

Pharyngeal Lipase and Digestion of Dietary Triglyceride in Man

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ABSTRACT Lipolytic activity was studied in esophageal and gastric aspirates obtained with a nasogastric tube from 14 healthy adult subjects. Samples were collected from esophagus, first at 30–35 cm and then at 40–45 cm from the nose, as the subject, after drinking 15–30 ml of a cream-milk mixture, swallowed small amounts of water. The samples from stomach were taken last and usually contained a small amount of cream-milk mixture. Lipolytic activity was assayed using chylomicron, milk, and corn oil triglyceride as substrate. Esophageal and gastric samples both contained lipolytic activity which hydrolyzed long-chain triglyceride to diglyceride, monoglyceride, and FFA, had a pH optimum of 5.4, and was not affected by either 0.5 M NaCl or 4 mM sodium taurodeoxycholate. The activity, expressed as nanomoles of chylomicron triglyceride hydrolyzed per milliliter per minute, ranged from 0 to 145 in upper esophageal, 5 to 303 in lower esophageal, and 50 to 357 in gastric samples. Only a trace of lipolytic activity was found at pH 5.4 in saliva collected from the parotid, submandibular, and sublingual glands, thus excluding those tissues as a source of the activity found in esophageal and gastric aspirates. The findings suggest that in man glands in or near the pharynx secrete a lipase that acts in the stomach to hydrolyze long-chain triglyceride to partial glycerides and FFA. It is proposed that this reaction is the first step in the digestion of dietary fat and that the amphi-

philic lipids formed by lipolysis facilitate the emulsification of triglyceride in the stomach.

INTRODUCTION

A potent lipase, which hydrolyzes triglyceride to diglyceride, monoglyceride, and FFA at pH 4.5–5.4, was recently found in serous glands of the tongue in rat (1). A similar activity was also found in the soft palate, anterior oral pharyngeal wall, and lateral oral pharyngeal glands. The studies also showed that dietary triglycerides are readily hydrolyzed in the stomach to partial glycerides and FFA and that this reaction is catalyzed by the lingual lipase (1).

Intragastric hydrolysis of long-chain triglycerides has also been observed in man (2), dog (3), and calf (4), but the source of the activity was determined only in calf. The lipolytic activity in calf, called "pregastric esterase," was found in glandular tissue of the tongue, pharynx, and upper esophagus and in ingesta from the lower esophagus (5). The present study demonstrates in man a lipolytic activity in esophageal aspirates, which resembles closely that in stomach and, also, that in rat tongue (1).

METHODS

Subjects. 11 Female (20–22 yr) and six male (20–54 yr) normal volunteers were subjects for this study. They were on a regular diet with a daily intake of 1,200–1,500 kcal for female and 2,500–3,500 kcal for male volunteers. Each subject was tested after an overnight fast.

Collection of samples. Esophageal and gastric aspirates were obtained with a rubber Levine tube, French gauge no. 16 (Argyle Scotsman Cat. no. MAR 2670-16, Health and Science Division, Brunswick Corp., Chicago), inserted through the nose. The tube was shortened by 9 cm, measured from the pointed end, so that it would have a single lateral opening 1 cm from the tip. The tip of the tube was positioned at 30–35 and at 40–45 cm from the nose for esophageal samples, and in the fundus for gastric samples. No topical anesthesia was used. Esophageal samples were

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TABLE I
Hydrolysis of Chylomicron Triglyceride In Vitro by Esophageal and Gastric Aspirates

Hydrolysis of chylomicron triglyceride									
At pH 5.4									
Source of sample	pH of sample	No. of subjects	Amount hydrolyzed	Products of hydrolysis				At pH 7.4	
				Diglyceride	Mono-glyceride	Glycerol	FFA (Calculated)	No. of subjects	Amount hydrolyzed
			<i>nmol/ml per min</i>	<i>mol% of glyceryl products</i>			<i>mol% of acyl products</i>		<i>nmol/ml per min</i>
Upper esophagus (30–35 cm from nose)	6.0–7.5	14	29 (0–145)*	77.9±2.7	20.8±1.4	1.3±0.9	39	6	0.8±0.5
Lower esophagus (40–45 cm from nose)	5.5–7.0	13	78 (5–303)	75.9±3.9	21.0±2.7	3.1±1.4	43	5	0.8±0.8
Stomach (60 cm from nose)	3.0–5.0	12	240 (50–357)	67.3±3.0	25.5±2.3	7.2±1.4	47	9	2.7±0.3

