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In Vivo and In Vitro Studies on the Stereoselective Hydrolysis of Tri- and Diglycerides by Gastric and Pancreatic Lipases

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Abstract—The stereoselectivity of dog gastric and dog pancreatic lipases was investigated both in vitro, under simulated physiological conditions, and in vivo, during the digestion of a liquid test meal. In vitro it was observed that although both lipases had a stereopreference for the *sn*-3 position in triglycerides, it was about three times higher in the case of the gastric lipase. On the other hand, both lipases clearly showed a comparable enantioselectivity for the *sn*-1 position when a racemic diolein was used as the substrate. In the case of pancreatic lipase, the enantiomeric excess of 1,2-*sn*-diolein generated in vitro by the hydrolysis of triolein was found to decrease significantly, and even to be slightly reversed, at high rates of hydrolysis (above 50%) due to the further stereoselective hydrolysis of diglycerides into monoglycerides. This finding may explain the low enantiomeric excess of the diglycerides observed in vivo during the early phase of intraduodenal digestion when pancreatic lipase plays a predominant role and the rate of triolein hydrolysis is already high. On the other hand, a large enantiomeric excess of 1,2-*sn*-diolein generated from triolein was always the fingerprint of the gastric lipase in vitro even at high hydrolysis rates. This fingerprinting of gastric lipase was observed during both the intragastric phase and the late intestinal phase of lipolysis. This feature was therefore taken as an index to determine the respective roles of gastric and pancreatic lipases during in vivo lipolysis. To the best of our knowledge, this is the first time that stereoselectivity has been used as a tool to discriminate between the activities of two enzymes hydrolyzing the same substrate in vivo.

Introduction

In order to evaluate quantitatively the relative physiological roles of gastric and pancreatic lipases (triacylglycerol hydrolases, EC 3.1.1.3), we recently developed experimental procedures for measuring in vivo the digestive lipase secretions as well as the lipolysis occurring during the digestion of a liquid test meal.[1,2]

In dogs equipped with gastric and duodenal cannulae,[1] the secretion of gastric lipase was found to be stimulated by food ingestion and was three times higher than the basal secretion rate during the first hour of digestion. On a weight basis, the ratio of the total secreted pancreatic to gastric lipase was around 3 (18.7 ± 1.2 mg and 7.2 ± 1.2 mg, respectively) during a 3-h period of digestion. In humans, this ratio was around 4 (88.2 ± 25 mg and 22.6 ± 8.1 mg, respectively).[2]

Based on HPLC analysis, it has been established that 10% of the acyl chains of meal triglycerides (TG) are released in the stomach by human gastric lipase, yielding 1,2(2,3)-*sn*-diglycerides (DG) as the main products of intragastric lipolysis.[2] Using both a specific enzymatic titration and an ELISA test, we reported for the first time that gastric lipase remains active in the intestinal contents[1,2] and may therefore act in synergy with pancreatic lipase as previously proposed.[3] In the duodenum, however, the contributions of gastric and pancreatic lipases to the lipolysis cannot be measured separately, since both lipases hydrolyse the same substrate and give rise to the same products (Fig. 1). The lipolysis products released cannot be quantified, since 2-monoglycerides (2-MG) and fatty acids (FA) are absorbed by the intestinal mucosa. The contributions to duodenal lipolysis of these two human lipases were therefore estimated based on their respective concentrations in the duodenal contents and their specific activities.[2] With this method of estimation, gastric lipase probably releases about 7.5% of the TG acyl chains in the duodenum. During the whole digestion period, gastric lipase may release up to 17.5% of the TG acyl chains. It is worth noting that only 66% of TG hydrolysis is required for them to be completely absorbed in the form of 2-MG and FA (Fig. 1) and that gastric lipase is therefore not a minor enzyme. The respective contributions of gastric and pancreatic lipases to duodenal lipolysis probably vary during the period of digestion with the pH of the duodenal contents and the respective amounts of lipases. The relative contribution of gastric lipase may increase in the late phase of intraduodenal lipolysis, due to the acidification of the duodenal contents.[1,2]

An analytical method developed by Rogalska et al.[4] can be used to separate and quantify the enantiomeric DG

