate-fat and high-fat diet periods (Fig. 3). Basal and postprandial pepsin concentrations and output were similar irrespective of whether the children were fed high-fat or moderate-fat diets (Fig. 4). Enzyme activity (units per milliliter or total units per kilogram of body weight) did not differ during the usual diet (Table 3),
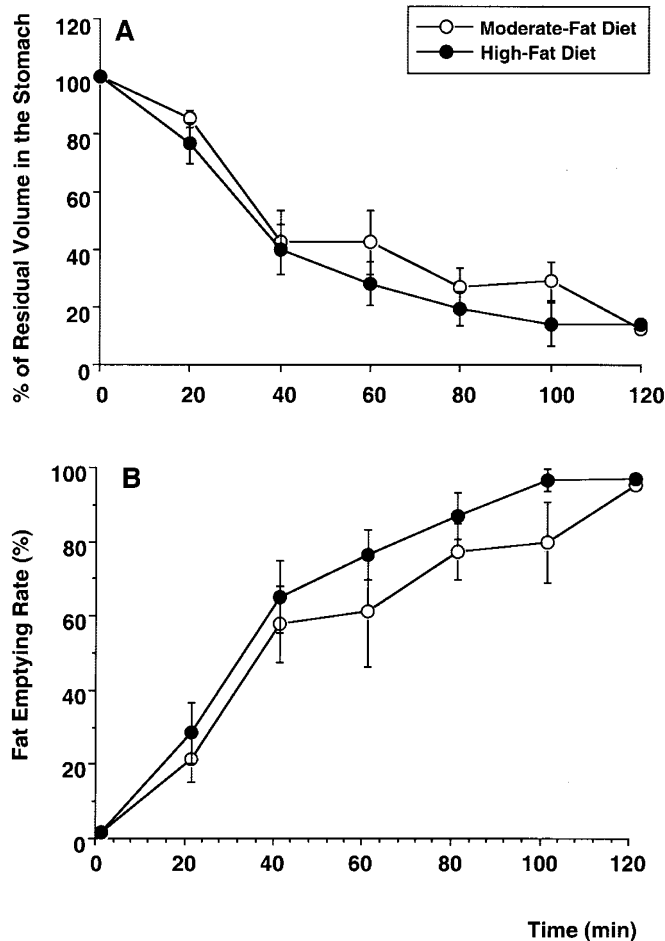
Figure 2. Effect of moderate-fat (○) or high-fat (●) diet on gastric volume (*A*) and fat emptying rates in the stomach (*B*). Data are mean ± SEM of four children with CF.
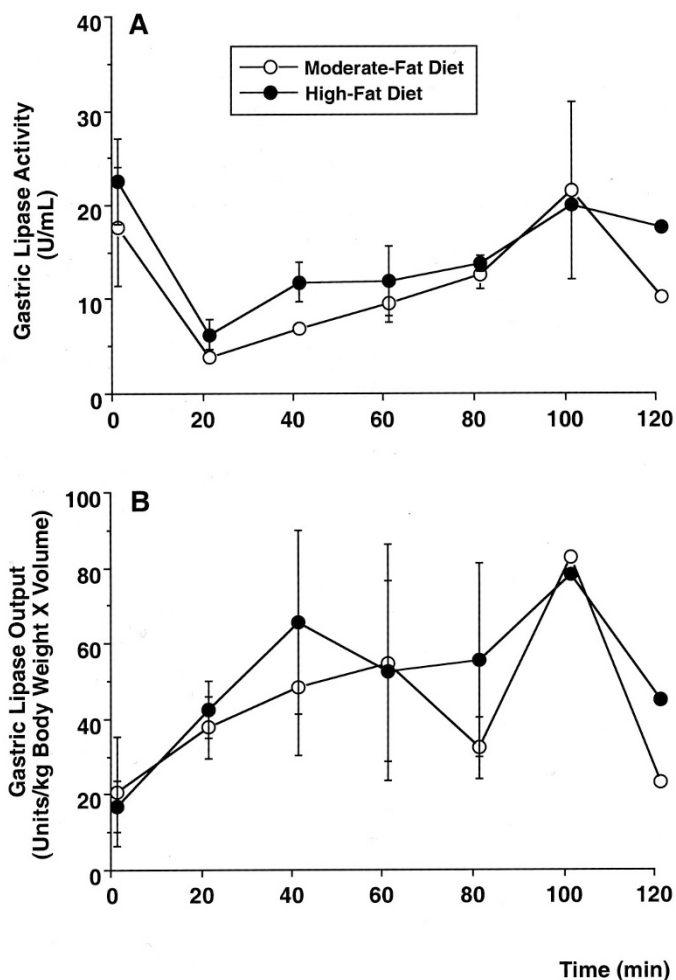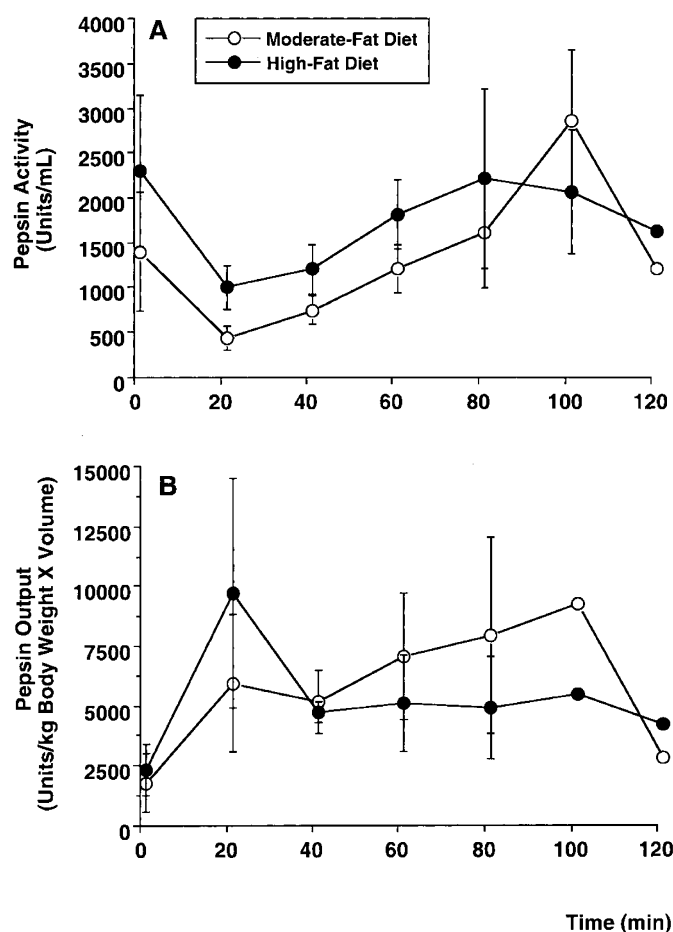