The esophageal samples were aspirated with a nasogastric tube while the subject, after drinking 15–30 ml of a cream-milk mixture, swallowed 15–45 ml of water; the samples analyzed were water-clear. The gastric samples, which were taken last, usually contained small amounts of cream-milk and had a triglyceride content of 1.5–5.0 $\mu\text{mol/ml}$. A mixture of 0.1–0.25 ml of sample, 0.25 ml of 10% albumin-buffer solution containing 2 μmol of doubly labeled chylomicron triglyceride ($[^3\text{H}]$ glycerol and $[^{14}\text{C}]$ palmitic acid), and water to a final volume of 0.5 ml was incubated 15–60 min at 38°C. The duration of incubation depended on the amount of activity present in the sample; gastric samples assayed at pH 5.5 were usually incubated 15 min, whereas the other samples were incubated 15 and 60 min. Values are means±SE. The procedures for measuring glycerides and glycerol, and for calculating the amount of FFA formed are given in the Methods section.

* Range of values observed in group.

obtained at each level while the subject swallowed 15–30 ml of a cream-milk mixture (half-and-half, purchased from local food markets) and then 15–45 ml of water, until the samples were clear; only clear esophageal samples were assayed for lipolytic activity. The gastric sample, which was taken last, usually contained a small amount of cream-milk and had a triglyceride content ranging from 1.5 to 5.0 $\mu\text{mol/ml}$. Samples were collected with the aid of a manually operated vacuum pump (Nalge Company, Division of Sybron Corp., Rochester, N. Y.) into 20 ml glass vials and stored on ice until assayed.

Secretions of the major salivary glands were collected simultaneously for 20–60 min with devices placed bilaterally

over the orifices of the parotid, submandibular, and sublingual ducts (6, 7). The glands were stimulated with application by the subject of small amounts of lemon juice to the tongue every 15 s. Secretions were collected and stored separately in ice-cooled tubes and assayed 30–60 min later.

Assay of lipolytic activity. Lipolytic activity in esophageal, gastric, and salivary samples was measured by the amount of chylomicron triglyceride hydrolyzed to diglyceride, monoglyceride, glycerol, and FFA (1). The assay mixture contained 100–250 μl of sample, 2 μmol of doubly labeled chylomicron triglyceride, 50 μl of 1 M sodium citrate- Na_2HPO_4 buffer solution (pH 3.6–7.6), 200 μl of 12.5% bovine serum albumin (Fraction V, Metrix, Armour

TABLE II
Hydrolysis of Chylomicron and Milk Triglyceride In Vitro by Secretions of Parotid, Submandibular, and Sublingual Glands

Source of sample	No. of subjects	Rate of secretion	pH of sample	Chylomicron triglyceride*				Milk triglyceride† Amount hydrolyzed at pH 5.4
				Amount hydrolyzed		Glycerol produced		
				At pH 5.4	At pH 7.5	At pH 5.4	At pH 7.5	
				<i>nmol/ml per min</i>		<i>mol% of glyceryl products</i>		<i>nmol/ml per min</i>
Parotid	6	0.2–0.5	7.0	1.8±0.5	4.2±0.5	47±3	58±4	0
Submandibular	6	0.1–0.3	7.0	2.0±0.7	3.5±0.7	50±8	58±7	0
Sublingual	5	0.003–0.05	7.0	0.7±0.3	2.0±0.3	44±10	66±4	0

Secretions were collected bilaterally from parotid and submandibular glands for 20–30 min and from sublingual glands for 30–60 min. Values are means±SE. * A mixture of 0.25 ml of sample, 1 μmol of doubly labeled chylomicron triglyceride, and 0.25 ml of 10% albumin-buffer solution were incubated 30–60 min at 38°C.

† A mixture of 0.5 ml of sample, 0.05 ml of cream-milk (1:1) containing about 5 μmol of triglyceride, and 0.5 ml of 10% albumin-buffer solution were incubated 60 min at 38°C.