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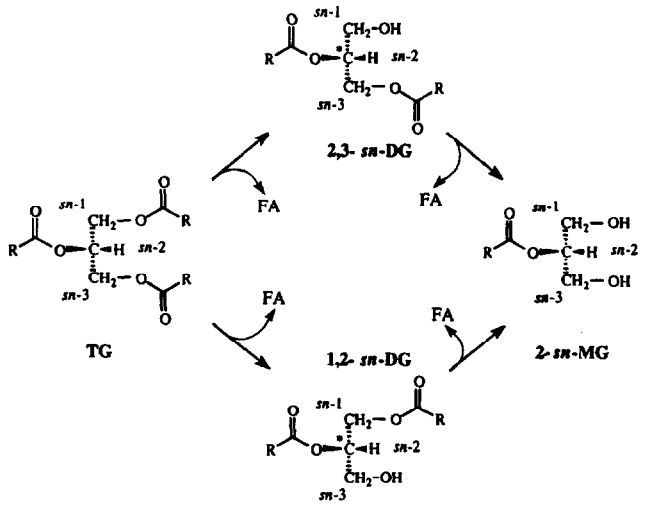

Figure 1. Chemical pathways of triolein and diolein hydrolysis by gastric and pancreatic lipases. The homogeneous prochiral triolein (TG) is converted into chiral diolein (2 enantiomers: 1,2-*sn*-DG or 2,3-*sn*-DG) during the first step of lipolysis by the hydrolysis of the ester bond at the *sn*-1 or *sn*-3 position. During the second step of lipolysis, diolein is further converted into monoolein (2-*sn*-MG) by the hydrolysis of the ester bond either at the *sn*-1 position in 1,2-*sn*-DG or at the *sn*-3 position in 2,3-*sn*-DG. In the present study, the HPLC analysis did not show the presence of either 1,3-*sn*-DG or 1(3)-*sn*-MG.

generated upon the hydrolysis of homogeneous prochiral TG. Using this method, in vitro investigations on the stereoselective hydrolysis of TG by digestive lipases clearly demonstrated the stereopreference of gastric lipase for the *sn*-3 position of TG, giving rise to a large enantiomeric excess (ee) of 1,2-*sn*-DG (50–70%).[5] On the other hand, pancreatic lipase was found to be poorly stereoselective.[5] Neither digestive lipase directly hydrolyses the ester bond at the *sn*-2 position of long chain TG; consequently, 1,3-*sn*-DG were never detected.[4] These findings suggest measuring the ee of 1,2(2,3)-*sn*-DG might be an original means of distinguishing the gastric from the pancreatic lipase activities in vivo, since these DG are nonabsorbable intermediary lipolysis products. To validate this method, we checked that the bile salt-stimulated lipase (BSSL) was not active towards oleoyl glycerides under our experimental conditions.

In the present in vitro and in vivo studies, we investigated the stereoselectivity of the hydrolysis of a prochiral TG (triolein) and 1,2-*rac*-diolein by dog gastric (DGL) and dog pancreatic (DPL) lipases. This is the first attempt so far to use stereoselectivity as a tool to discriminate between the activity of two enzymes acting simultaneously on the same substrate.

Results and Discussion

In vivo experiments

A large 40–60% ee of 1,2-*sn*-diolein was observed throughout the intragastric phase of lipolysis (Fig. 2A). It is worth noting that the level of intragastric lipolysis became significant when the pH of the gastric contents reached values approximating 4.0 (Fig. 2C), which is the optimum pH for DGL activity upon long-chain TG.[6] The TG hydrolysis level then remained stable and quite low (7–10%, Fig. 2B). We checked (data not shown) that no pancreatic lipase contamination had occurred during this experiment by measuring the absence of pancreatic lipase activity in gastric samples, as previously described.[1,2]

On the other hand, a very low ee (around 5%) of 2,3-*sn*-diolein was observed during the first 2 h of the intraduodenal phase of digestion (Fig. 2D). The apparent stereopreference was then reversed and a high ee (20–45%) of 1,2-*sn*-diolein was measured

