

## Lingual lipase in cystic fibrosis. Quantitation of enzyme activity in the upper small intestine of patients with exocrine pancreatic insufficiency.

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### Research Article

We have measured the level of lingual lipase activity in gastric and duodenal aspirates of five patients with cystic fibrosis (CF) and pancreatic insufficiency. Lingual lipase activity (measured *in vitro* by the hydrolysis of long-chain triglyceride, tri-[3H]olein, at pH 4.2 and expressed in nanomoles FFA released per milliliter aspirate per minute) and pH in gastric and duodenal aspirates were measured at 10-min intervals during a 30-min basal period and at 15-min intervals during a 2-h period after the ingestion of a test meal. In gastric aspirates, lingual lipase activity decreased from basal levels of  $200 \pm 34$  nmol FFA released per milliliter per minute (similar to values reported previously in normal subjects (Hamosh M., H. L. Klaeveman, R. O. Wolf, and R. O. Scow, 1975, *J. Clin. Invest.*, 55:908-913) to  $79 \pm 15$  nmol FFA/ml per min during the first postprandial hour and returned to basal levels during the second postprandial hour, ( $206 \pm 39$  nmol FFA/ml per min). Duodenal aspirates, obtained during basal conditions, had lingual lipase activity similar to that in the stomach,  $178 \pm 63$  nmol FFA/ml per min. Enzyme activity levels were  $56 \pm 14$  and  $113 \pm 29$  during the first and second postprandial hours. Measurements of total lipase activity delivered to the ligament of Treitz showed that lingual lipase amounted to  $91.22 \pm \dots$

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# A

**Abstract.** We have measured the level of lingual lipase activity in gastric and duodenal aspirates of five patients with cystic fibrosis (CF) and pancreatic insufficiency. Lingual lipase activity (measured in vitro by the hydrolysis of long-chain triglyceride, tri-[<sup>3</sup>H]olein, at pH 4.2 and expressed in nanomoles FFA released per milliliter aspirate per minute) and pH in gastric and duodenal aspirates were measured at 10-min intervals during a 30-min basal period and at 15-min intervals during a 2-h period after the ingestion of a test meal. In gastric aspirates, lingual lipase activity decreased from basal levels of  $200 \pm 34$  nmol FFA released per milliliter per minute (similar to values reported previously in normal subjects

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Parts of this work are derived from a dissertation to be submitted to the Graduate School, University of Maryland, by Cynthia K. Abrams in partial fulfillment of the requirements for the Ph.D. degree in Nutritional Sciences.

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## Lingual Lipase in Cystic Fibrosis

### Quantitation of Enzyme Activity in the Upper Small Intestine of Patients with Exocrine Pancreatic Insufficiency

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(Hamosh M., H. L. Klaeveman, R. O. Wolf, and R. O. Scow, 1975, *J. Clin. Invest.*, 55:908-913) to  $79 \pm 15$  nmol FFA/ml per min during the first postprandial hour and returned to basal levels during the second postprandial hour, ( $206 \pm 39$  nmol FFA/ml per min). Duodenal aspirates, obtained during basal conditions, had lingual lipase activity similar to that in the stomach,  $178 \pm 63$  nmol FFA/ml per min. Enzyme activity levels were  $56 \pm 14$  and  $113 \pm 29$  during the first and second postprandial hours. Measurements of total lipase activity delivered to the ligament of Treitz showed that lingual lipase amounted to  $91.22 \pm 4.06\%$  of the total lipase activity in the upper small intestine during the 150-min study period. The basal and postprandial gastric pH levels in the five CF patients studied ( $3.2 \pm 0.44$ ,  $4.0 \pm 0.16$ , and  $4.4 \pm 0.4$  for basal and first and second postprandial hours, respectively) did not differ from previously reported values for normal subjects. The pH of duodenal aspirates was however significantly lower ( $P < 0.001$ ) in CF patients, both under basal conditions ( $5.0 \pm 0.26$ ) and during the first and second postprandial hours ( $4.9 \pm 0.13$  and  $4.4 \pm 0.36$ , respectively), than in normal subjects. The low postprandial duodenal pH enables lingual lipase to act not only in the stomach but to continue the hydrolysis of dietary fat in the upper small intestine of CF patients. The data presented show that lingual lipase remains fully active in CF and accounts for  $>90\%$  of total lipase activity in the upper small intestine. We suggest that, because of low intestinal pH in CF, enzyme replacement therapy containing lingual lipase could improve fat absorption in CF patients to a greater extent than the pancreatic preparations now in use.

