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Fat Digestion in the Stomach: Stability of Lingual Lipase in the Gastric Environment

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Summary

Digestion of dietary fat starts in the stomach, where lingual lipase hydrolyzes triglycerides to free fatty acids and partial glycerides at pH 3.0–6.0. Lingual lipase is secreted continuously from lingual serous glands and accumulates in the stomach between meals, when gastric pH is <3.0. We have, therefore, examined the resistance of lingual lipase to low pH and its possible protection by dietary components present in the stomach contents. Partially purified rat lingual lipase (7–15 μg enzyme protein) was preincubated at 37°C for 10–60 min at pH 1.0–6.0 before incubation for assay of lipolytic activity, hydrolysis of tri-[^{3}H]olein at pH 5.4. The data show that partially purified rat lingual lipase preparations are stable at 37°C in the pH range of 2.5–6.0. Enzyme activity, however, is rapidly and irreversibly lost during preincubation at pH 1.0–2.4 for 10–30 min. Protein (gelatin 1% or albumin 1% or 2.5%) cannot prevent the inactivation of lingual lipase at low pH. The large molecular species (molecular weight >500,000) of lingual lipase (thought to be an aggregate of enzyme with lipids) is slightly more resistant to inactivation than the 46,000 dalton preparation, suggesting that lipids might protect the enzyme from inactivation. Indeed, about 60% of the initial lipase activity is preserved during incubation at pH 2.0 in the presence of 50 mM lecithin or 10 mM triolein. The data indicate that triglycerides which are hydrolyzed by this enzyme as well as phospholipids that are not hydrolyzed can prevent the inactivation of the enzyme. Lingual lipase is resistant to peptic digestion, even at high pepsin concentrations. Because of the special importance of lingual lipase in neonatal fat digestion, we compared the stability of the lipase in gastric aspirates of newborn infants to that of rat lingual lipase. The lipase in human gastric aspirates is more resistant to inactivation than the rat enzyme. There are however individual variations in enzyme stability: from preservation of 100% of original activity to the loss of 80% of activity during incubation for 30 min at pH 1.6. The data suggest that substances within the newborn infant's stomach stabilize the enzyme at low pH. We suggest that the extensive intragastric hydrolysis of dietary fat (in formula or breast milk) is related to the stability of the lipase in the stomach of newborn infants.

Abbreviations

BSA, bovine serum albumin
FFA, free fatty acids

Digestion of dietary fat by lingual lipase is initiated in the stomach of the newborn (4, 28, 29, 40, 63) and the adult (10, 26, 28, 51). Intragastric lipolysis results in the hydrolysis of 10–30% of dietary fat to partial glycerides and FFA (4, 26, 28, 29, 40, 57, 63). The products of intragastric lipolysis are amphiphilic substances (62) that facilitate fat emulsification (26) and enhance the intestinal hydrolysis of fat by pancreatic lipase (6). Recent studies show that the site of action of lingual lipase, an enzyme with optimal activity at pH 3.0–6.0, is not limited to the stomach, but continues in the duodenum (1, 12), especially in conditions of physiologic (9, 10, 37, 47, 69) and pathologic (46, 53, 55, 59) pancreatic insufficiency, characterized by low duodenal pH (12–14, 19). In these cases, the enzyme is probably responsible for the digestion and absorption of as much as 70% of dietary fat (55, 59).

Lingual lipase is secreted from von Ebner glands (67) located at the proximal site of the tongue beneath the circumvallate papillae (24, 28). Enzyme preparations from the supernatant (100,000 *g*) fraction of lingual serous gland homogenates have a molecular weight >500,000 daltons (25). Further purification by acetone extraction at low temperature (−20°C) converts the enzyme to a low molecular form of 46,000 daltons (25). The latter molecular weight is identical to that of the lipase purified from gastric juice of newborn (27) or adult humans (8, 65). The data suggest that the high molecular weight species results from aggregates of enzyme and cell membrane lipids during the process of tissue homogenization.

There is good evidence that lingual lipase is secreted continuously and accumulates in the stomach between meals (1, 26). Because the stomach pH decreases from postprandial levels of 4.5–6.0 to 2.0–2.5 between meals (32), we have investigated the effect of low pH on the activity of the high and low molecular weight forms of lingual lipase in order to assess whether the enzyme will remain active after exposure to low pH.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats, weighing approximately 250 g and maintained on Purina Laboratory Chow and water *ad libitum*, were used in these studies. The animals were anesthetized by IP injection of 50 mg/kg of sodium pentobarbital (Abbott Laboratories, North Chicago, IL), exsanguinated, and the tongues were removed and placed on ice.

Tissue preparation. The proximal area of the tongue was sliced to expose the lingual serous glands. The entire area beneath the circumvallate papilla was carefully dissected to remove the serous glands free from contamination by muscle. The tissue was homogenized in ice cold 0.9% NaCl and centrifuged at 100,000 *g* at 4°C for 60 min. Part of the supernatant was frozen at −70°C for later use and the rest was extracted with acetone at −20°C as described previously (25). Protein concentrations were determined by the method of Lowry *et al.* (43) using BSA (Fraction V, Miles Laboratories, Inc., Elkhart, IN) as standard. Seven to 15 μg of enzyme protein were used for the assay of lipolytic activity.