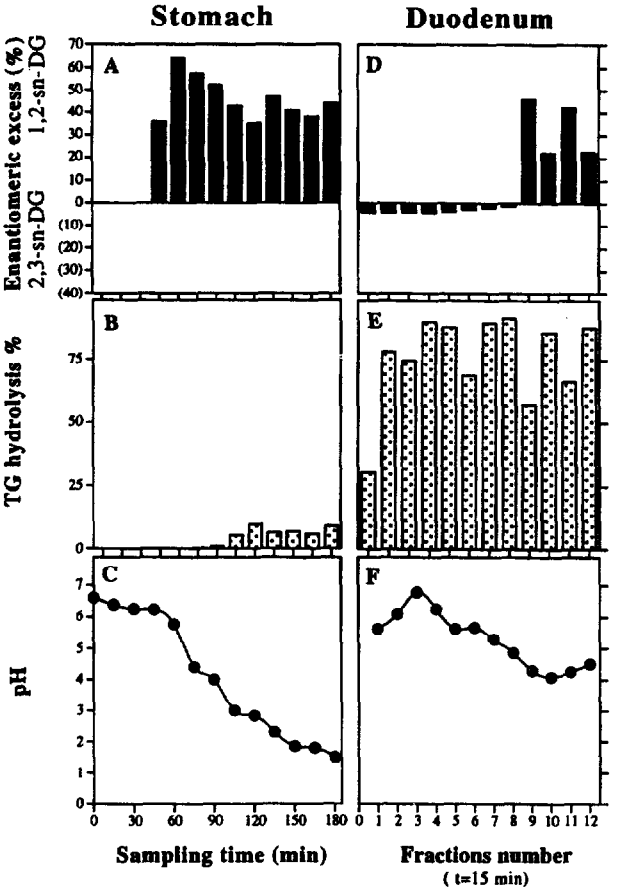

Figure 2. Gastric and duodenal parameters recorded during the in vivo digestion of a test meal. **A** and **D**, DG ee (%) in gastric and duodenal contents, respectively. **B**, TG hydrolysis levels in gastric contents. Based on the HPLC analysis of the residual glycerides present in the gastric contents, the TG hydrolysis level (%) in the stomach was expressed as the molar ratio (DG)/(TG + DG). No significant amounts of MG were detected in the present experiment. **E**, TG hydrolysis levels (%) in duodenal fractions collected every 15 min. With each fraction, the TG hydrolysis level was calculated based on the TG pyloric output and the HPLC analysis of the residual TG collected through the duodenal cannula. The intraduodenal TG hydrolysis level (%) was expressed as the molar ratio (TG pyloric output — TG recovered through the duodenal cannula)/(TG pyloric output). As previously described,[2] the intraduodenal hydrolysis level of TG reflects a 15 min period of lipolysis occurring in each duodenal fraction withdrawn, whereas the intragastric TG hydrolysis level reflects the variation in the course of lipolysis in the total gastric contents, during digestion (see ref 2 for further details). **C** and **F**, pH variations during the period of digestion in gastric and duodenal contents, respectively.

during the last hour of the intraduodenal phase of lipolysis. The duodenal rates of TG hydrolysis (Fig. 2E) were always found to be very high (60–90%), except during the first period (15 min) of collection. The pH of the duodenal contents was found to vary between 6.8 and 4.0 during this experiment (Fig. 2F).

In vitro stereoselective hydrolysis of pure triolein

In order to determine the stereoselectivity of each lipase with a prochiral TG as substrate, the hydrolysis of pure triolein by DGL or DPL was studied in vitro, using the minimum additives required to be able to measure a significant level of catalytic activity. The experiments were always stopped after a very slight increase in the rate of hydrolysis to ensure that the measured ee mainly reflected the stereoselectivity of the TG hydrolysis into DG and was not significantly influenced by the selective disappearance of the enantiomeric DG formed. As indicated in Table 1, although both lipases showed a stereopreference for the *sn*-3 position in triolein, it was much higher in the case of DGL than in that of DPL. In the presence of bile salts, the stereoselectivity of these two lipases was not significantly affected by the pH variations (Table 1). In the absence of bile salts, however, the stereoselectivity of both lipases increased significantly with increasing pH (Table 1).

In vitro stereoselective hydrolysis of triolein under simulated duodenal conditions

The meal constituents, including triolein, were diluted with water and dog bile in order to simulate the natural duodenal conditions throughout the period of digestion. As shown in Table 2, and in line with previous experiments (Table 1), DGL and DPL showed a stereopreference for the *sn*-3 position of triolein, which was much higher in the case of DGL. The stereoselectivity of neither lipase was affected by the pH values, as previously observed in experiments performed in the presence of bile salts (Table 1).