## Introduction

Exocrine pancreatic insufficiency, resulting in steatorrhea, is found in at least 85% of cystic fibrosis (CF)<sup>1</sup> patients (1–3). 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. Ross (4) found fat absorption to be between 26 and 81% in a group of children with CF, although most of them did not have detectable pancreatic lipase activity. These findings were supported by Muller et al. (5), who reported 50% absorption of dietary fat in a patient with congenital absence of pancreatic lipase activity, and by Lapey et al. (6) who found >50% fat absorption in 15 patients with exocrine pancreatic insufficiency secondary to CF, without any oral enzyme supplementation. Ross and Sammons (7) have postulated the presence of compensatory lipolytic activity in pancreatic insufficiency, but were unable to find the source of that activity.

We propose that lingual lipase (8) provides the extrapancreatic lipolytic activity seen in CF. Lingual lipase, an enzyme secreted from lingual serous glands (8, 9), hydrolyzes dietary triglycerides at pH 3.0–6.5 and thus initiates the digestion of fat in the stomach. Since the lipase in preparations of human (8) and rat (9, 10) lingual serous glands, as well as in esophageal (11) and gastric aspirates (11–13) of healthy adults and infants (14–16), has similar characteristics (molecular weight, substrate specificity, pH optimum) and because only very low lipase activity has been reported in rat gastric mucosa (9), we assume that lingual lipase is the main digestive enzyme in the stomach. This lipase (8–16) differs from pancreatic lipase (17) in that it is relatively acid resistant, does not require bile salts for activity, and has a low pH optimum. Recent animal studies have shown that hydrolysis of fat in the stomach is essential for normal fat absorption, even under conditions of normal pancreatic function. Several studies have demonstrated that after diversion of oral secretions by means of an esophageal fistula, there is not only a marked fall in intragastric lipolysis (9), but also a decrease in intestinal fat digestion as well as a rise in fat and bile salt excretion (18). It appears that fat digestion in the stomach may be a much more important process in conditions of physiologic or pathologic pancreatic insufficiency. Adequate fat absorption in the newborn is directly related to high levels of lingual lipase activity (14, 15) and gastric lipolysis (14, 16), suggesting that the latter can successfully compensate for the low pancreatic lipase activity (19–21) and bile acid levels (21–25) of early infancy. Lingual lipase could play a similar role in CF, a disease characterized by impaired pancreatic acinar function (26) (and thus absence of pancreatic enzymes), impaired pancreatic ductular function (leading to impaired water and bicarbonate secretion [27]), as well as decreased duodenal bile acid concentration (28–33). Furthermore, because of low bicarbonate secretion, the low

duodenal pH in the postprandial phase (4–6) would be compatible with continued activity of lingual lipase.

The aim of this study was to (a) measure intraluminal pH in the stomach and duodenum in basal and postprandial conditions; (b) measure lingual lipase activity in the stomach and duodenum in the basal and postprandial period; and finally (c) to quantitate the contribution of lingual lipase activity to the total lipolytic activity in the duodenum.

## Methods

Five male CF patients with pancreatic insufficiency participated in this study. The study was approved by the Institutional Review Committees of the National Institutes of Health, University of Maryland Hospital and Georgetown University Medical Center. Informed consent was obtained from all patients before the study.

Adult male patients (age 21–29 yr) were selected because of the availability of data from control subjects (11, 34) and from subjects with pancreatic insufficiency secondary to chronic alcoholic pancreatitis (34) who had been studied previously. The diagnosis of CF was based on clinical evaluation and a sweat chloride > 60 meq/liter. Pancreatic insufficiency was previously documented by the absence of pancreatic digestive enzymes (trypsin, chymotrypsin, lipase, and amylase) in duodenal aspirates and abnormal quantitative fecal fat in all patients. All patients had evidence of pulmonary disease and their NIH clinical score (35) ranged from 50 to 88. Three of the five patients were receiving continuous oral antibiotic coverage for treatment of their respiratory disease. All subjects had been receiving pancreatic extracts, vitamin E (Aquasol E), and multivitamins. The brand of pancreatic extracts used by each patient was different and the dosage used was based on control of their gastrointestinal symptomatology as judged by the patients or their referring physicians. No change in the subjects' clinical status or medications prescribed occurred during the study period. The subjects were electively hospitalized at the National Institutes of Health Clinical Center during the time the study was conducted. Clinical data regarding the subjects are given in Table I.

*Collection of specimens.* After an overnight fast, the patients were asked to swallow a triple-lumen polyvinyl tube (each lumen 1.2 mm i.d.) with a mercury bag at the tip. The polyvinyl tube was placed under fluoroscopic control, in such a way that the perfusion site was located at the ampulla of Vater, and the distal opening for duodenal sample aspiration at the ligament of Treitz. The most proximal opening was placed in the antrum for aspiration of gastric samples. After proper placement of the tube, the duodenum was perfused with normal saline containing [<sup>14</sup>C]polyethylene glycol (PEG) (10 µCi/liter, New England Nuclear, Boston, MA) at a rate of 2 ml/min. Before collection of baseline samples, the duodenum was perfused for 30 min for equilibration. Base-line samples were aspirated from the stomach and duodenum at 10-min intervals for half an hour. The patients were then asked to ingest a standardized test meal. The meal consisted of 8 oz orange juice, 8 oz vanilla Ensure (Mead Johnson Co., Evansville, IL), and 4 oz vanilla ice cream, and contained 500 calories, 59% carbohydrate, 30% fat, and 11% protein. The test meal was eaten without supplemental pancreatic enzyme administration. Postprandial specimens were sampled at 15-min intervals for 2 h.