For the experiments described in this study, we used two batches of lingual lipase preparation. For each batch of lingual lipase (low and high molecular weight enzymes) we used lingual serous glands pooled from 10–15 rats.

Collection of gastric aspirates from newborn infants. Gastric aspirates were obtained from term infants delivered at Columbia Hospital for Women, Washington, D.C., as part of routine neonatal care. The specimens were frozen immediately and stored at −70°C until analysis. Lipase activity is stable at −70°C for years (27). The protocol was approved by the Institutional Review Board, and informed consent was obtained.

Determination of lipase activity. Lipolytic activity in preparations of rat lingual lipase or in specimens of gastric aspirates of newborn infants was measured with long chain triglyceride (triolein) as substrate. A stable triglyceride emulsion was prepared by mixing 200 μmol of triolein (Sigma Chemical Company, St. Louis, MO) with tri-[9, 10 n^{3}H]oleate (Amersham, TRA-191, Arlington Heights, IL) and 15 μmol of phosphatidylcholine in chloroform. After evaporating the solvent, 3.3 ml of anhydrous glycerol (Baker Chemical Corp, Phillipsburg, NJ) was added and the mixture was sonicated in a Polytron PCU-2-110 (Brinkman Instruments, Westbury, NY) at setting 7 for two periods of 1 min each. The emulsion was stored at room temperature and was stable for 3 mo. The triglyceride concentration of the substrate emulsion was quantitated by the hydroxamic acid method of Rapport and Alonzo (52).

Lipase assay system. The assay system contained in a final volume of 200 μl: 1 μmol triolein (specific activity, 400,000 dpm/μmol); 10 μmol of sodium citrate-Na_2HPO_4 buffer, pH 5.4; 7 mg bovine serum albumin and enzyme, rat lingual lipase preparation or human gastric aspirate (7–15 μg protein). Incubation was for 30 min at 37°C in a Dubnoff shaking bath. The reaction was stopped by the addition of 3.25 ml of a mixture of methanol:chloroform:heptane (1.41:1.25:1.00, v/v/v). The FFA produced were separated from the glycerides (partial glycerides produced during the incubation and the unhydrolyzed triglyceride substrate) by the addition of 1.05 ml of potassium carbonate buffer, 0.05 M, pH 10.0 (5). Aliquots (0.5 ml each) of the aqueous phase were transferred to 5.0 ml of Ready-Solv (Beckman Comp. Fullerton, CA) and the radioactivity measured in a Beckman Scintillation Counter (model LS 7500, Beckman Instruments, Inc., Fullerton, CA) using internal standards for quench correction. Of the total amount of FFA, 75–80% was present in the alkaline upper aqueous phase. The effect of variable conditions during enzyme preincubation studies, such as variations in pH from 1.0–6.0, addition of lecithin (5–50 mM), proteins (BSA or gelatin) or pepsin, on the partition of FFA between the non-aqueous and aqueous phases was tested by determining the partition coefficient of [^{3}H]oleic acid in the two phases. The partition of FFA to the aqueous phase was not affected by any of the preincubation conditions tested, except for lecithin. In the latter case, appropriate controls were run with each experiment.

Analysis of reaction products formed during in vitro *or* in vivo *hydrolysis of triglycerides.* In these experiments, gastric aspirates were obtained at timed intervals after feeding. The lipids were extracted with chloroform:methanol (2:1) (15) and the glycerides and FFA were separated by column chromatography (28); the glycerides were quantitated by analysis of ester bonds (52) and the FFA by microtitration (11). For *in vitro* experiments, the reaction was stopped by the addition of 5 ml of chloroform:methanol (2:1). The labeled reaction products (partial glycerides and FFA) and the unhydrolyzed triglyceride substrate were separated by thin layer chromatography and quantitated by liquid scintillation counting (28).

Preincubation studies. For stability studies, aliquots of rat lingual lipase preparations (100,000 *g* supernatants of lingual serous gland homogenates, which are high molecular weight species, and acetone extracts of this preparation, which are low molecular weight enzyme species), were incubated for 0, 10, 30, and 60 min at 37°C at pH 1.0–6.0. The buffers for the various pH ranges were glycine-HCl (12.5 mM), pH 1.0–2.0; and citrate-phosphate buffer (12.5 mM), pH 2.5–6.0. At the end of the preincubation period, the pH was rapidly adjusted to 5.4 and aliquots were incubated in the lipase assay system described above.

RESULTS

Comparison of hydrolysis of fat in the stomach with in vitro *hydrolysis of triolein by rat lingual lipase and human gastric aspirates.* The extent of hydrolysis and the nature of the reaction products formed during *in vivo* lipolysis or during incubation of lingual lipase with triolein substrate are very similar, indicating that fat hydrolysis in the stomach is catalyzed by lingual lipase (Table 1). The data presented in Table 1 suggest, therefore, that the stability of lingual lipase in the gastric milieu could be investigated *in vitro* by simulating the conditions in the stomach between and after meals.