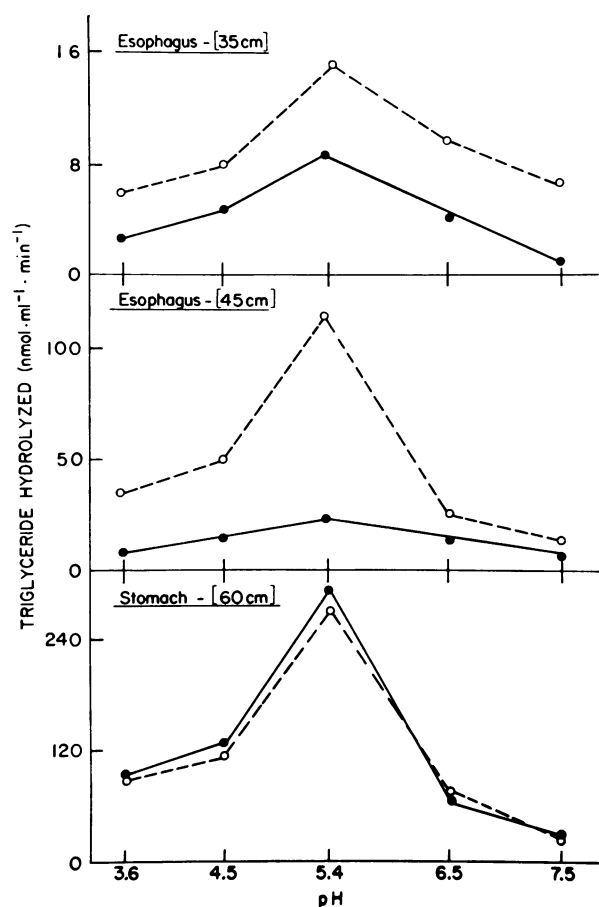


FIGURE 1 Effect of pH on the hydrolysis of chylomicron triglyceride by aspirates obtained from the esophagus and stomach of two normal subjects. Samples were incubated 15 min at 37°C.

Pharmaceutical Company, Chicago, Ill., lot G36912) solution, and water to a final volume of 500 μ l. The chylomicrons were isolated by centrifugation from thoracic duct chyle of fasted rats tube fed corn oil containing [14 C]-palmitic acid and trioleoyl-[2 - 3 H]glycerol and suspended in 4% albumin solution (1). The assay mixture was incubated in polypropylene tubes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) at 38°C for 15–60 min in a Dubnoff shaking bath. Lipids in the incubation mixture were extracted by a modification of the procedure of Dole and Meinertz (8), substituting hexane for heptane (9). Lipids in the hexane extract were separated by thin-layer chromatography (10) and analyzed for 3 H and 14 C content (1). The amount of glycerol formed was calculated from either the decrease in ratio of 3 H to 14 C in the hexane extract of the assay mixture (11) or from the amount of [3 H]glycerol produced during incubation (1). The proportions of tri-, di-, and monoglyceride in lipid samples were calculated from the distribution of [3 H]glycerol among the three classes of lipids. The amount of FFA formed was calculated from the amount of triglyceride (TG) hydrolyzed and the relative amounts of diglyceride (DG), monoglyceride (MG), and glycerol (G) formed during lipolysis (Table

I): FFA formed (μ mol) = TG hydrolyzed (μ mol) \times (% DG + [% MG \times 2] + [% G \times 3]).

When nonradioactive lipids were used, as in Table II and III, the hexane extract of lipids was carefully washed with blank aqueous phase (isopropanol-water) to remove short-chain fatty acids and other acidic contaminants (8). FFA in the hexane extract were measured by titration (9), and glycerides were separated by column chromatography (1) and measured by the method of Rapport and Alonzo (12).

RESULTS

Lipolytic activity in esophageal and gastric aspirates.

Esophageal and gastric aspirates were obtained with a nasogastric tube from normal subjects fasted overnight. Samples for analyses were collected from esophagus, first at 30–35 cm and then at 40–45 cm from the nose, as the subject, after drinking 15–30 ml of a cream-milk mixture, swallowed small amounts of water. Usually only 10–20%, but sometimes up to 50%, of the water swallowed was recovered from the esophagus. The esophageal samples used for analyses were water-clear. Samples from stomach, which were taken last, contained small amounts of the cream-milk mixture and had a triglyceride content of 1.5–5.0 μ mol/ml. The pH of samples from the upper esophagus ranged 6.0–7.5, from the lower esophagus, 5.5–7.0, and from stomach, 3.0–5.0 (Table I).

Aspirates from both esophagus and stomach contained lipolytic activity that hydrolyzed chylomicron triglyceride at pH 5.4 to mostly partial glycerides and FFA (Table I). The glyceryl products formed averaged 67–78% diglycerides, 21–26% monoglycerides, and <8% glycerol. The lipolytic activity in samples from the upper esophagus hydrolyzed 0–145, from the lower esophagus, 5–303, and from stomach, 50–357 nmol of chylomicron triglyceride/ml per min (Table I). Since milk triglyceride in gastric samples probably competed with chylomicron triglyceride for enzyme, the rate observed with chylomicrons (Table I) may be an underestimate of the lipolytic activity in samples from the stomach. The lipolytic activity in esophageal and gastric aspirates was not affected by either 0.5 M NaCl or 4 mM sodium taurodeoxycholate. The results obtained in two subjects show that the lipolytic activity in esophageal and gastric samples had the same pH optimum, 5.4 (Fig. 1).