Figure 3. Effect of moderate-fat (○) or high-fat (●) diet on gastric lipase activity (*A*) and gastric lipase output (*B*) at baseline conditions (time 0) and during 120 min after feeding. Data are mean ± SEM of four children with CF.

and no relationship between activity and fat intake (3.5–8.8 g fat/kg per day) was observed.

Fat digestion in the stomach. As the stomach was almost empty 100 min after feeding (78–95% of the fat in the test meals had left the stomach), lipid analysis is presented until 80 min after feeding (Table 4). TG concentrations decreased, and the products of lipolysis, FFA, and DG increased throughout digestion. Production of DG rather than MG is owing to the stereoselectivity of gastric lipase (50). Gastric lipolysis, expressed as percentage decrease from initial TG minus percentage TG in the stomach at each sampling time, increased linearly for both diets until 40 min of digestion and then started to level off. Lipolysis rates 80 min after feeding were 36 and 20% during the moderate-fat and high-fat diets, respectively (Fig. 5). The extent of lipolysis during the usual diet (9.1 ± 1.2%, 19.1 ± 0.5%, 20.3 ± 3.1%, and 25.0 ± 3.8% at 20, 40, 60, and 80 min of digestion, respectively) was close to the values obtained during the high-fat diet.

DISCUSSION

Current dietary practice for patients with CF is to advocate energy-dense foods to maximize growth potential and improve

Figure 4. Effect of moderate-fat (○) or high-fat (●) diet on pepsin activity (*A*) and pepsin output (*B*) at baseline (time 0) and during 120 min after feeding. Data are mean ± SEM of four children with CF.

pulmonary function (21–23). Because of exocrine PI, CF patients depend on pancreatic enzyme supplements. However, the use of high doses of pancreatic enzymes does not always

normalize fat absorption and may lead to fibrosing colonopathy (51). This led to an interest in increasing the level of gastric lipase (the only endogenous digestive lipase not affected by CF) by nutritional adaptation as previously reported in animals (24–27) and in healthy adults (28).