On the contrary, when both lipases were added simultaneously the stereoselectivity was clearly dependent on the pH of the incubation medium (Table 2). At pH 6.0, the ee (12.8%) was approximatively that recorded with DPL alone (16.4%). When the experiment was carried out at pH 4.0, the ee increased to 42.6%, which is roughly the value obtained with DGL alone (53.1%). These results can be explained by the fact that DPL is a more active enzyme than DGL at pH 6.0, whereas DGL predominates at pH 4.0 (see their respective specific activities in Table 2). The ee values reflect the respective contributions of the two digestive lipases, in a mixture containing both enzymes (see Table 2).

Table 1. In vitro stereoselective hydrolysis of pure triolein by DGL and DPL

Enzyme	pH	$ee_{1,2}$ (+NATDC)	$ee_{1,2}$ (−NATDC)
DGL	4.0	51.4	54.4
	5.5	59.6	76.6
DPL + colipase	5.5	14.2	7.4
	8.0	12.2	12.7

Both lipases were tested at the average pH value of 5.5 recorded in duodenal contents as well as at their respective pH optimum for long-chain TG hydrolysis (4.0 and 8.0 with DGL and DPL, respectively). Each value is an average based on two experiments, with triolein hydrolysis levels not exceeding 5%. See Experimental for the amounts of lipases and substrate concentrations used.

Table 2. In vitro stereoselective hydrolysis of triolein by DGL and DPL alone, and by a mixture of DGL+DPL, under conditions simulating duodenal lipolysis

pH	DGL		DPL		DGL+DPL
	$ee_{1,2}$ (%)	U/mg	$e_{1,2}$ (%)	U/mg	$ee_{1,2}$ (%)
6.0	53.3	4.8	16.4	39	12.8
5.0	53.5	n.d.	12.9	n.d.	24.2
4.0	53.1	27	16.6	2.8	42.6

The liquid test meal constituents used for in vivo experiments were diluted with dog bile and water as described in the Experimental, and the pH was adjusted to values (4.0, 5.0, and 6.0) overlapping the pH range existing in duodenal contents during digestion. Each value is an average based on two experiments with triolein hydrolysis levels not exceeding 5%. See Experimental for the amounts of lipases and the substrate concentrations used. The specific activities [unit (U) per mg of enzyme] of DGL and DPL were calculated based on the HPLC measurement of oleic acid [one unit (U)=1 μmol of oleic acid released per min]. It should be noted that the specific activities of both DGL and DPL were very low under simulated duodenal conditions in comparison with the specific activities determined under optimal conditions with purified enzymes.[6,7] Similar results have previously been obtained with human lipases.[2]

Comparisons between in vivo and in vitro experiments using triolein as substrate

In the stomach, DGL is the only lipase present and the ee observed in vivo (Fig. 2A) were in good agreement with the in vitro data (Tables 1 and 2). A high 1,2-*sn*-diolein ee was also observed in vivo during the late phase of intraduodenal lipolysis (Fig. 2D). The possibility that gastric DG emptied into the duodenum may have contributed to this high ee can be ruled out due to the low level of hydrolysis occurring in the stomach (Fig. 2B). In the duodenum, the contribution of DGL to the late phase of duodenal lipolysis is probably higher than that of DPL, due both to the acidification of the duodenal contents (Fig. 2F) and to the higher amounts of DGL present in the intestine.[1]

The in vitro TG hydrolysis experiments (Tables 1 and 2), showing a *sn*-3 preference in the case of both lipases, cannot explain the low ee of 2,3-*sn*-diolein observed in vivo during the first 2 h of the intraduodenal phase of lipolysis (Fig. 2D). In the duodenum, however, the rate of TG hydrolysis was very high (60–80%, Fig. 2E) and the second step of lipolysis (i.e. the hydrolysis of DG into MG; Fig. 1), might therefore also contribute to the ee of DG. To clarify this point,

Table 3. In vitro enantioselective hydrolysis of 1,2-*rac*-diolein by DGL and DPL under simulated duodenal conditions

Enzyme	pH	*t* (min)	DG hydrolysis (%)	$ee_{2,3}$ (%)
DGL	6.0	60	1.3	0.18
		120	2.58	0.44
	4.0	30	15.7	3.17
		60	31.3	7.73
DPL + colipase	4.0	30	6.0	1.64
		60	12.0	3.46
	6.0	5	11.9	3.23
		10	23.9	7.28

The rate of DG hydrolysis is the percentage initial DG hydrolysed into MG; t is the incubation time (min). See Experimental for the amounts of lipases and substrate concentrations used.

the hydrolysis of racemic DG (1,2-*rac*-diolein) was also studied in vitro as described below.

