In Vitro Gastrointestinal Lipolysis: Replacement of Human Digestive Lipases by a Combination of Rabbit Gastric and Porcine Pancreatic Extracts

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Abstract Various combinations of digestive lipases were tested in vitro under conditions simulating the earlier phases of gastrointestinal lipolysis in the stomach and the duodenum. A solid/liquid test meal was mixed first with either human gastric juice or a solution containing gastric lipase, followed by either the addition of human pancreatic juice and bile or the addition of a solution containing pancreatic lipase, colipase, and bile salts. The rate of lipolysis and the composition of the lipolysis products were assessed as a function of time after lipid extraction and analysis by thin-layer chromatography coupled to flame ionization detection. The lipolytic potential of a crude rabbit gastric extract (RGE) associated with porcine pancreatic extract (PPE) was assessed and compared with the rates of lipolysis of the meal triacylglycerols by human digestive lipases recorded under the same in vitro conditions. RGE combined with PPE appeared to be a good substitute for human gastric and pancreatic lipases. RGE and PPE could therefore be used to simulate the gastrointestinal lipolysis of various foods and emulsions in vitro, as well as that of pharmaceutical lipid formulations.

Keywords Fat digestion · Fatty acids · Lipase · Lipid analysis · Lipolysis · Solid meal

Introduction

Human gastric and pancreatic lipases are the main enzymes involved in the digestion of dietary triglycerides. These lipases have by now been thoroughly biochemically characterized [7, 13, 38], and their secretion levels, their specific activities, and their respective contributions to the lipolytic processes occurring during a meal have been clearly established in healthy volunteers [6, 8, 10–12]. Based on these in vivo data, a two-step gastrointestinal in vitro digestion model has been developed for studying the lipolysis of test meals containing various fats [10] to predict the efficacy of the lipase inhibitor Orlistat [11, 12] and to study the effects of lipolysis on the solubility of hydrophobic drugs formulated with lipids [21].

It is difficult, however, to obtain native or recombinant human gastric lipase (HGL) for performing these experiments. A good alternative for human gastric lipase would be native rabbit gastric lipase (RGL) recovered from rabbit stomachs [31, 32]. The specific activities of RGL and HGL on various triglycerides are known to be in the same range [9, 23, 31, 32]. It addition, the rabbit stomach is known to be an abundant source of gastric lipase, which is resistant and active under acidic pH conditions, and crude rabbit gastric extracts (RGE) can be easily prepared [27, 31, 32].

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It is also difficult to obtain native or recombinant human pancreatic lipase (HPL) for performing experiments of this kind at a reasonable cost, although HPL can be produced in the form of a recombinant enzyme in insect cells [37] and yeast [3]. Porcine pancreatic lipase (PPL) and HPL are known, however, to have similar properties [38], and porcine pancreatic extracts (PPE) containing PPL and colipase are already being used in in vitro digestion studies [1, 2, 16, 20, 21, 24, 25, 36, 40].

The aim of this study was to compare the lipolytic potential of crude animal digestive lipases (RGE and PPE) with that of human gastric and pancreatic juices and that of purified human digestive lipases. For this purpose, the in vitro lipolysis of a complete solid/liquid test meal by these lipases was performed under conditions mimicking the physiological conditions observed in humans during the digestion of a test meal [10].

Materials and Methods

Chemicals

1,2-O-Dioleoyl-rac-glycerol (1,2-diolein) was purchased from Larodan Fine Chemicals (Malmö, Sweden). Glycerol trioleate (triolein); 1-monooleoyl-sn-glycerol (1-monoolein); oleic acid; anhydrous magnesium sulfate ($MgSO_4$); HCl; porcine pancreatic extracts (PPE), also named pancreatin (P7545; 8× USP); and bovine bile extract (B3883) were purchased from Sigma-Aldrich (St Quentin-Fallavier, France). All organic solvents were purchased from SDS (Peypin, France) and were of HPLC grade. O-Cholesteryl ethylene glycol (CholE1), the internal standard (IS) used for lipid extraction and thin-layer chromatography with flame ionization detection (TLC-FID) analysis [14], was synthesized by Dr. Dominique Lafont (Laboratoire de Chimie Organique II-Glycochimie, ICBMS UMR 5246, CNRS, Université Claude Bernard Lyon 1, Villeurbanne, France).