Since the early 1980s, patients with CF have been encouraged to consume a diet, which provides 120–150% of the normal RDA for energy for age (22, 23). This high-energy intake can be achieved by the use of oral supplements in addition to the three principal meals. A high intake of fat is encouraged and should provide 40% of total energy intake (23). A high protein intake is also recommended, *i.e.* 15–20% of total daily caloric intake from protein as long as a high-energy intake is obtained (23). The children in this study on the usual diet consumed 110 ± 6% of the RDA for energy and a high-fat diet (44 ± 4% of energy) that covers 163 ± 20% of the RDA for fat. During the high-fat diet 113 ± 8% of the RDA for energy and 176 ± 13% of the RDA for fat was achieved, and the moderate-fat diet provided 78 ± 8% of the RDA for energy and 73 ± 10% for fat. These data are in agreement with previous reports on the dietary intake of children with CF (23, 52–55) showing that, in practice, few CF patients consume more than the RDA for age. Furthermore it is difficult for CF patients to maintain a high-energy intake, despite nutritional counseling, when dietary fat is restricted (56). Protein consumption was in the recommended range (23) and close to values previously reported (54, 55).

In our study, CF basal and postprandial levels of gastric lipase and pepsin activity were higher when compared with those in normal subjects consuming a low-fat (23% of energy as fat) or a high-fat (53% of energy as fat) diet (28). In the study by Armand *et al.* (28), gastric lipase activity was 5.7 ± 0.5 or 9.9 ± 1.5 U/mL at baseline and 5.2 ± 1.3 or 7.5 ± 1.5 U/mL after hormonal stimulation during a low-fat or a high-fat diet, respectively (28). In CF patients, gastric lipase activity averaged 17.0 ± 6.3 or 21.9 ± 4.5 U/mL at baseline and 10.1

**Table 3.** *Gastric enzyme activity and output in CF children during usual diet*

Patient number	Enzyme concentration (U/mL)		Enzymes output (total U/kg)		Fat consumption	
	Gastric lipase*	Pepsin*	Gastric lipase*	Pepsin*	% Fat	Fat intake (g · kg^{-1} · d^{-1})
1	13.06 ± 2.18	1 034 ± 189	72.8 ± 8.0	5 659 ± 637	46.5	8.8
2	10.64 ± 0.83	1 058 ± 105	26.1 ± 8.1	2 442 ± 635	37.5	4.5
3	12.57 ± 2.07	1 558 ± 250	41.3 ± 8.1	4 884 ± 915	35.4	3.9
4	6.83 ± 0.20	639 ± 104	36.44 ± 2.7	4 258 ± 2 405	60.0	8.0
5	11.94 ± 3.02	1 519 ± 456	28.6 ± 3.6	7 984 ± 2 478	39.9	5.2
6	13.33 ± 4.30	1 519 ± 456	27.5 ± 8.6	3 269 ± 1 015	43.4	3.5

* Data represent average values for a 20-min period obtained after a high-fat test meal during 120 min of digestion.

Table 4. *Fat content in the stomach (mM)*

Time (min)	Moderate-fat diet			High-fat diet		
	FFA	DG	TG	FFA	DG	TG
20	7.3 ± 2.1	5.5 ± 1.1	35.5 ± 7.0*	12.4 ± 2.8	10.2 ± 1.3	55.6 ± 3.5
40	10.1 ± 4.7	5.3 ± 2.6*	14.7 ± 7.4*	22.5 ± 3.2	14.8 ± 2.3	43.6 ± 9.2
60	19.8 ± 4.5	10.0 ± 2.4	21.4 ± 5.7*	24.9 ± 5.6	14.6 ± 3.0	49.9 ± 8.0
80	27.3 ± 6.0	11.3 ± 3.4	17.8 ± 6.0	26.7 ± 12.4	12.8 ± 5.5	34.7 ± 7.9

Data are mean ± SEM of four CF children. * Indicates significant differences between diets for a lipid class and at a given time (ANOVA for paired values, $p < 0.05$). MG was only present during the high-fat diet at 80 min after feeding (1.8 ± 1.1).
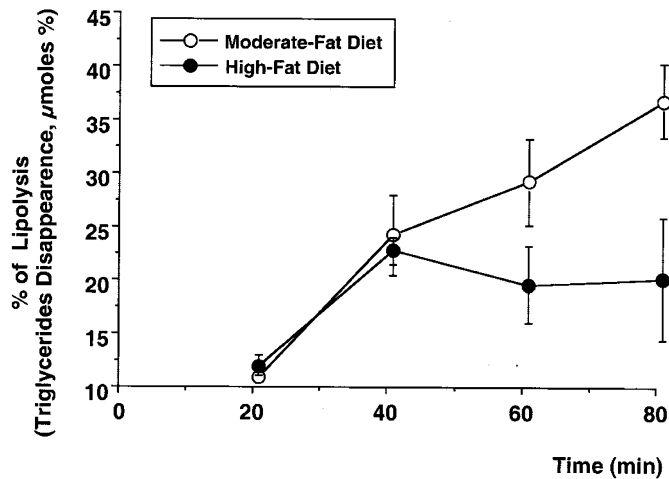
Figure 5. Lipolysis in the stomach after feeding moderate-fat (○) or high-fat (●) diet. A moderate-fat or a high-fat test meal, respectively, was fed to each diet group, and fat digestion was quantified during 80 min after feeding. Data are mean ± SEM of four children with CF.