In vitro enantioselective hydrolysis of 1,2-*rac*-diolein under simulated duodenal conditions

The hydrolysis of racemic diolein was investigated using the same simulated duodenal conditions as those used previously for TG hydrolysis (see Experimental). The rate of hydrolysis and the ee of DG were measured as a function of time, at two pH values (4.0 and 6.0). As shown in Table 3, the ee of 2,3-*sn*-diolein increased as a function of the hydrolysis level, with both DGL and DPL. The two digestive lipases both clearly show a comparable stereopreference for the *sn*-1 position in DG, which contrasts with the *sn*-3 stereopreference observed with triolein (Tables 1 and 2). A similar reversal of the stereopreference was previously observed using medium chain glycerides such as tricaprin and dicaprin.[8]

Variations in the diolein ee during the in vitro hydrolysis of triolein under simulated duodenal conditions

Based on the above findings, the ee of 1,2-*sn*-diolein, resulting from the initial hydrolysis of the *sn*-3 position in triolein, was expected to decrease in the course of hydrolysis. To check this prediction, we carried out an in vitro simulation of duodenal lipolysis, up to high hydrolysis levels (Fig. 3). The results of these experiments clearly show that a decrease in the DG ee occurred with increasing levels of hydrolysis in the case of both lipases. With DGL, the 1,2-*sn*-DG ee remained at a high level due to the predominance of the first step of lipolysis (Fig. 1), which is much more stereoselective than the second one. On the contrary, with DPL, the first step of lipolysis is not as stereoselective as in the case of DGL and therefore, at high levels of hydrolysis (above 50%), the DG ee is reversed (Fig. 3). The above in vitro experiments demonstrate that the combination of the DPL stereoselectivity for the *sn*-3 position in TG and its reverse stereoselectivity for the *sn*-1 position in DG is the reason for the low 2,3-*sn*-DG ee observed in vivo during the first 2 h hours of duodenal lipolysis (Fig. 2D).

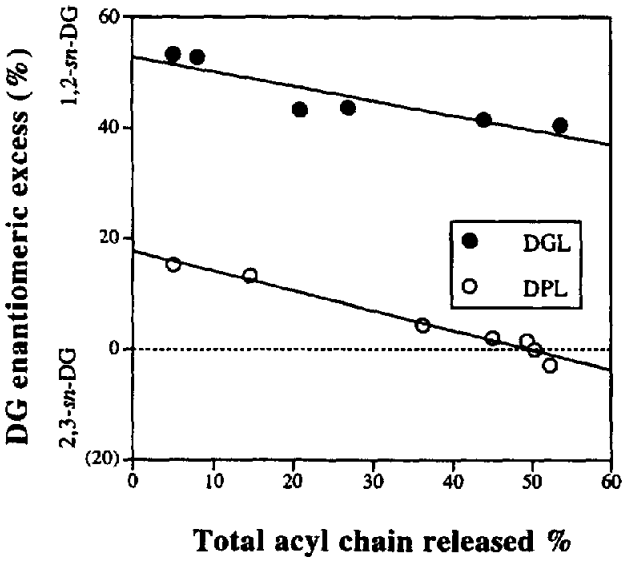

Figure 3. Variations in the diolein ee during the in vitro hydrolysis of triolein under simulated duodenal conditions. The rate of hydrolysis is expressed as the percentage total acyl chains (FA) released from the initial triolein substrate (TG_0), as measured by HPLC analysis. Hydrolysis % = 100.FA/3.TG_0.