Human Digestive Juices and Bile

Digestive juice and bile samples were collected from healthy volunteers with no digestive pathologies at the Sainte-Marguerite Hospital (Marseille), under the supervision of Professor René Laugier. Gastric juice was aspirated during 60 min via a gastric tube, after stimulating the gastric secretion by injecting pentagastrin subcutaneously (6 μg/kg Peptavlon. I.C.I. Pharma, Gery, France). HGL activity was measured in all the samples using a tributyrin emulsion substrate [23], and selected samples were pooled to obtain an average value of 100 U/mL of HGL (equivalent 100 μg/mL of active HGL), which is the mean HGL concentration recorded in basal human gastric juice with pH ranging from 1 to 1.5 [11, 12,

26, 39]. Three-milliliter samples of gastric juice were then frozen and stored at −20 °C before use.

Human pancreatic juice was obtained by performing retrograde endoscopic catheterization on the main pancreatic duct. The pancreatic juice samples were immediately mixed with a solution of protease inhibitors, phenylmethane sulfonyl fluoride and benzamidine, each at a final concentration of 2 mM. The samples were then lyophilized and stored at −20 °C before use. The pool of bile samples contained 60 mM bile salts, and 5-mL samples were frozen at −20 °C and stored before use.

Preparation of the Human Pancreatic Juice/Bile Mixture

In each in vitro experiment with digestive juices, an 11-mL mixture of human pancreatic juice and bile was prepared as follows: 400 mg of lyophilized human pancreatic juice containing 1.7% (w/w) HPL (i.e., 6.75 mg HPL) was mixed with 2 mL of human bile and water to obtain a total volume of 11 mL.

Preparation of Solutions of Purified Human Lipases and Porcine Colipase

Native HGL and HPL were purified at our laboratory as described previously [17, 30]. Porcine colipase was purified from fresh porcine pancreas as previously described in [15]. The fraction obtained is mainly composed of the proform of colipase (99% [35]). Colipase can be obtained from the tryptic cleavage of the N-terminal pentapeptide of procolipase, but the respective effects of procolipase and colipase on pancreatic lipase activity can be hardly differentiated in vitro under the present assay conditions and in the absence of large amounts of phospholipids (unpublished data from CNRS-UPR9025 laboratory). To replace the gastric juice with pure HGL, a 100-μg/mL HGL solution was prepared in 150 mM NaCl. To replace the pancreatic juice with pure HPL + colipase, a solution containing 6.75 mg HPL, 1.35 mg porcine procolipase, and 2 mL human bile was prepared in 150 mM NaCl (11 mL final volume) prior to each experiment. HPL and colipase were mixed in a 1:1 molar ratio, which is that found in the human duodenal contents [5].

Preparation of a Solution of Rabbit Gastric Extracts for Replacing Gastric Juice

A crude RGE containing RGL was prepared as described by Moreau et al. [33]. To replace human gastric juice, a solution of RGE was prepared by mixing 21 mg of powdered RGE (containing 1.41% (w/w) of RGL) with 3 mL of 10 mM MES and 150 mM NaCl buffer, pH 6.0, in order to obtain a gastric lipase concentration of 100 μg/mL, which is that found to occur in basal human gastric juice.

Preparation of a Mixture of Porcine Pancreatic Extracts and Bovine Bile

PPE were found to contain 1.1% (*w/w*) of PPL. The bovine bile was reconstituted by dissolving 68 mg of bovine bile powder (Sigma ref. 3883) in 1.6 mL of 10 mM Tris and 150 mM NaCl, pH 6.0. The total bile salt concentration was 60 mM (see the individual bile salt proportions in Table 1). To replace the human pancreatic juice–bile mixture, 500 mg of PPE (5.5 mg PPL) and 1.6 mL bovine bile solution were mixed with 150 mM NaCl to a final volume of 9.1 mL. The pH of this solution was adjusted to 6.25. The bile salt and PPL concentrations in this solution were 10.5 mM and 604 μg/mL, respectively. The final PPL and bile salt concentrations in the incubation vessel were 250 μg/mL and 4.4 mM, respectively, during the simulation of the duodenal digestive phase.