Immediately after collection of each sample, pH was measured by glass electrode (pH meter, Radiometer, Copenhagen), and the specimens

1. Abbreviations used in this paper: CF, cystic fibrosis; PEG, polyethylene glycol.

were transferred to polypropylene tubes, capped, and kept on dry ice until transfer to the laboratory. All specimens were stored at  $-70^{\circ}\text{C}$  until assay. Under these storage conditions, lipolytic activity is stable for years (15, 36).

**Assay of lipase activity.** The specimens were thawed rapidly and the pH measured again with the aid of pH paper. The lipolytic activity in all specimens was measured using a long-chain triglyceride substrate, tri-[ $^3\text{H}$ ]olein. A stable preparation of the triglyceride was made by emulsification of the labeled and carrier triolein (200  $\mu\text{mol}$ ) with phosphatidylcholine (15  $\mu\text{mol}$ ) and anhydrous glycerol (3.3 ml) as described in detail elsewhere (37). The assay mixture contained in a final volume of 200  $\mu\text{l}$ : 1  $\mu\text{mol}$  of labeled triglyceride, 15  $\mu\text{mol}$  of sodium citrate buffer, pH 4.2 or Tris-HCl pH 8.1, 5.6 mg of bovine plasma albumin (fraction V, Armour Pharmaceutical Co., Tarrytown, NY, lot N 50402) and 50  $\mu\text{l}$  gastric or duodenal aspirate. Incubation was for 30 min at  $37^{\circ}\text{C}$  in a Dubnoff shaking bath. The reaction was stopped by the addition of 3.25 ml of a mixture of methanol/chloroform/heptane (1.41:1.25:1, vol/vol/vol). FFA were separated from glycerides by the addition of 1.05 ml of potassium carbonate buffer, 0.05 M, pH 10.0, as described by Belfrage and Vaughan (38). Aliquots of 0.5 ml from the aqueous phase were transferred to 5.0 ml Aquasure scintillation fluid (New England Nuclear) and the radioactivity was measured in a Beckman model LS 7500 scintillation spectrometer (Beckman Instruments, Inc., Fullerton, CA), using internal standards for quench correction.

Of total FFA, 70–80% were present in the alkaline upper phase. Mixtures of nonradioactive triglyceride emulsion and [ $^3\text{H}$ ]oleic acid were used to determine the partition coefficient of FFA in the two phases. The partition of FFA between the nonaqueous and aqueous phases was not affected by the following changes in the reaction mixture: type of buffer, pH of medium, or albumin concentration (10). Partial glycerides (mono- and diglycerides) are retained in the nonaqueous phase and do not interfere with the quantitation of FFA (10).

The triglyceride concentration of the substrate emulsion was quantitated by the hydroxamic acid method of Rapport and Alonso (39).

Lipase activity was expressed as nanomoles of FFA produced per milliliter aspirate per minute.

Total lipase activity delivered to the ligament of Treitz was calculated according to Malagelada et al. (40), as follows:

$$\text{Lipase (IU/15 min)} = (\text{Concentration of } [^{14}\text{C}] \text{PEG in infusate}) / (\text{Concentration of } [^{14}\text{C}] \text{PEG in aspirate}) \times \text{lipase activity of duodenal aspirate} \times \text{collection time (min)} \times \text{infusion rate (ml/min)}.$$

Because an earlier report (42) indicated that PEG in the range of 1–5 mg/ml inhibits the activity of pancreatic lipase (0–50% inhibition), we have tested the effect of PEG on lingual lipase. PEG was added to purified rat lingual lipase and to human gastric and duodenal aspirates collected without the marker and lipase activity was measured in the assay system described above. These tests showed that PEG does not inhibit lingual lipase activity in the range reported to affect pancreatic lipase (1–5 mg/ml PEG).

Statistical analysis was conducted using the *t* test (41).