Because lingual lipase is secreted continuously from lingual glands (1, 26) and probably accumulates in the stomach between meals (1), we investigated the effect of low pH on lipase activity. In the experiments described below, rat lingual lipase preparations were first preincubated without substrate under various experimental conditions, followed by pH adjustment of 5.4 (optimum for lingual lipase activity), addition of labeled triolein substrate, and measurement of lipase activity. We used two preparations of rat lingual lipase: a large molecular weight species, which is probably an aggregate of the enzyme with polar lipids, and a low molecular weight species, prepared by delipidation of the former with acetone. The smaller enzyme species is identical in molecular weight (44–48,000 daltons) to the lipase in gastric aspirates of newborn infants (27) and healthy adults (8, 65). We showed previously that the two enzyme preparations (large and small) have the same level of activity (25); however, we did not know whether they differed in stability.

Effect of preincubation in the pH range of 1.5–6.0 on the activity of lingual lipase. A comparison between the stability of the two forms of lingual lipase to low pH is given in Figures 1 and 2. Both enzyme preparations are stable in the pH range of 2.5–6.0: the large molecular weight species retaining 90–95% of the original activity (Fig. 1), and the low molecular weight species retaining 75–85% of the initial activity (Fig. 2) after preincubation for 10–60 min at 37°C. At low pH (1.5–2.0), both enzyme preparations are rapidly inactivated; however, the large molecular weight species retains more activity at pH 2.0 (58% after 10 min, 35% at 30 min, and 14% after 60 min of preincubation) than the low molecular weight species (14% of original activity after 30 min and only 5% after 60 min of preincubation at pH 2.0). The loss of activity at low pH is irreversible because raising the pH to 5.4 does not return enzyme activity to the level before

Table 1. *Fat digestion in the stomach: comparison of* in vivo *lipolysis with* in vitro *hydrolysis of triglyceride by lingual lipase*

		Products* of Lipolysis mol % of total lipid			
Species	Study	TG	DG	MG	FFA
Human†	*in vivo*	54.4	15.0	3.2	29.2
		±4.2	±3.0	±0.4	±4.7
	in vitro	67.6	9.3	3.7	14.2
		±2.6	±1.1	±0.7	±2.9
Rat‡	*in vivo*	77.3	16.4	2.3	7.3
		±3.2	±2.8	±0.2	±1.2
	in vitro	77.7	9.2	2.8	10.3
		±4.8	±2.4	±0.4	±2.0

* TG, triglycerides; DG, diglycerides; MG, monoglycerides and FFA, free fatty acids.

† *In vivo* studies, the products of intragastric lipolysis were quantitated in gastric aspirates of eight infants (gestational age 28.9 ± 0.4 wk, birth weight, 1010 ± 67 g; age at study, 6.0 ± 0.8 wk) obtained 15 min after gavage feeding of an infant formula similar to the Enfamil premature formula but containing 91% long-chain triglyceride (Mead-Johnson Co.). *In vitro* assay of purified preparations of rat lingual lipase or of infant gastric aspirates was as described in "Materials and Methods." Data are mean ± SEM.

‡ Adult rats were fed a mixture of milk and cream by stomach tube and the products of lipolysis were analyzed 30 min after feeding.

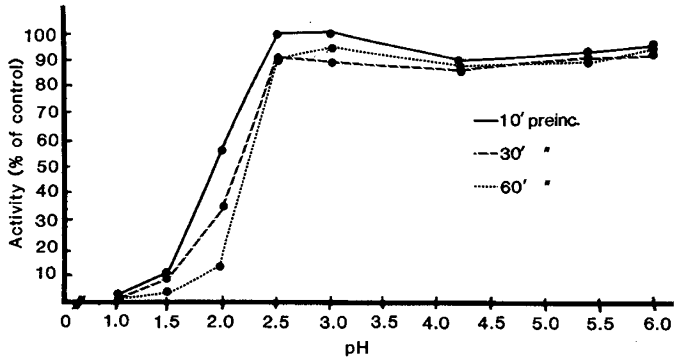

Fig. 1. Effect of preincubation at pH 1.0–6.0 on the activity of high molecular weight (>500,000 daltons) preparations of lingual lipase. 100,000 *g* supernatant preparations of rat lingual serous glands were preincubated for 10, 30, and 60 min at 37°C and pH 1.0–6.0. After preincubation, lipase activity was measured in the assay system at pH 5.4 as described in the "Materials and Methods" section. Lipolytic activity is expressed as percentage of control activity. The enzyme in the control tubes was exposed to the entire range of pH levels while on ice for less than 1 min; the pH was then immediately adjusted to 5.4 and lipolytic activity quantitated. Values are means of at least three experiments.