Test Meal

The test meal was prepared by mixing 80 g string beans, 90 g grilled beef meat, 70 g French fries, 10 g butter, and 20 g sunflower oil. All these ingredients were purchased at local grocery stores (in Marseille, France). The butter and sunflower oil were used for cooking the beans and the fries, respectively. After all the ingredients had been cooked separately, they were mixed and passed through a mincer with a 2-mm grid in order to obtain a homogeneous paste. The paste was then divided in 5.8 g aliquots, which were stored at −20 °C before use. In each lipolysis assay, 5.8 g of

Table 1 Bile acid composition of bovine bile extracts and human duodenal contents

Bile acids (% mol/mol)		Bovine bile	Human duodenal contents
TUDCA	Tauroursodeoxycholic acid	nd	0.8
THDCA	Taurohyodeoxycholic acid	0.1	0.1
TCDCA	Taurochenodeoxycholic acid	2.1	14.6
TDCA	Taurodeoxycholic acid	9.0	7.8
THCA	Taurohyocholic acid	nd	nd
TCA	Taurocholic acid	42.5	19.1
GLCA-3S	Glycolithocholic acid 3-sulfate	nd	0.1
TLCA-3S	Taurolithocholic acid 3-sulfate	nd	0.1
UDCA-3S	Ursodeoxycholic acid 3-sulfate	nd	nd
CDCA-3S	Chenodeoxycholic acid 3-sulfate	nd	nd
DCA-3S	Deoxycholic acid 3-sulfate	nd	nd
LCA-3S	Lithocholic acid 3-sulfate	nd	nd
CA-3S	Cholic acid 3-sulfate	nd	nd
TUDCA-3S	Tauroursodeoxycholic acid 3-sulfate	nd	nd
GUDCA-3S	Glycoursodeoxycholic acid 3-sulfate	nd	nd
GLCA	Glycolithocholic acid	0.1	0.4
GUDCA	Glycoursodeoxycholic acid	0.1	2.3
GHDCA	Glycohyodeoxycholic acid	0.1	0.4
GCDCA	Glycochenodeoxycholic acid	2.8	26.2
GDCA	Glycodeoxycholic acid	7.9	12.6
GHCA	Glycohyocholic acid	nd	nd
GCA	Glycocholic acid	24.8	15.0
TLCA	Taurolithocholic acid	0.2	0.3
LCA	Lithocholic acid	nd	nd
UDCA	Ursodeoxycholic acid	nd	nd
HDCA	Hyodeoxycholic acid	nd	nd
CDCA	Chenodeoxycholic acid	0.5	nd
DCA	Deoxycholic acid	1.3	nd
MCA	Muricholic acid	nd	nd
HCA	Hyocholic acid	nd	nd
CA	Cholic acid	8.5	nd
Total		100.0	100.0

Bile acid analysis was performed using LC-MS. Bovine bile was purchased from Sigma-Aldrich (reference B3883). Human duodenal contents were collected from healthy volunteers in the framework of a previous clinical study [8]

this paste was first mixed with 9.2 mL water in order to reconstitute 15 mL of the test meal used in previous clinical studies [10–12].

In Vitro Gastrointestinal Lipolysis Experiments

Each experiment was performed in a 50-mL thermostated (37 °C) vessel equipped with a pH electrode and a 1-cm magnetic bar rotating at a speed of 1,000 rpm (Fig. 1). At time 0, the test meal (15 mL) was mixed with either 3 mL human gastric juice, 3-mL HGL solution, or 3-mL RGE solution. Whatever the mixture used, the gastric lipase concentration was 17 μg/mL. The pH was immediately adjusted to 5.5, and a 1-mL sample was collected for lipid extraction and analysis. In a blank experiment without any enzyme, the initial triacylglycerols (TAG) concentration was found to be 36.2±4.3 g/L, which is equivalent to 40.9± 4.9 mM. One-milliliter samples were collected at $t=15$ and $t=29$ min for immediate lipid extraction and analysis in the case of experiments with human digestive lipases. In the experiments with animal lipases, three other samples were collected at times $t=0$, $t=5$, and $t=10$ min. The pancreatic enzyme/bile mixture (11 mL of human juices/solutions or 9.1 mL of PPL/bile solution) was then added at $t=30$ min, and the pH was immediately adjusted to 6.25. The total volume at $t=30$ min was 27 mL in the human lipase experiments and 25.1 mL in the animal lipase experiments, and the pancreatic lipase and bile salt concentrations were 250 μg/mL and 4.4 mM, respectively. The meal + gastric juice dilution factor was 1.7, and the gastric lipase concentration was then decreased to 10 μg/mL. One-milliliter samples of the reaction mixture were collected at $t=35$, 40, 45, 60, and 90 min for immediate lipid extraction and analysis. The meal dilutions used to simulate the gastric and duodenal digestion steps were based on the in vivo data recorded in previous clinical studies [10]. Fifty-microliter

samples were also collected during the experiments for measuring the residual lipase activities (see "Lipase Activity Measurements" section).