## Results

The clinical data for the five CF patients studied are given in Table I. The diagnosis of cystic fibrosis was made at birth (one patient), in infancy (three patients), or at the age of 13 yr (one patient). Pancreatic insufficiency was documented by absence of pancreatic enzymes in duodenal aspirates and steatorrhea in

Table I. Clinical Data of CF Patients

Patient No.	1	2	3	4	5
Age (yr)	22	29	28	22	23
Height (cm)	182	173	178	172	182
Weight (kg)	55.0	72.8	52.8	59.0	63.9
NIH clinical score (35)	61	79	50	88	81
Fat excretion*					
(% of fat intake)	8.4	27.6	45.1	28.4	30.4
Serum cholesterol‡					
(mg/dl)	88	160	96	114	60
Serum carotene§					
( $\mu\text{g}/\text{dl}$ )	22	31	26	24	17
Age at time of CF diagnosis	13 yr	8 mo	9 mo	2 mo	Birth

\* Fat excretion was quantitated while the patients received 4 Cotazyme capsules (Organon Pharmaceuticals, W. Orange, NJ: 8,000 USP lipase U/capsule) per meal. Fat intake (grams per day) was 237, 115, 150, 240, and 235 for patients 1–5, respectively.

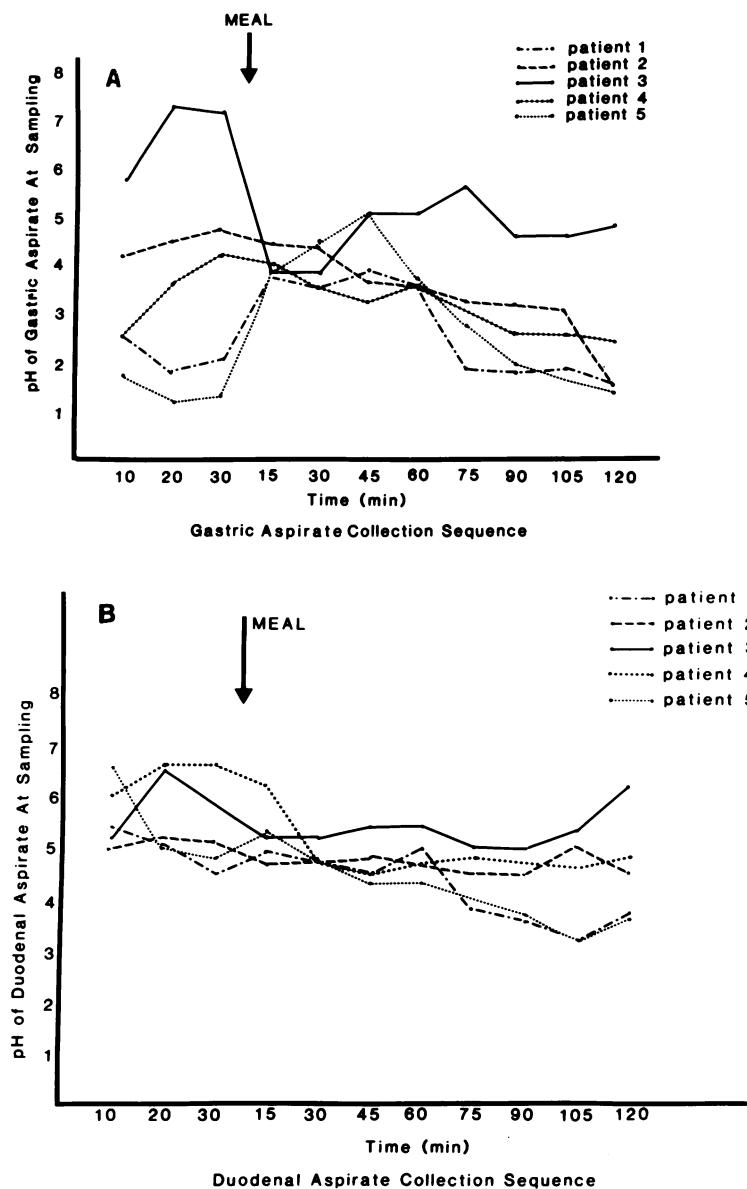
‡ Range of normal values: 163–263 mg/dl.

§ Range of normal values: 50–300  $\mu\text{g}/\text{dl}$ .

all patients. A 72-h stool collection (defined by excretion of stool markers given 72 h apart) for quantitation of fecal fat while on an ad lib. but recorded diet was performed and showed various degrees of fat malabsorption (Table I). Steatorrhea was present in all patients, even while they were receiving four Cotazyme capsules/meal.

**pH of gastric aspirates in CF patients.** Data for the baseline and postprandial pH in gastric aspirates are given in Fig. 1 A and Table II. There was wide variation in the pH values during the 30-min base-line collection period. The pH range was narrower during the first hour after ingestion of the test meal (pH 3.2–5.0) and widened again during the second postprandial hour (pH 1.3–5.5). The mean basal pH in gastric aspirates of CF patients ( $3.2 \pm 0.44$ ) was not different from that previously reported by one of us (Dr. M. Hamosh) for 10 healthy, age-matched, volunteers ( $3.5 \pm 0.26$ ) (11). The postprandial pH of the gastric aspirates of the five patients (mean value for the first hour after the meal  $4.0 \pm 0.16$ ) did also not differ from the postprandial gastric pH of normal controls ( $4.4 \pm 0.4$ ) (11).