To test whether the major salivary glands could be a source of the lipolytic activity present in esophageal and gastric aspirates, secretions were collected from the parotid, submandibular, and sublingual glands of six subjects and tested for lipolytic activity (Table II). Salivary secretions had only a trace of lipolytic activity at pH 5.4, hydrolyzing <2 nmol of chylomicron triglyceride/ml per min and 0 nmol of milk triglyceride. The lipolytic activity in saliva, in contrast to that in

TABLE III
Hydrolysis of Milk and Corn Oil Triglyceride in the Stomach

Test meal and sample	Time after test meal	pH	Volume	Glyceride concentra- tion	Lipid composition			
					Triglyceride	Diglyceride	Mono- glyceride	FFA
					<i>mol% of total glyceride</i>			<i>mol% of total fatty acids</i>
	<i>min</i>		<i>ml</i>	<i>mM</i>				
Cream-milk mixture A								
Meal	—	7.0	—	110	97.7	2.4	0	0
Gastric contents								
Subject I	0	5.0	20	2	—	—	—	—
	1	5.0	15	55	86.3	12.7	1.0	3.3
	10	5.0	30	9	90.9	7.7	1.3	1.7
Subject II	0	4.0	25	0.4	—	—	—	—
	1	4.5	20	15	84.4	14.0	1.6	4.5
	10	4.5	35	4	89.0	10.0	1.0	3.0
Cream-milk mixture B								
Meal	—	6.8	—	130	95.0	4.3	0.7	0.9
Gastric contents								
Subject III	0	5.0	15	0.2	—	—	—	—
	3	5.0	20	19.0	92.5	6.9	0.4	1.5
	5	5.0	20	26.0	91.0	7.5	0.8	1.7
Subject IV	0	5.5	12	0.1	—	—	—	—
	1	5.8	18	5.4	87.6	11.0	0.8	4.5
	5	5.8	10	3.7	83.5	14.6	1.6	6.0
Corn oil-water mixture*								
Meal	—	3.0	—	109	94.0	5.9	0.8	0.3
Gastric contents								
Subject V	0	5.5	10	0.5	—	—	—	—
	1	3.0	18	15.7	91.0	8.1	0.8	0.8
	3	3.0	20	9.3	89.5	9.0	1.3	2.5
Subject VI	0	6.0	6	0.1	—	—	—	—
	3	4.0	15	7.2	89.0	9.3	1.2	1.6
Subject VII	0	5.8	40	0.3	—	—	—	—
	4	3.5	30	2.8	88.0	9.2	2.4	4.3
	6	3.5	30	2.9	87.0	10.0	2.6	4.6

Subjects I–VI drank 30 ml, and subject VII drank 60 ml of the test meal after fasting overnight. Gastric samples were aspirated with a nasogastric tube before and at various times after the meal. The gastric samples taken from subjects I and II were placed immediately on ice, and aliquots were taken for analyses within 15 min after collection, whereas aliquots of samples collected from the other subjects were put immediately into hexane-isopropanol for analyses (9).

* Lemon juice was added to the corn oil-water mixture to make it more palatable. Incubation at 38°C for 1 h had no effect on the FFA content of the mixture.

esophageal and gastric aspirates, was slightly higher at pH 7.5 than at pH 5.4, and produced proportionally more glycerol, >44 vs. <8 mol% of glyceryl products (Tables I and II). Thus, it seems unlikely that salivary glands are the source of the lipolytic activity found in esophageal and gastric aspirates.

Intragastric lipolysis of milk and corn oil triglyceride was studied in seven subjects fasted overnight. Samples of gastric contents were taken immediately before and at various times up to 10 min after the subjects drank 30 or 60 ml of a liquid test meal containing 11–13% triglyceride. The results show that 3–12% of the tri-

glyceride was hydrolyzed within 4 min to diglyceride, monoglyceride, and FFA, and that hydrolysis did not continue appreciably beyond that time (Table III). The results also show that corn oil triglyceride was hydrolyzed in the stomach as quickly as milk triglyceride. Proportionally less monoglyceride was formed by lipolysis in the stomach in vivo (Table III) than in gastric aspirates assayed in vitro (Table I). This difference, as well as the limitation of lipolysis in vivo with time, may be due to the lack of FFA acceptor, such as albumin, in the test meals.