Assay of BSSL towards oleoyl glycerides under simulated duodenal conditions

The BSSL, also named carboxylic ester lipase or cholesterol esterase, has been shown to hydrolyse triolein, diolein, and monoolein in vitro, and accordingly, BSSL might play a significant role in triglyceride hydrolysis in vivo. This enzyme could act in synergy with gastric and pancreatic lipases for the digestion of milk triglycerides.[10,11] However, the specific activity of purified BSSL towards glycerides is very low under optimized assay conditions as compared to DGL[6] and DPL.[7] To validate our experimental protocol on the respective roles of DGL and DPL, it was important to address the potential role of BSSL. The hydrolysis of triolein by BSSL was investigated under the same simulated duodenal conditions as those used previously for DGL and DPL. The lipolysis reactions were carried out at pH 6.5 so that a significant fraction (50%) of free oleic acid was ionized and directly titrated using the pH-stat technique (see Experimental). The results presented in Table 4 clearly show that BSSL is inactive towards triolein, whereas DPL used as a control displays a specific activity similar to that determined by HPLC analysis, for a pH value of 6.0 (Table 2). Using a mixture of oleoyl glycerides (triolein, diolein, and monoolein) as substrate instead of triolein, BSSL is also found to be inactive, whereas the specific activity of DPL is increased (Table 4).

Furthermore, an in vitro study on the substrate specificity of BSSL by Wang et al.[12] clearly showed the nearly simultaneous appearance of 1,2-*sn*-, 2,3-*sn*-, and 1,3-*sn*-diolein in the course of triolein hydrolysis by BSSL. These authors thus demonstrated that BSSL possesses neither positional specificity nor stereospecificity. From samples collected in vivo, we were

Table 4. Assay of BSSL under conditions simulating duodenal lipolysis

Substrate	BSSL specific activity (U/mg)	DPL specific activity (U/mg)
Triolein	0	34.4
Mixture of oleoyl glycerides	0	47.0

The liquid test meal constituents, including either triolein or a mixture of oleoyl glycerides (triolein, diolein, and monoolein) as substrate, were diluted with dog bile and water as described in the Experimental. The lipolytic activities of BSSL, or DPL as a control, were measured by titration of oleic acid at pH 6.5 using the pH-stat technique [one unit (U) = 1 μmol of oleic acid released per min]. The average specific activities (U per mg of enzyme) of BSSL and DPL were determined from lipolysis experiments carried out over a 15 min period, with a total hydrolysis level not exceeding 5%.

never able to detect the presence of 1,3-*sn*-diolein, which would have been the fingerprinting of BSSL activity.

Even though BSSL displays its highest specific activity towards triglycerides (preferentially, short-chain triglycerides), this enzyme also hydrolyzes a large variety of lipidic substrates and its real physiological role remains probably to be elucidated.

Conclusions

We have demonstrated that a high ee of 1,2-*sn*-DG was the fingerprint of a predominant contribution by gastric lipase to the hydrolysis of triglycerides. This fingerprinting was observed in vivo both during the intragastric phase of lipolysis and the late phase of intraduodenal lipolysis. This finding is in agreement with our previous conclusions that gastric lipase continues its activity in the duodenum.[1,2] From our results, one can imagine a sequential as well as a specific release of free fatty acids from dietary fat (i.e. milk triglycerides) upon the stereoselective action of gastric and pancreatic lipases.

Herein, we have demonstrated that by measuring the DG ee in the samples collected during digestion in the dog, it is possible to evaluate in vivo the respective contributions to lipolysis of gastric and pancreatic lipases. This method should be particularly valuable for precisely assessing the role of gastric lipase in pathological situations involving pancreatic deficiency in humans.

Experimental

Lipases

DGL (M_r 49000) was purified as previously described by Carrière et al.[6] DPL (M_r 50000) was purified as previously described by Cudrey et al.[7] Porcine colipase (M_r 10000) was purchased from Boehringer Mannheim, Germany. Pure human BSSL was a generous gift from Professor Lars Bläckberg (Umeå University, Sweden).

Test meal

In previous studies,[1,2] Shak Iso (Sopharga Laboratories, Puteaux, France) was chosen as a complete liquid test meal for in vivo experiments on humans and dogs. This meal contained 14 g protein, 52 g carbohydrate, and 12.5 g lipid as the major constituents. The lipids were mainly heterogeneous long-chain TG from butter, maize, and soybean oils. It appeared impossible to separate the 1,2- and 2,3-*sn*-DG generated upon lipolysis and to recover them from the gastric and duodenal content samples throughout the digestion period. In order to study the stereoselectivity of digestive lipases in vivo, we prepared a similar complete meal containing only a homogeneous prochiral TG (12.5 g triolein) as the lipid source. The other constituents were 14 g Alburone, 9.3 g saccharose, 9.3 g lactose, and 33.4 g maltodextrine. The final volume was adjusted to 500 mL and the pH was 6.6. The triolein concentration was 25 mM. A nonabsorbable marker (0.5 mg/mL Phenol Red) was added to the meal to monitor the gastric emptying, as previously described.[1]