**pH of duodenal aspirates in CF patients.** The basal duodenal pH was significantly ( $P < 0.001$ ) lower (range 3.0–6.7) in the five CF patients studied (Fig. 1 B and Table II) than previously reported by one of us (Dr. S. K. Dutta) for seven normal subjects (pH range 6.2–7.5) (34). The postprandial duodenal pH level was also significantly lower in CF patients than in normal subjects. In four patients, the duodenal pH remained  $<5.0$  throughout the 2-h postprandial period studied, whereas in the fifth patient, the duodenal pH fluctuated between 5.0 and 5.4 and only one specimen had a pH of 6.2. For most of the postprandial period, the duodenal pH in control subjects was between 5.5 and 7.2 (34). The mean duodenal pH values during basal, and



**Figure 1.** (A) pH of gastric aspirates in CF. The pH of gastric aspirates was measured at 10-min intervals for 30 min before and at 15-min intervals for 120 min after ingestion of a test meal of 500 calories containing 59% carbohydrate, 30% fat, and 11% protein. Data for five patients. (B) pH of the luminal contents from the upper small intestine in CF. Basal (30-min at 10-min intervals), and postprandial pH measurements (at 15-min intervals for 120 min after ingesting a test meal) in the duodenal aspirates of five CF patients.

the first and second postprandial hours were significantly lower ( $P < 0.001$ ) in CF patients than in control subjects. The duodenal pH remained in the acid range for the entire 2 h following ingestion of the meal.

**Relationship between pH and lingual lipase activity in gastric and duodenal aspirates.** The low pH of the duodenal aspirates suggested that lingual lipase, an enzyme with a pH optimum of 3.0–6.5, might remain active in the upper small intestine in CF. The relationship between pH and lingual lipase activity in all the specimens studied, gastric as well as duodenal aspirates, is shown in Fig. 2. The pH of most of the aspirates was in the range of 3.0–6.0, thus coinciding with the optimal pH range of lingual lipase activity. The data in Fig. 2 show that there was no linear relationship between lipase activity and the pH of the

aspirate and that aspirates with a pH < 2.0 had only very low or no lipase activity. There was no significant difference in the level of lipase activity in the gastric or duodenal aspirates.

**Lingual lipase activity in the stomach and duodenum.** Basal lipase activity levels in the stomach of five CF patients (Table II) were similar ( $200 \pm 34$  nmol FFA released/ml aspirate per min) to values previously reported by us for normal subjects ( $197 \pm 31$ , [11]). Lingual lipase activity in gastric aspirates decreased significantly ( $P < 0.05$ ) during the first hour after ingestion of the test meal (mean  $79 \pm 15$  nmol FFA released/ml aspirate per min) and returned to basal levels during the second postprandial hour ( $206 \pm 39$ ). The decrease in enzyme activity after ingestion of the meal is probably due to dilution of gastric contents by the meal and to increased gastric secretions. It is

Table II. pH and Lingual Lipase Activity\* in Gastric and Duodenal Aspirates in CF

	Gastric aspirate		Duodenal aspirate	
	pH	Lingual lipase activity	pH	Lingual lipase activity
Basal (fasting)‡	3.2±0.44 (1.2-7.2)	200±34 (0-572)	5.0±0.26 (3.0-6.9)	178±63 (0-534)
Postprandial§				
1st h	4.0±0.16 (3.2-5.0)	79±15 (0-219)	4.9±0.13 (4.3-6.2)	56±14 (0-137)
2nd h	2.7±0.58 (1.3-5.5)	206±39 (0-457)	4.4±0.36 (3.2-6.2)	113±29 (5-269)

\* Lingual lipase activity; nanomoles FFA released per milliliter aspirate per minute.

‡ 15-16-h fast.

§ 1st and 2nd h after ingestion of test meal (500 calories, 59% carbohydrate, 30% fat, 11% protein). The data are mean±SEM (range of values in parenthesis) of five patients studied.

also possible that the lipase binds rapidly to the food in the stomach and that less remains free in gastric juice. Return to basal levels during the second postprandial hour suggests con-