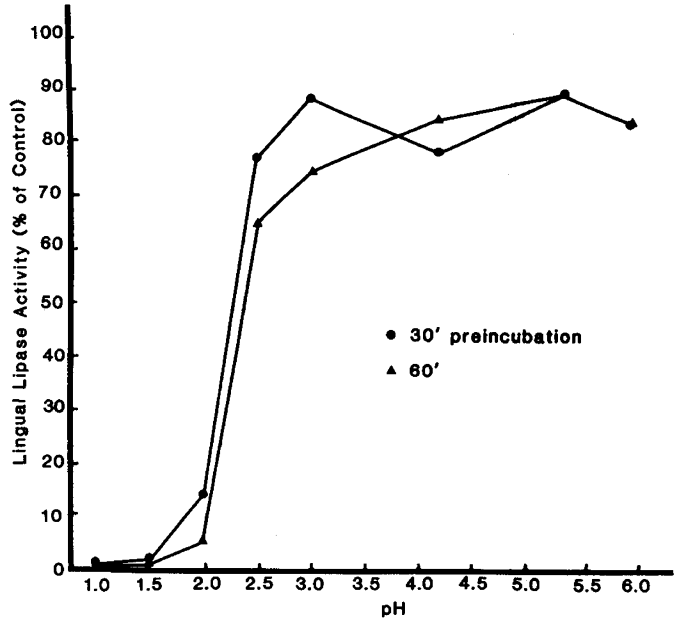

Fig. 2. Effect of preincubation at pH 1.0–6.0 on the activity of low molecular weight (46,000 daltons) preparations of rat lingual lipase. Acetone-extracted preparations of rat lingual serous glands were incubated for 30 and 60 min at 37°C and pH 1.0–6.0. Preincubation and assay conditions as described in Figure 1. Values are means of at least three experiments.

preincubation. Inactivation, in the pH range of 1.5–2.5, is shown in greater detail in Figure 3. In this experiment, we also investigated whether the nature of the preincubation buffer might affect the extent of enzyme inactivation. The large molecular weight enzyme is stable at pH above 2.4 during preincubation for 10 or 30 min at 37°C. Below pH 2.4, lipase activity is rapidly lost. There was a slight difference in the rate of inactivation during preincubation in the two buffers tested (12.5 mM Na_2 HPO_4-acetic acid buffer at pH 1.5–2.2 and glycine-HCl); slightly more enzyme activity was retained during preincubation in phosphate-acetic acid buffer at pH 1.5–2.2 than in glycine-HCl buffer.

Effect of protein and lipids on the inactivation of lingual lipase at low pH. In the series of experiments described below, we investigated whether proteins or lipids might prevent the loss of lipase activity at low pH. Preincubation of rat lingual lipase at

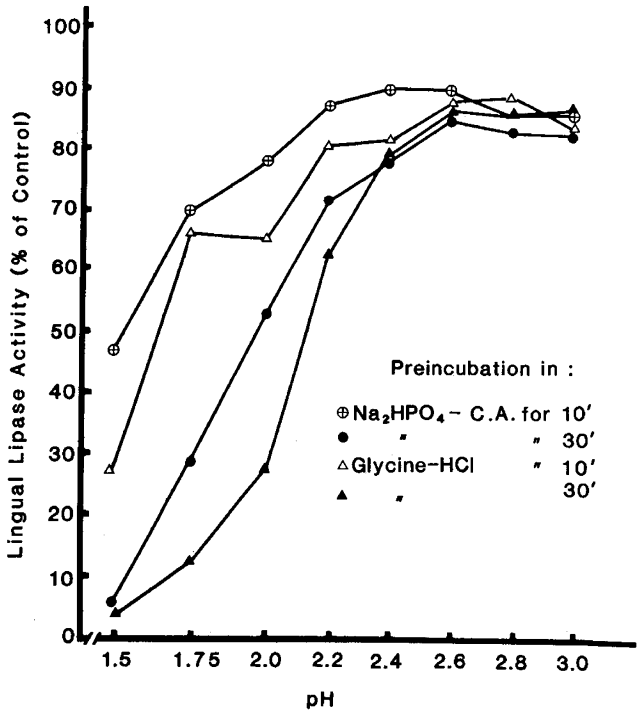

Fig. 3. Effect of preincubation at low pH, 1.5–3.0, on the activity of the high molecular weight species of rat lingual lipase. Preincubation was carried out in two separate buffers Na_2HPO_4-citric acid (12.5 mM) or glycine-HCl (12.5 mM) for 10 and 30 min followed by assay of lipolytic activity at pH 5.4. Values are means of at least three experiments.

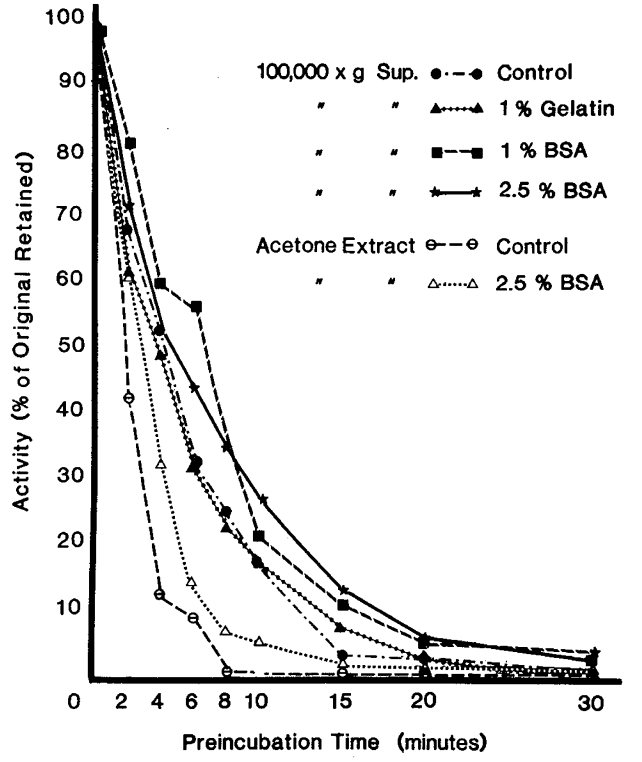

Fig. 4. Effect of protein-bovine serum albumin and gelatin on the inactivation of lingual lipase at low pH. Low molecular weight preparations (acetone-treated 100,000 *g* supernatant) of rat lingual lipase were preincubated at 37°C in glycine-HCl buffer pH 1.8, alone (control) or in the presence of 2.5% bovine serum albumin for 0–30 min. High molecular weight rat lingual lipase (100,000 *g* supernatant of rat lingual serous glands) was likewise preincubated alone (control) or in the presence of 1% gelatin or 1% and 2.5% bovine serum albumin for 0–30 min. Lipolytic activity was then determined at pH 5.4. Values are means of at least three experiments.