The effect of simulated products of intragastric

TABLE IV
Effect of Simulated Lipolytic Products on the Emulsification of Glyceride at pH 5.4 in 1% Gelatin Solution

Lipid composition				Total fatty acid concentration mM	Lower half of suspension 15 min after mixing		
Triolein	Diiolein % of total glyceride	Monoolein	Oleic acid % of total fatty acids		Turbidity OD at 550 nm	Glyceride fatty acid concentration* mM	% of total fatty acid concentration
100	0	0	0	11.7	0.32±0.05	0.71±0.17	6.1±1.4
86	11.6	2.3	3.7	11.7	0.86±0.01	1.84±0.09	15.7±0.8
71	24.1	4.9	7.6	11.7	1.15±0.13	2.52±0.3	21.5±2.6

The lipids were added in the amounts indicated to 1% gelatin in phosphate buffer solution at pH 5.4 in disposable glass test tubes, warmed to 38°C, mixed for 1 min with a Vortex shaker set at 5 (Vortex-Genie, model K-550-G, Scientific Industries, Inc., Springfield, Mass.), and left standing at room temperature for 15 min. Turbidity was then measured in a Beckman model B spectrophotometer (Beckman Instruments, Inc., South Pasadena, Calif.) and an aliquot (3 ml) equal to half of the suspension was taken from the lower part of the tube for analyses (12).

* Column chromatography (1) showed that the glyceride composition was the same as that of the total suspension.

lipolysis on the emulsification of triglyceride was examined by replacing triglyceride with various amounts of partial glycerides and FFA in 1% gelatin solution at pH 5.4. The quantitative relationship between diglyceride, monoglyceride, and FFA added to the mixture was the same as that found in stomach contents (Table III). Emulsification was measured by the degree of turbidity and the amount of glyceride fatty acid present in the lower half of the aqueous suspension 15 min after mixing with a Vortex shaker. The results show that replacement of tryglyceride with partial glycerides and FFA aids dispersion of the lipid in an aqueous solution at pH 5.4 (Table IV). These findings indicate that products of intragastric lipolysis could be expected to facilitate emulsification of triglyceride in the stomach.

DISCUSSION

Although it is generally thought that enzymic digestion of fat begins in the duodenum (13, 14), several studies have shown that dietary triglyceride is readily hydrolyzed in the stomach to FFA and partial glycerides (1-3, 15, Table III). Furthermore, gastric juice collected free of bile from subjects fasted overnight contains lipolytic activity that hydrolyzes at pH <6 long-chain triglycerides of milk, various vegetable oils, and animal fats to partial glycerides and FFA (16, 17). Similar lipolytic activity was found in gastric juice of infants (15) and in gastric contents of suckling and adult rats (1, 15). Lipolytic activity has been demonstrated histochemically in rat gastric mucosa (18), but the activity found in homogenates of the mucosa had very little effect on long-chain triglyceride (19).

Our studies show that esophageal aspirates obtained from adult subjects contain lipolytic activity (Table I)

similar to that found in homogenates of tongue and other pharyngeal tissues of the rat (1). The activity hydrolyzed at pH 5.4 long-chain triglyceride to diglyceride, monoglyceride, and FFA (Table I) and was not affected by either 0.5 M NaCl or 4 mM sodium taurodeoxycholate. Lipolytic activity was negligible at pH 5.4 in secretions collected from the parotid, submandibular, and sublingual glands (Table II), excluding those tissues as a source of the lipolytic activity present in esophageal aspirates. Lipolytic activity was similarly absent from the major salivary glands in rat (1). The findings suggest that glands in or near the pharynx in man secrete a lipase that hydrolyzes triglyceride to partial glycerides and FFA at pH 5.4.

Lipolytic activity in gastric contents is markedly reduced when pharyngeal secretions are excluded from the stomach in rats (1) and calves (4). The close similarity in lipolytic activity between esophageal and gastric aspirates in our adult subjects (Table I) suggests that the source of gastric lipolytic activity in man may also be the pharynx. The above findings suggest that a lipase is secreted by pharyngeal tissues which hydrolyzes in the stomach long-chain triglyceride to partial glycerides and FFA. It is proposed that this reaction is the first step in the digestion of dietary fat in man and that the amphiphilic lipids (20) formed, particularly monoglyceride and FFA, facilitate the emulsification of triglyceride in the stomach (13).

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