± 2.3 or 12.9 ± 1.4 U/mL postprandially after the moderate-fat or the high-fat diet, respectively. In healthy adults, pepsin activity averaged 942 ± 120 or 1333 ± 115 U/mL at baseline and 718 ± 147 or 1042 ± 204 U/mL after stimulation during low-fat or high-fat diets, respectively (28). In the CF patients, pepsin activity averaged 1335 ± 668 or 2242 ± 838 U/mL under basal conditions and 1279 ± 328 or 1591 ± 432 U/mL postprandially after the moderate-fat or the high-fat diet, respectively. Thus, gastric lipase activity was 1.7 to 3.0 times higher than in healthy subjects, similar to data reported by Balasubramanian *et al.* (57), and the pepsin activity was 1.4- to 1.8-fold higher. Because of similarities in the techniques used, comparisons can also be made with the values obtained in other cases of PI on a high-fat diet, such as premature babies (40). Thus, it appears that in CF patients gastric lipase activity is close to or sometimes even higher than in premature infants, as gastric lipase activity was 9.1 ± 0.7 U/mL at baseline and 3.3 ± 0.2 U/mL after feeding for the premature infants (40). The pepsin profile was quite different, *i.e.* much lower activity in premature infants than in CF, with 183 ± 16 U/mL at baseline and 92 ± 4 U/mL after feeding. This may be related to the low

pepsinogen secretion in premature infants compared with full-term infants (58, 59). Gastric enzyme activity expressed as units per milliliter is dependent on the volume of gastric secretions and on dilution by the test meal; therefore, comparison of enzyme output (expressed as total units per kilogram body weight) is more pertinent (Table 5). In summary of Table 5 and taking into account the difference in collection timing, gastric lipase output was at least seven- to 20-fold higher in our CF patients compared with healthy subjects (28) and approximately 2.5-fold higher compared with premature infants (40). Pepsin output was approximately five- to eightfold or eight- to 17-fold higher in CF *versus* healthy adults or premature infants, respectively.

Inasmuch as gastric lipase and pepsin are secreted at high rates in CF patients, we were looking for a relationship between enzyme secretion and fat consumption. In fact, no correlation was found between daily fat intake (grams per kilogram of body weight) and gastric lipase or pepsin activity (units per milliliter or output). This is probably because of the nutritional adaptation of these enzymes as observed in healthy adult humans: activity increased when daily fat intake increased from 0.71 to 2.03 g/kg of body weight (28). In fact, the daily fat intake in CF patients was within the range of 2.0 to 8.8 g/kg of body weight. This finding agrees with earlier studies in animals showing that gastric lipase levels increased rapidly after a moderate increase of fat in the diet and did not increase further with additional increments in the amount of dietary fat (25, 26). The data suggest that it might be impossible to increase endogenous gastric lipase activity in CF patients who consume more than 2 g fat/kg of body weight per day, because at this fat intake the nutritional adaptation of the lipase is already maximal. In light of these data, we suggest that the reason for different levels of gastric lipase activity [similar (18, 60), higher (57, 61) or lower (62)] in CF patients compared with controls might be related to differences in fat consumption in the different patient populations (23, 52, 55, 63).

Furthermore, our study shows that gastric function is normal in children with CF. The pH profiles at baseline and after feeding were similar to values reported for CF patients (57, 60, 64) and for healthy adults (32). Gastric emptying rates were normal and similar to earlier reports for these types of test

Table 5. *Gastric enzyme output in healthy adults, CF children, and premature infants during low-fat and high-fat diets*