Protocol for in vivo experiments

The experimental device (Fig. 4) for sampling the gastric and duodenal contents of dogs during the digestion of a liquid test meal has been previously described.[1] The in vivo lipolysis of meal TG was studied using a 3-year-old male beagle dog showing no symptoms of pancreatic disorder, equipped with chronic gastric and duodenal cannulae. After checking that no residual nutrients were present in the digestive

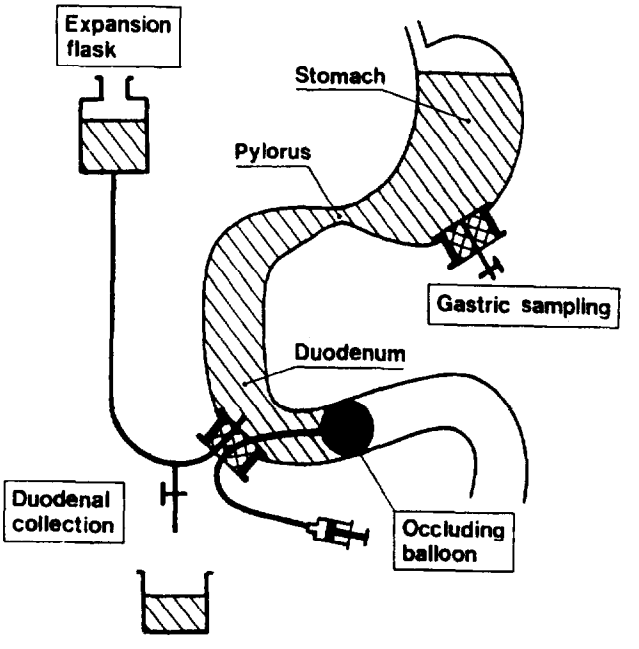

Figure 4. Experimental device for measuring the gastric and duodenal rates of lipolysis during the digestion of a liquid test meal in the dog. (See in vivo experiment design in the Experimental and ref 1).

tract, the dog was fed orally (2–3 min) and the subsequent digestion process was studied for 3 h.

Gastric samples (3 mL) were collected every 15 min from the tap connected to the gastric cannula (Fig. 4). In order to account for any phase separation and to ensure homogeneous gastric sampling, the gastric contents were gently mixed before each sampling by aspiration using a 50 mL syringe. The duodenal fluid was collected stepwise every 15 min and the duodenal tap was kept closed meanwhile. Recovery of the total duodenal fluid was achieved using the occluding balloon inflated at the ligament of Treitz (Fig. 4). In order to prevent any increase in the duodenal fluid pressure from affecting gastric emptying,[1] the duodenal cannula was connected to an expansion flask (Fig. 4).

In each gastric and duodenal sample, the volume, pH, and Phenol Red concentration were measured directly. One milliliter of each sample was immediately acidified to pH 1–2 with 1 N HCl in order to stop the lipolysis, and the lipids were extracted and further analyzed by HPLC.

From the total duodenal Phenol Red recovered and its concentration in the residual gastric contents, the variation with time in the volume of the gastric contents was calculated.[1] This information was used to calculate the variations with time in the amounts of TG and lipolysis products remaining in the stomach and their corresponding pyloric outputs.[2] The amounts of lipids not absorbed in the duodenum were directly determined by performing HPLC analysis on the duodenal samples.

In vitro lipolysis of pure triolein

The progress of triolein lipolysis with DGL and DPL (+colipase) was monitored with a pH-stat under the following conditions: 10 mL of 150 mM NaCl, 4 mm NATDC, 0.5 mL of triolein, and varying quantities of DGL (60–120 μg) or DPL (30–60 μg) were vigorously agitated in a pH-stat vial at a temperature of 37 °C. With DGL, the pH was 5.5 or 4.0. With DPL, the pH was 5.5 or 8.0, and colipase was added in excess to the vial before DPL. The mixtures were maintained at constant pH by titrating the released fatty acids with 0.5 M NAOH and the lipolysis was stopped before triolein hydrolysis had reached 5% by adding 10 mL of chloroform. The lipolysis products present in the organic phase were analysed.