Lipid Extraction

Lipid extraction was performed using the procedure of Folch et al. [22] as previously described [8], with some in-house changes [14]. One milliliter of the reaction mixture was mixed with 5 mL chloroform–methanol (2:1 v/v) containing 0.600 mg CholE1 (IS) and mixed vigorously in a 15-mL glass tube with a screw cap. After phase separation, the lower organic phase was collected using a Pasteur pipette and transferred to a 15-mL test tube, in which it was dried over anhydrous MgSO$_4$. The clear dried organic lipid extract (nearly 3.50 mL) was transferred to a 5-mL vial with a screw cap, and the vial was kept at −20 °C until the lipid analysis was performed.

TLC-FID Analysis of Lipolysis Products

TAG, diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA), and IS were separated on silica-coated quartz rods (SIII Chromarods) and quantified TLC-FID using a MK-5 Iatroscan apparatus (Iatron, Japan). The hydrogen flow rate was 160 mL·min^{-1}, the airflow rate was 2,000 mL·min^{-1}, and the scanning speed was 35 s per chromarod burned. Data acquisition and processing were performed using a compatible PC (processor 486) equipped with a ChromStar®*light* software program for Iatroscan (Bionis, France). Using a 2-μL Hamilton syringe, 1 μL of each lipid extract was spotted onto the chromarod, and the elution was performed with n-heptane/diethyl ether/formic acid (55:45:1 $v/v/v$) as the migration solvent. After 20 min, the chromarod holder (ten chromarods) was removed from the TLC tank, and the chromarods were dried at 150 °C for

Fig. 1 Scheme of the experimental device and lipolysis product analysis. The chromatogram shows the triacylglycerols (*TAG*), free fatty acids (*FFA*), diacylglycerols (*1,2(2,3)-DAG*), internal standard (*CholE1*), and monoacylglycerols (*MAG*) separated and analyzed by performing TLC-FID
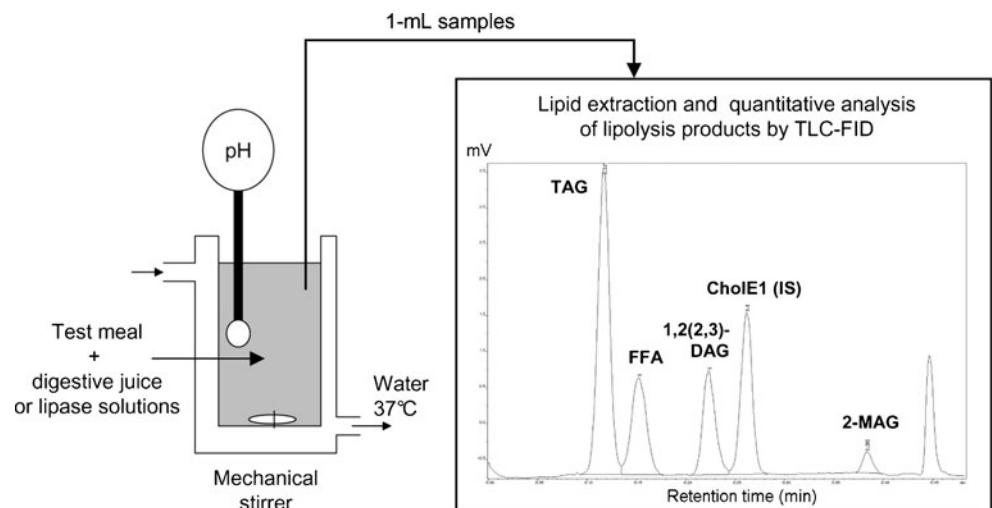
15 min. The chromarod holder was then placed in the Iatroscan MK5 apparatus, and each chromarod was scanned with the FID to detect and quantify the compounds separated on silica rods (Fig. 1). The ionization current generated by burning a compound in the hydrogen flame was taken to be proportional to the mass of this compound. Calibration curves (peak area vs. mass) were drawn up by spotting various amounts (0.1 to 10 μg) of triolein, oleic acid, diolein, monoolein, and IS onto the chromarods; 10 mg/mL stock solutions of triolein, 1,2-diolein, 1-monoolein, oleic acid, and IS in methanol were used to prepare standard and quality control samples.