Patients	CF children*		Healthy adults†		Premature infants‡	
	Baseline	Postprandial	Baseline	Postprandial	Baseline	Postprandial
Low-fat diet						
Gastric lipase output (U/kg)	19.1 ± 14.4	44.9 ± 8.6	1.9 ± 0.5	3.7 ± 0.5	ND	ND
Pepsin output (U/kg)	1 544 ± 1 217	6 117 ± 925	336 ± 98	565 ± 123	ND	ND
Fat intake (g · kg^{-1} · d^{-1})	2.90 ± 0.43		0.71 ± 0.09		ND	
High-fat diet						
Gastric lipase output (U/kg)	15.1 ± 6.7	54.8 ± 5.5	3.1 ± 0.6	5.6 ± 0.8	6.0 ± 1.0	23.6 ± 1.6
Pepsin output (U/kg)	2 088 ± 1 091	5 444 ± 819	458 ± 105	838 ± 188	124 ± 18	653 ± 36
Fat intake (g · kg^{-1} · d^{-1})	6.31 ± 1.53		2.02 ± 0.30		5.83 ± 0.10	

Outputs are expressed as average of total units/kg/10-min collection time for baseline and as average of total units/kg/20-min collection time after administration of a moderate-fat or a high-fat test meal during 120 min of digestion.

* Outputs are expressed as average of total units/kg/15-min collection time in baseline and posprandially under pentagastrin stimulation. Weight was 61.3 ± 4.7 kg during the low-fat diet and 61.4 ± 4.7 kg during the high-fat diet 28.

‡ Outputs are expressed as average of total units/kg/20-min collection time. Weight was 1.56 ± 0.03 kg 40.

ND, not determined.

meals in healthy adults and in patients with CF (32, 61, 64). Furthermore, as reported in healthy humans (65), an overall strong relationship was found between lipase and pepsin outputs independent of diet and time of sampling ($r^2 = 0.80$; $p < 0.0001$).

The gastric phase of lipolysis in CF patients supports the digestion of 20–36% of the TG ingested during 80 min. These lipolysis rates are higher [10% at 45 min after feeding (57)] or lower [33% at 13 min (61)] than previously reported in CF patients. This discrepancy could be a result of differences in test meals or in study subjects. The nonlinear rates of gastric lipolysis can be explained by a feedback inhibition of gastric lipase activity by the progressive release of protonated fatty acids (31). This phenomenon has been shown to be dependent on the lipid surface area (31, 32). A fine emulsion sizing of approximately 0.7 μm increases in size while in the human digestive tract with a consequent decrease in the surface area from 20.3 to 2.5–8.2 m^2/g of fat (32). We can speculate that the test emulsions in this study had the same physicochemical pattern. Taking into account the total surface area of the emulsion, the fatty acid concentrations could have reached values shown previously to be inhibitory (32). Estimated total lipid surface areas during digestion were 23.5, 12.8, 11.8, and 7.1 m^2 and 26.3, 13.2, 9.0, and 5.3 m^2 at 20, 40, 60 and 80 min during feeding of moderate-fat and high-fat diets, respectively. Thus, fatty acid concentrations reached 63, 80, 172, and 248 or 87, 164, 185, and 236 μmol/m^2 surface at 20, 40, 60, and 80 min after feeding moderate-fat and high-fat diets, respectively. In healthy humans, gastric lipolysis reaches a plateau for a concentration of 121–128 μmol FFA/m^2 (32). Inhibiting FFA concentrations were reached 60 min after feeding the moderate-fat test meal (172 μmol) and 40 min after feeding the high-fat test meal (164 μmol). This explains why lipolysis seems to stop more rapidly with the high-fat test meal; 22% lipolysis 40 min after feeding of the high-fat test meal (16 g of fat) led to the release of more fatty acid than a 24% lipolysis of the moderate-fat test meal (8.8 g of fat) 40 min after feeding. On the whole, the quantity of TG digested in the stomach is quite similar with the two diets, as it reached 3.2 g 80 min after feeding the moderate-fat test meal and 3.5 g 40 min after feeding the high-fat test meal. Once the duodenum is reached, this inhibition should be prevented by absorption of the fatty acids, which might allow lipolysis by gastric lipase to proceed, especially given the low pH in the upper small intestine in CF (18).

CONCLUSIONS

In conclusion, the data suggest that gastric lipase level is already high in children with CF who have a daily fat intake equal to or greater than 2 g/kg body weight. A high-fat diet might benefit CF patients not only by providing higher energy and essential fatty acid intake but also by increasing the gastric lipase potential for fat digestion. As gastric lipase level is already high and does not increase further in the ranges of fat consumed by CF patients, the supplementation of exogenous recombinant gastric lipase might be the only way to increase the gastric lipase level and consequently gastric lipolysis. Fat

digestion by gastric lipase could also be increased by feeding specific formulas with optimal physicochemical properties of the lipid emulsion.

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