In vitro simulation of duodenal lipolysis

From previous in vivo experiments on dogs,[1] we determined the duodenal concentrations of DGL and DPL and the mean TG duodenal concentration was estimated to be 25 mM. We obtained this TG concentration in vitro by solubilizing the triolein and the other meal constituents with a water:dog bile (95:5, v:v) mixture containing bile salts at a final concentration of 4 mM. The lipolytic activities of (a) pure DGL (15 μg/mL final concentration), (b) pure DPL+colipase (36 μg/mL+8 μg/mL final concentrations), and (c) a mixture of both lipases and colipase were tested at 37 °C and at three different pH values (4.0, 5.0, and 6.0) covering the duodenal pH range observed during the in vivo experiments. The lipolysis was stopped by acidification after variable intervals of time, depending on the level of hydrolysis required, and lipids were subsequently extracted and analysed by HPLC.

A similar experiment was performed using 1,2-*rac*-diolein (25 mM final concentration) as the substrate instead of triolein. All the other constituents of the experimental mixture remained unchanged. DGL and DPL (+colipase) lipolytic activities upon diolein were tested at pH 4.0 and 6.0.

Under simulated duodenal conditions, we also checked the activity of BSSL (50 μg/mL final concentration) towards either triolein, or a mixture of oleoylglycerides (monoolein from Fluka containing 16–18% triolein and 38–40% diolein). The release of oleic acid was monitored using a pH-stat (TTT80 Radiometer, Copenhagen). The experiments were carried out at pH 6.5, for which only 50% of the free long chain fatty acids are ionized and can be titrated by addition of sodium hydroxyde. The reaction volume was 15 mL and the substrate concentration was 25 mM.

Extraction and analysis of residual TG and lipolysis products

Neutral lipids were recovered from gastric and duodenal samples or after the lipolysis simulated in vitro, using a two-step extraction procedure with (a) n-hexane:n-butanol (3:2) and (b) n-hexane:isopropanol (3:2) mixtures. This procedure, which was previously described by Carrière et al.[6] makes it possible to specifically recover TG, DG, 2-MG, and free FA. They were separated by HPLC on a silica column (Beckman Ultrasphere 5 μ, 4.6×250 mm) using a linear gradient of tetrahydrofuran in heptane:acetic acid (99:1) ranging from 10 to 100% (v/v) in 15 min. The HPLC (Beckman, Gold system software package) was coupled to a light-scattering detector (Cunow DDL 11). The HPLC mass detection data were converted into moles using the respective molecular masses of triolein (884 Da), diolein (620 Da), monoolein (356 Da), and oleic acid (282 Da).

In order to determine the ee of 1,2(2,3)-*sn*-diolein, a fraction of the crude lipid extracts was lyophilyzed and further reacted with R-(+)-phenylethylisocyanate (20 μL/mg of lipids) in heptane (100 μL/mg of lipids) under stirring in a sealed vial at room temperature, for 48 h. The mixture of enantiomeric DG was thus derivatized to give two diastereomeric carbamates (1,2-dioleoyl-*sn*-glycero-3-[(*R*)-l-phenylethyl] carbamate and 2,3-dioleoyl-*sn*-glycero-1-[(*R*)-1-phenylethyl] carbamate), which were subsequently separated by HPLC on a silica column (Beckman Ultrasphere 5 μ, 10×250 mm) in heptane:ethyl alcohol (99.6:0.4) as described by Rogalska et al.[4] The enantiomeric excess percent

(ee%) was calculated by integrating the corresponding chromatographic peaks as follows:

$$ee_{1,2}\% = \frac{1,2\text{-DG} - 2,3\text{-DG}}{1,2\text{-DG} + 2,3\text{-DG}} \times 100\%$$

Chemicals

All solvents were purchased from SDS (Peypin, France) and were reagent or HPLC grade. Sodium taurodeoxycholate (NaTDC), triolein, and 1,2-*rac*-diolein were from Sigma (St Quentin Fallavier, France). Monoolein, Phenol Red, *R*-(+)-1-phenylethylisocyanate were from Fluka (Mulhouse, France). Lactose and saccharose were from Merck AG (Darmstadt, Germany). Alburone powder was from Sopharga Laboratories (Puteaux, France). Maltodextrins were from Jacquemaire Santé (Villefranche-sur Saône, France).

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