pH 1.8 was performed with or without 1% gelatin and at 1 and 2.5% BSA. In addition to the large molecular weight species, we tested the effect of 2.5% BSA on the low molecular weight species. The data (Fig. 4) show that proteins cannot prevent the inactivation of the two enzyme preparations at low pH. All proteins tested maintained slightly higher activity during short preincubation only (<10 min). Although enzyme protection was slight, 2.5% albumin was the most effective in maintaining enzyme activity during the first few minutes of preincubation. Lipids, on the other hand, protected the enzyme very efficiently from inactivation at low pH. We tested lecithin, a phospholipid that is not hydrolyzed by lingual lipase (Fig. 5) and triolein, the substrate of the enzyme (Fig. 6). Triolein seems to be more efficient in preventing the loss of activity than lecithin. Lecithin at a concentration of 50 mM preserves 60% of lipase activity after 30 min of preincubation at pH 2.0. Protection decreases at lower lecithin concentrations, 35% at 25 mM and only 2–3% at 5–10 mM. When lingual lipase is preincubated at pH 2.0 with 10 mM triolein, 94% of the original activity is preserved after 15 min and 66% after 30 min of preincubation.

Effect of pepsin on lipase activity. Because pepsin is active at low pH, considerable proteolytic activity is present in the stomach between meals. We have tested the effect of pepsin (porcine, Sigma Chemical Comp, St. Louis, MO, EC 3.4.2.3.1) on lingual lipase during preincubation at pH 2.0 for 30 min. At low pepsin concentrations (25 U/ml), lingual lipase activity decreases at rates similar to that of the controls; however, high pepsin concentrations (18,750 U/ml) protect the lipase from inactivation (Fig. 7). Because pepsin at the high concentration amounts to 22 mg/ml of enzyme protein, we assume that its protection is similar to that of serum albumin. The pepsin preparations were tested for proteolytic activity (35) and found to be fully active. It is possible that the hydrophobic nature of lingual lipase (49, 54) prevents its denaturation by pepsin.

Effect of low pH on the lipase in gastric aspirates of newborn infants. Because of the similarity in enzyme characteristics, we compared the stability to low pH of the lipase in four specimens of gastric aspirates obtained at birth, with the stability of rat

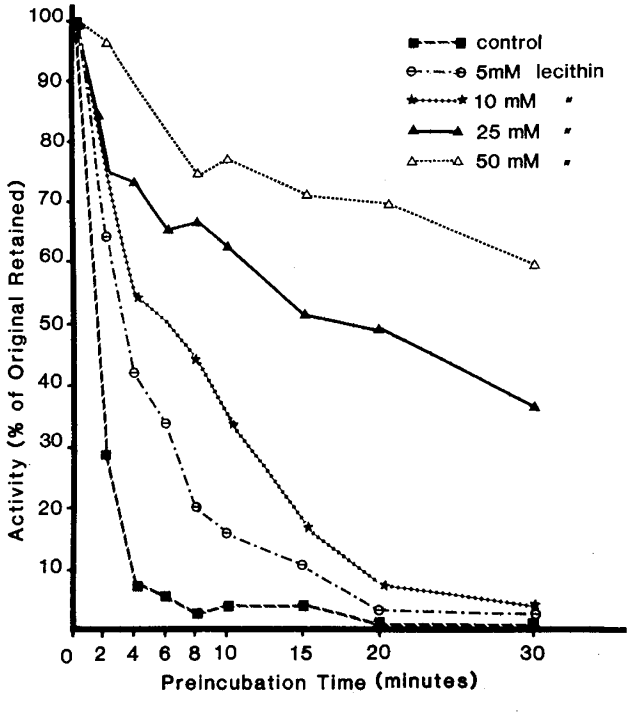

Fig. 5. Effect of lecithin on the inactivation of rat lingual lipase at low pH. Low molecular weight preparations of rat lingual lipase were preincubated alone (control) at 37°C in glycine-HCl buffer, pH 2.0 or in the presence of various concentrations of lecithin. Lipolytic activity was determined, after preincubation, at pH 5.4. Values are means of at least three experiments.