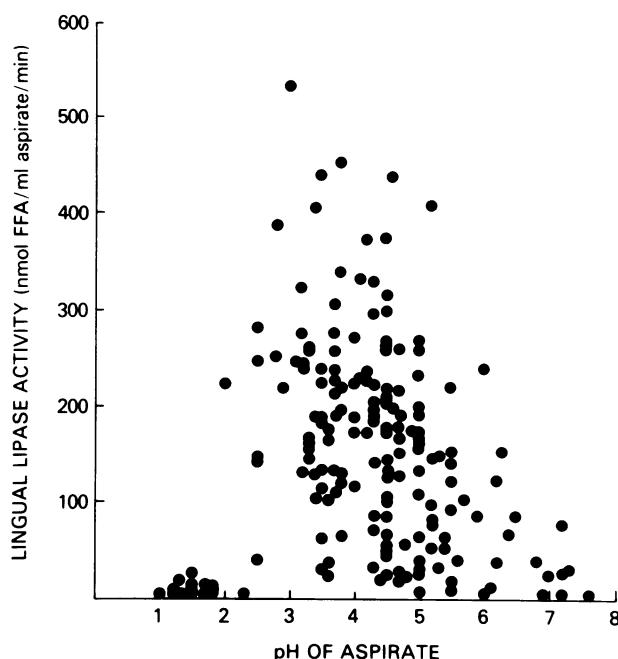


Figure 2. Relationship between the pH of gastric and duodenal aspirates and the level of lingual lipase activity. Each dot represents the level of lingual lipase activity (ordinate) and pH (abscissa) for each specimen of gastric or duodenal aspirate from CF patients.

tinuous enzyme secretion, probably independent of oral stimulation by ingestion of food.

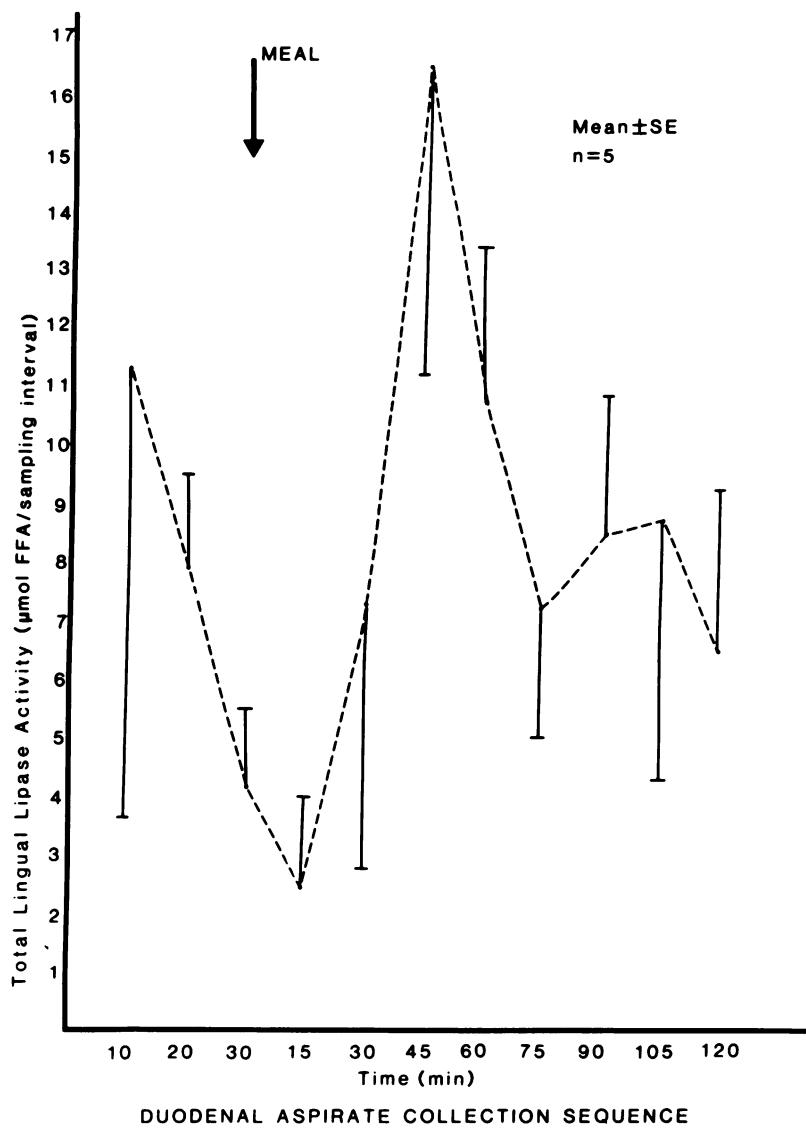
Basal lingual lipase activity levels in the duodenum of CF patients (178±63) were similar to fasting enzyme activity levels in the stomach (Table II). Lingual lipase activity was present in duodenal aspirates of all five patients throughout the 2-h postprandial period studied. Although enzyme activity appeared to decrease after eating, the difference between basal and postprandial activity levels was not statistically significant. The data suggest that, because of the persistent low postprandial duodenal pH (Fig. 2 and Table II), lingual lipase continues to be active for several hours after ingestion of food in the upper small intestine of CF patients.