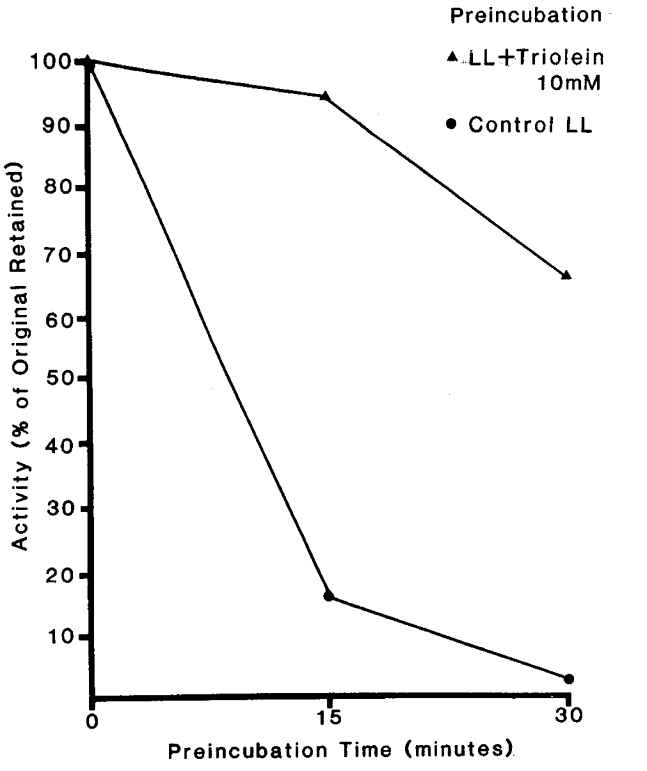

Fig. 6. Effect of triolein on the inactivation of rat lingual lipase at low pH. Low molecular weight preparations of rat lingual lipase were preincubated alone (control) at 37°C in glycine-HCl buffer pH 2.0, or in the presence of 10 mM triolein. Lipolytic activity was determined, after preincubation, at pH 5.4. Values are means of at least three experiments.

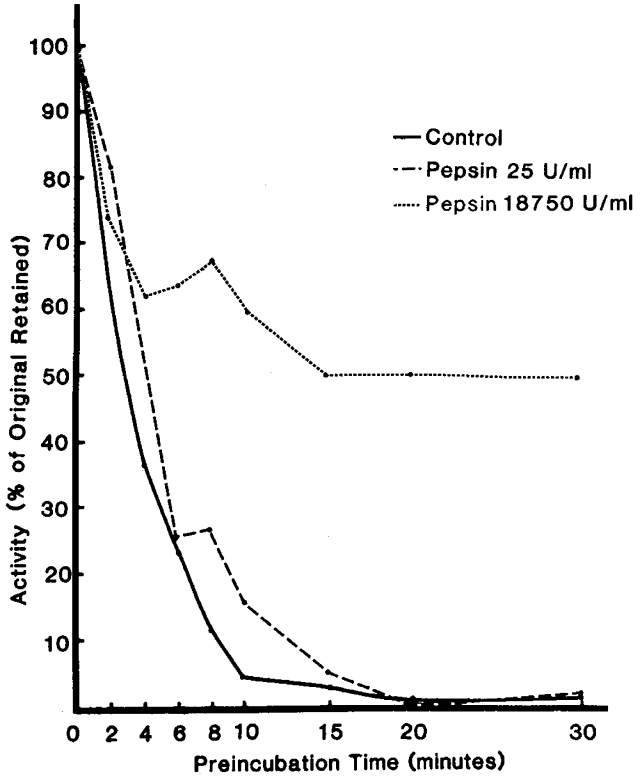

Fig. 7. Effect of pepsin on the activity of lingual lipase. Low molecular weight preparations of lingual lipase were preincubated alone (control), or in the presence of various concentrations of pepsin, in glycine-HCl buffer, pH 2.0. Lipolytic activity was determined at pH 5.4. Values are means of at least three experiments.

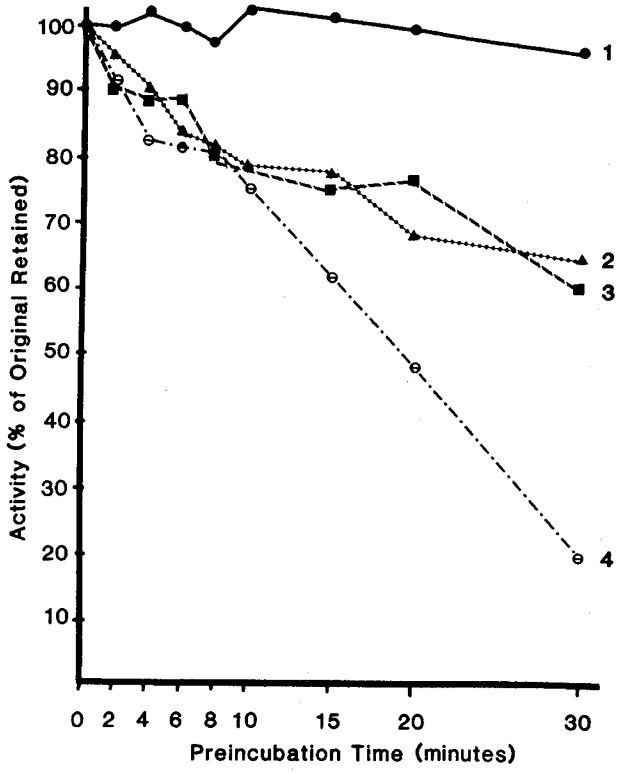

Fig. 8. Effect of low pH on the lipolytic activity in gastric aspirates of newborn infants. Gastric aspirates obtained at birth from term infants were preincubated at pH 1.6 (glycine-HCl buffer) followed by quantitation of lipase activity at pH 5.4.

lingual lipase described above. As can be seen, in Figure 8, there is marked variation in the loss of activity during preincubation at pH 1.6 for 30 min. Whereas the lipase activity in one specimen was stable during the entire 30-min period, two specimens lost about 35 and 40% of their original lipase activity and the fourth specimen retained less than 20% of its original activity after 30 min of preincubation at low pH. In view of our findings that rat lingual lipase is protected from inactivation by lipids and to a lesser extent by protein, we have quantitated the amount of lipid and protein in the gastric aspirates, but found no marked differences among the four specimens. There might, therefore, be additional components in the gastric aspirates of newborn infants that protect the lipase from inactivation at low pH.

DISCUSSION

The digestion and absorption of dietary fat is the process of transport of water insoluble molecules from one water phase, the intestinal lumen, to another water phase, the lymph and plasma. During this process, triglycerides (the major component of dietary fat) are first hydrolyzed, followed by dispersion of the products of lipolysis into absorbable forms. Whereas the major part of lipid absorption occurs in the intestine—with the exception of short and medium chain fatty acids, which can be absorbed directly through the gastric mucosa (4, 30)—hydrolysis of triglyceride starts in the stomach (7, 8, 28–30, 40, 42, 51, 58, 66). Intragastric lipolysis is necessary for normal digestion and absorption of dietary fat. Recent animal studies have shown that after diversion of oral secretions from the stomach, there is a marked fall not only in fat hydrolysis in the stomach but also in the duodenum, jejunum, and ileum (51). Concomitantly, there is a marked increase in the excretion of fat and bile salts (57). Fat digestion in the stomach becomes a much more important process in conditions of physiologic and pathologic pancreatic insufficiency. At birth, the levels of pancreatic lipase (9, 37, 47, 69) and bile salts (47, 68) are low, indicating that the major components of intestinal fat digestion are not sufficiently developed for the normal digestion of fat, the major energy source of the newborn (20).

Newborn infants, even very premature ones, absorb 85–95% of dietary fat (3, 48, 60), suggesting that fat digestion proceeds by mechanisms different from those in the adult. Likewise, in cystic fibrosis, a genetic disease resulting in severe pancreatic insufficiency (50), as much as 50–80% of dietary fat is absorbed in the absence of pancreatic lipase (38). Recent studies show that lingual lipase is well developed at birth [as early as 26 wk gestation (27)] and that it remains fully active in persons with cystic fibrosis (1, 56). Furthermore, because of low bicarbonate secretion in pancreatic insufficiency (14, 19), the upper small intestine maintains a low basal and postprandial pH (1, 13), compatible with the activity of lingual lipase. Although under normal conditions, the stomach is its major site of activity, lingual lipase probably continues to digest fat in the intestine of the newborn as well as in other cases of pancreatic insufficiency. Studies that compare the characteristics of lingual lipase with those of pancreatic lipase suggest that the former is a much less complex enzyme (21, 23, 33) and might, therefore, be better suited for the conditions in the immature gastrointestinal tract. Present evidence suggests that lingual lipase is secreted continuously from the serous glands and therefore that it might accumulate in the stomach between meals (1, 27, 28). The enzyme secreted during or immediately after feeding reaches the stomach when the pH is in the range of 4.0–6.0. The enzyme that reaches the stomach 2–3 hr postprandially is exposed to low pH and might therefore be inactivated by the acidity and peptic activity (64) in the stomach. The present study was, therefore, designed to assess the stability of lingual lipase in the gastric environment. We have shown previously that during the purification of lingual lipase one obtains a high molecular weight species, which seems to be an aggregate of enzyme with cell membrane lipids, and a low molecular weight species, which is probably the form in which the enzyme is secreted because it has an identical molecular weight (44–48,000) as that of the lipase in human gastric aspirates (8, 27, 65). Although these two forms hydrolyze triglyceride at similar rates, we had no previous information on their stability to low pH. Our present study shows that both forms of enzyme are stable at pH > 2.5 and that at low pH there is irreversible loss of activity (Figs. 1–3). The high molecular weight form is however slightly more resistant to low pH, suggesting that lipids might protect the enzyme. Indeed, as seen in Figs. 5 and 6, loss of activity is reduced markedly when lipids are present during preincubation of the low molecular form of lingual lipase at low pH. The substrate of the enzyme, triolein, protects the enzyme at lower concentrations (10 mM) than lecithin (25–50 mM), a lipid that is not hydrolyzed by the enzyme (41). Proteins, on the other hand, have only little protective action (Fig. 4). Lingual lipase does not seem susceptible to peptic denaturation (Fig. 7); thus, at low concentrations [similar to pepsin levels in the stomach of newborn infants (2)], loss of enzyme activity is not greater than during incubation at low pH in the absence of pepsin. Very high pepsin concentrations, sufficient to raise the protein concentration in the incubation system to 2.2% seem to protect the enzyme from inactivation at low pH. The protective action of pepsin is much higher than that of similar concentrations of gelatin or albumin, and might be related to the properties of the enzyme (pepsin) protein. Although we do not know what prevents the peptic digestion of lingual lipase, it is possible that because it is a highly hydrophobic protein (54), it is much more resistant to proteolysis than pancreatic lipase.