*Quantitation of total lipase activity delivered to the ligament of Treitz under basal and postprandial conditions.* The total amount of lipase delivered to the ligament of Treitz was quantitated with the aid of <sup>14</sup>C-labeled PEG (10 µCi/liter) infused at a rate of 2 ml/min (40). All specimens of duodenal aspirate were analyzed for lingual lipase (assay at pH 4.2) and nonlingual lipase (assay at pH 8.1 with and without 4 mM sodium taurocholate) for quantitation of the lipase activity delivered to the duodenum under basal and postprandial conditions. The data are presented in Table III and Fig. 3. Lingual lipase amounted to 91.22±4.06% of the total lipase activity delivered to the ligament of Treitz during the 150-min study period. Only one patient had lingual lipase activity of <90% of total lipolytic activity. There was no difference between basal and postprandial sampling periods, or between the first or second hour after ingestion of the test meal (Table III). At all times tested, lingual lipase activity was >90% of total lipase in the upper small intestine.

Table III. Basal and Postprandial Levels of Lingual Lipase Activity Delivered to the Ligament of Treitz

Patient No.	1	2	3	4	5	Mean±SE
<i>Lingual lipase activity percent of total lipase activity</i>						
Total collection period (150 min)	96.6	93.8	92.0	97.0	76.7	91.22±4.06
Basal (30 min)	97.5	96.5	91.9	98.9	88.8	94.72±1.4
Postprandial						
1st h	96.3	98.4	89.5	96.3	78.4	91.8±4.0
2nd h	96.5	87.5	96.5	97.8	73.2	90.3±4.6
<i>Percent of lingual lipase activity delivered to ligament of Treitz during sampling interval</i>						
Basal (30 min)	11.9	40.8	72.8	25.3	14.2	33.00±12.8
1st h	59.0	26.0	15.8	50.9	36.8	37.70±8.6
2nd h	29.9	32.9	11.3	23.7	48.9	29.15±7.2

\* Lingual lipase activity percentage of total lipase activity delivered to the ligament of Treitz during the entire collection period (150 min: 30 min basal, 120 min after test meal).



**Figure 3.** Quantitation of lingual lipase activity delivered to the ligament of Treitz during basal (first 30 min after an overnight fast) and postprandial conditions (during 120 min after a test meal) in CF patients.

Although the mean values for the amounts of lingual lipase delivered to the duodenum were similar during the initial 30-min basal study period, during the first hour after ingestion of the test meal and during the second postprandial hour, the individual values fluctuated widely (Table III). Thus, while in patients 1 and 5 the amounts of lipase activity delivered to the ligament of Treitz during the basal period were 11.9 and 14.2%, respectively, in patient 3, 72.8% of lipase activity was delivered to the duodenum during this initial study period.

#### Discussion

The data presented suggest that lingual lipase might be responsible for the hydrolysis of dietary fat in CF patients with exocrine pancreatic insufficiency. The changes in the intestinal milieu in CF, low postprandial pH (Fig. 1 B and Table II), and low con-

centration of bile salts (28–33) actually enable lingual lipase to act not only in the stomach (its normal site of action) but to continue the hydrolysis of dietary fat in the duodenum. High levels of lingual lipase activity in the stomach and in the duodenum during basal as well as postprandial conditions (Table II and III, Fig. 3), suggest that this enzyme might hydrolyze as much as 40–70% of dietary fat, the amount absorbed by patients who do not receive pancreatic enzyme supplements (6).

There is good evidence from both human and animal studies that as much as 70% of dietary fat is absorbed in the absence of pancreatic lipase activity. Several earlier studies have shown that children with congenital or acquired absence of pancreatic lipase (43, 44) absorb as much as 70% of ingested fat. Detailed animal studies to establish the extent of fat digestion and absorption in the absence of pancreatic secretions were carried out in calves by Gooden and Lascelles (45). Their studies show

that 47% of milk fat entered the ileum digested to partial glycerides and FFA, compared with 60% in intact calves. Furthermore, 70% of long-chain triglyceride fatty acids was absorbed in the absence of pancreatic juice. Thus, as much as 70% of dietary fat can also be absorbed in ruminants lacking pancreatic lipase, the chief compensatory enzyme being pregastric esterase (46–48), an enzyme of similar origin and characteristics as lingual lipase in man (8, 11, 15).

Measurements of lingual lipase activity in gastric aspirates, by *in vitro* assay of hydrolysis of long-chain triglyceride, show similar basal levels of activity in CF patients (Table II) and in age-matched normal subjects (11). A similar comparison of the hydrolysis of tributyrin by the lipase in gastric aspirates, has shown higher lipolytic activity in CF patients than in controls (49). Although the difference between our data and data reported by Roulet et al. (49) could be due to the different substrates used to quantitate lipase activity (triolein vs. tributyrin), it is more likely that the higher activity in the CF group was associated with the significantly lower age than that of the control group. Lingual lipase activity in gastric aspirates is higher at younger age both in man (11, 15) and rat (50, 51).