Conditions in the stomach of the newborn may further stabilize lingual lipase (Fig. 8). We do not yet know why the lipase in some gastric aspirates is stable at pH 1.6, whereas in others there is a variable loss of activity. The data in this study suggest that in the breast-fed infant, lingual lipase might remain fully active in the stomach between meals because gastric pH rarely falls below 3.5 (44). Salivary amylase, another digestive enzyme of oral origin, is able to pass through the stomach and retain

sufficient activity to provide 15–40% of total duodenal amylase activity in man (61).

Because of the relatively high pH in the stomach of breast-fed infants, the digestive enzymes in human milk, alpha-amylase (31, 34) and bile salt-stimulated lipase (16, 17, 45) retain sufficient activity to hydrolyze carbohydrates and fats in the duodenum.

Enzymes of extrapancreatic origin provide alternate mechanisms for nutrient digestion in the newborn (18, 22, 37). These mechanisms differ from those in the adult, but seem equally efficient for the digestion and absorption of food. There might indeed be advantages in the slow development of pancreatic function that could be important for the structural and functional development of the intestine. The "wear and tear" by pancreatic proteolytic enzymes would be less disruptive to the immature intestinal mucosa, and probably is one of the reasons for lower turnover of intestinal epithelial cells in the newborn (18, 22). Furthermore, intestinal brush-border enzymes, important in neonatal digestion (39), would not be degraded as rapidly at low levels of pancreatic enzymes (36).

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Longitudinal Studies on Metabolic Rate, Heat Loss, and Energy Cost of Growth in Low Birth Weight Infants

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Summary

Longitudinal studies on total and resting metabolic rate and total heat loss were made in 14 LBW infants, age 1–58 d. Metabolic rate was calculated from indirect calorimetry, heat loss was measured by direct calorimetry. Total metabolic rate and total heat loss were lowest during the first week of life, 178.9 ± 18.0 and 171.8 ± 15.5 kJ · kg^{-1} · 24 h^{-1}, respectively. During the age period of 8–58 d both total metabolic rate and heat loss showed a very slight increase with age: mean total metabolic rate was 278.8 ± 2.6 and mean total heat loss 257.0 ± 3.4 kJ · kg^{-1} · 24 h^{-1}. Resting metabolic rate was 171.0 ± 25.2 kJ · kg^{-1} · 24 h^{-1} during the first week of life and 248.0 ± 2.5 during d 8–58.

The energy cost of components of new tissue was calculated from the energy balance equation. $E_{components}$ during the second week of life was 25.5 ± 4.9 kJ/g weight gain and 11.9 ± 0.4 kJ/g weight gain over subsequent weeks. The net energy cost of tissue synthesis, calculated from the difference between indirect and direct calorimetry was 3.2 ± 1.1 kJ/g weight gain during the second week and 1.1 ± 0.1 kJ/g weight gain in the following weeks. A neonate who receives a caloric intake of 535 kJ · kg^{-1} · 24 h^{-1} and is growing at a rate of 17 g · kg^{-1} · 24 h^{-1} will use 42% of the caloric intake for maintenance and thermoregulation, 6% for activity, 38% for the components of new tissue, 4% for tissue synthesis and 10% for loss in faeces and urine.

Abbreviations

BWT, birth weight
GA, gestational age
LBW, low birth weight
VLBW, very low birth weight

The prognosis for survival of VLBW infants is improving (30). The quality of outcome will depend on adequate growth and caloric intake during the critical postnatal period of development as well as on adequate circulatory and ventilatory support. Studies concerning caloric intake and growth in VLBW infants are therefore necessary. The energy balance equation makes it possible to calculate the energy cost of growth and to give guidelines for the caloric intake of these infants (6, 8, 11, 20, 24).

The energy balance equation has been defined as (24, 27):

$$\text{Energy}_{\text{intake}} = \text{Energy}_{\text{excreted}} + \text{Energy}_{\text{expended}} + \text{Energy}_{\text{components}}$$

$\text{Energy}_{\text{intake}}$ represents the energy in the food. $\text{Energy}_{\text{excreted}}$ occurs mainly via the faeces in the postnatal period. $\text{Energy}_{\text{expended}}$ includes *1*) the energy used in maintenance, *2*) the energy used for thermoregulation, *3*) the energy used in activity, and *4*) the energy used for the synthesis of new tissue. $\text{Energy}_{\text{expended}}$ is equal to the metabolic rate and can be measured by indirect calorimetry. $\text{Energy}_{\text{components}}$ includes the energy present in the components of new tissue. The $\text{Energy}_{\text{cost of growth}}$ is comprised of the energy required for the synthesis of new tissue and the energy stored in the components of this new tissue:

$$\text{Energy}_{\text{cost of growth}} = \text{Energy}_{\text{components}} + \text{Energy}_{\text{synthesis}}.$$

The cost of tissue synthesis represents energy required for the organisation of the components of new tissue (6, 9, 27, 29) and for the formation of complex proteins, lipids, carbohydrates, and combinations thereof (14, 15). All energy used for maintenance, thermoregulation, and activity is given off as heat. Part of the energy consumed for tissue synthesis is converted to heat and given off. Another part of the energy for tissue synthesis is not