The pH of gastric aspirates during basal and postprandial conditions was similar in the five CF patients studied (Table II, Fig. 1 A) to that of 10 age-matched healthy subjects studied previously (11). Although it is not clear whether CF patients with pancreatic insufficiency have normal (52), hypo- (53) or hypersecretion (54) of gastric acid, the pH in the stomach of the CF patients studied was in the optimal range for lingual lipase activity (8, 11, 15). Seldom was the pH in the stomach <2.0 (Fig. 1 A). In gastric aspirates with pH <2.2 (as we have previously seen in gastric aspirates of healthy newborn infants [15]), lipase activity was undetectable. We do not yet know the reason for the loss of activity at pH 2.0, i.e., whether it is the result of denaturation of the lipase or of peptic digestion (55) of the enzyme at low pH.

The normal values of basal and postprandial pH in the stomach of CF patients are in sharp contrast to the significantly lower pH in the duodenum throughout the entire study period (Fig. 1 B and Table II). Earlier studies have shown that secretion of bicarbonate is markedly impaired in children suffering from CF (27). Most of the bicarbonate in pancreatic secretions is secreted by cells lining the pancreatic ducts, and indeed, it has been shown that "ductular activity" is more severely impaired in CF than in exocrine pancreatic insufficiency of other origin (27). There is good evidence that patients with significant exocrine pancreatic disease have an acid pH in the upper small intestine, under both fasting and postprandial conditions (34), because of impaired ability to neutralize even small levels of acid in the duodenum (56). The present study shows that in CF patients with pancreatic insufficiency the pH measured at the ligament of Treitz both before and after a test meal, remains <5.5 for most of the test period of 150 min. It was suggested previously (56) that the relatively acidic intraluminal environment in the upper small intestine in pancreatic insufficiency may have significant influence on the absorption of nutrients

and drugs. Thus, enhanced folate absorption in patients with pancreatic insufficiency (57) is probably due to the low intraluminal pH (<6.0), which is optimal for maximal folate uptake (58). The low postprandial pH in the upper small intestine might also be associated with higher activity of lingual lipase, an enzyme active at pH 3.0–6.5 (Fig. 3, Table III, and reference 59). Our study indeed shows that throughout the 2-h postprandial period, lingual lipase activity amounted to >90% of total lipase activity delivered to the ligament of Treitz in CF patients. A comparison of our data on lingual lipase in CF patients with the data reported for pancreatic lipase in healthy subjects by Di Magno et al. (60) shows that the total amount of lingual lipase delivered to the ligament of Treitz, during the first and second postprandial hours (Fig. 3), amounts to ~25–40% of the maximal amount of pancreatic lipase released in response to cholecystokinin-pancreozymin stimulation in healthy adults (60).

Although the relationship between lingual lipase activity, hydrolysis of fat, and extent of steatorrhea was not the aim of this study, the data indicate a possible correlation between lingual lipase output and fat absorption: high lipase activity (51 KU/h) was associated with low fat excretion (8.4% of intake, patient 1), whereas (in patient 3) the highest fat excretion (45.1% of intake) was associated with the lowest level of lingual lipase (11.3 KU/h); the other patients had intermediate levels of both lipase activity (18–20 KU/h) and fat excretion (27–28% of intake). The reason for the presence of steatorrhea despite considerable lipolytic activity in these patients, remains to be investigated. Resection of part of the intestine (documented in one patient, but unknown for the other patients) might be one of the reasons. We do not yet know to what extent the enzyme might be active in the duodenum of normal subjects.

In addition to lingual lipase activity, duodenal aspirates of all five patients contained low levels (mean 8.7% of total activity) of a lipolytic activity with an alkaline pH optimum (Table III). Since the patients have repeatedly been tested for many years and found to be completely deficient of pancreatic lipase, we do not know the nature or origin of the lipolytic activity that we measure at pH 8.1. It is possible that this lipase activity is similar to a lipase described previously in the intestinal mucosa of several species (61–64), and that it remains active in CF. To what extent this lipase might be able to act in the acidic environment of the upper small intestine of CF patients is not known at present.

Malabsorption in CF patients with pancreatic insufficiency is generally treated by administration of exogenous pancreatic extracts. Several reports suggest, however, that pancreatic enzyme supplements do not normalize fat absorption in CF (65–67). Inactivation in the stomach and low duodenal pH are the main reasons that only as little as 8% of the lipase given in pancreatic supplements reaches the ligament of Treitz (65–67). The effectiveness of pancreatic supplements improves after reduction of acid secretion by cimetidine (68–71), but even under these conditions there is marked inactivation of pancreatic lipase (72, 73). Our study suggests that lingual lipase might provide better

therapy for the prevention of steatorrhea in CF. Lingual lipase supplements would remain fully active in the acid environment of the stomach and intestine and furthermore would not be affected by low levels of bile salts or absence of colipase. Further studies are needed to explore the possibility of treating pancreatic insufficiency with enzyme preparations with high lingual lipase activity.

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