The final results were expressed in milligrams per milliliter of each lipolysis product present in the initial aqueous phase, as well as in millimolars using the following molar masses estimated from the fatty acid composition of the test meal: 885.5 g/mol with TAG, 621.0 g/mol with DAG, 356.6 g/mol with MAG, and 282.5 g/mol with FFA. This method was validated as previously described [14].

Calculation of the Lipolysis Level

The chemically relevant expression of TAG lipolysis level (L1%) is percentage of the total meal TAG (TAG_0) acyl chains converted into FFA. It is given by the following equation, where TAG_t, DAG_t, MAG_t, and FFA_t are the amounts (in millimoles) of residual triglycerides and lipolysis products recovered at a given time t during the hydrolysis process:

$$L1\% = 100 \times FFA_t/(3 \times TAG_0)$$
$$= 100 \times (FFA_t)/(3 \times TAG_t + 2 \times DAG_t + MAG_t + FFA_t)$$

Since the complete absorption of TAG requires only their conversion into MAG, which corresponds to the release of two FFA from one TAG molecule and 66.6 L1%, it is also convenient to express a more physiologically relevant lipolysis level (L2%) corresponding to the percentage of the total meal TAG acyl chains converted into "intestinally absorbable" acyl chains, i.e., FFA and MAG, and defined by the following equation:

$$L2\% = 100 \times (MAG_t + FFA_t)/(3 \times TAG_0)$$
$$= 100 \times (MAG_t + FFA_t)/(3 \times TAG_t + 2 \times DAG_t + MAG_t + FFA_t)$$

Calculation of Glycerol Production

The amounts of glycerol (G; millimoles), produced during the lipolysis reaction, were not measured, but they were calculated from the levels of the other lipolysis products measured at a given time t, according to the following equation:

$$G_t = (FFA_t - DAG_t - 2 \times MAG_t)/3$$

Lipase Activity Measurements

To monitor the stability of lipases in the course of test meal lipolysis, the residual lipase activities were measured potentiometrically using a pH-stat (TTT 80 Radiometer, Copenhagen, Denmark), in a thermostated reaction vessel (37 °C) containing a mechanically stirred emulsion of 0.5 ml of tributyrin (Fluka, Paris, France) in 14.5 ml of the assay buffer. The specific assay conditions were those previously reported for measuring gastric lipase [6] and pancreatic lipase [19] activities, respectively.

Analysis of Bovine Bile and Human Duodenal Bile Acids by LC-MS

The bile acids present in bovine bile and human duodenal contents were analyzed as previously described [18]. The bile acids were separated chromatographically with an Agilent 1100 HPLC apparatus (Massy, France) equipped with a Restek C18 Pinnacle II (250×3.2 mm, 5 μm particle size) analytical column (Restek, Lisses, France). The HPLC was in series with the turbo ion spray source of a QTRAP 2000 mass spectrometer (Applied Biosystems-SCIEX, Concord, ON, Canada).

Results and Discussion

Design of the In Vitro Experiments

The experimental conditions used to simulate the in vitro gastrointestinal lipolysis of a meal were based on data obtained in previous in vivo studies [6, 10–12]. The pH values, lipase concentrations, and meal-to-digestive juice ratios used were those observed at 50% gastric emptying of a test meal in both the stomach and the lower duodenum.

A mixed solid–liquid test meal was first mixed with human gastric juice for 30 min and then with human pancreatic juice and bile for a further 60 min. Samples were collected at various times to measure the concentrations of the lipolysis products and to determine the lipolysis levels. Similar experiments were performed with purified HGL and HPL, as well as with solutions of RGE and PPE replacing the gastric and pancreatic juices, respectively. In the latter experiments, human bile was replaced by bovine bile. The bile acid composition of commercial bovine bile extract, determined by LC-MS analyses, was found to be

qualitatively similar to that of the human duodenal contents in terms of the main molecular species (Table 1), but the levels of hydrophobic bile acids in the tauro- and glyco-conjugated series were found to be lower in bovine bile. Since it was shown that the different conjugated bile acids have similar effects on lipase activity [4], these differences in bile acid composition are not expected to have a major impact on the lipolysis experiments.

In Vitro Lipolysis of the Test Meal by Lipases from Human Gastric and Pancreatic Juices

When the test meal was incubated with human gastric juice under conditions mimicking the fed conditions (pH 5.5), the lipolysis level reached a maximum value of $1.6\pm0.7\%$ (Table 2; Fig. 2) at the end of the gastric phase ($t=29$ min). When human pancreatic juice and bile were added (at $t=30$ min) to the test meal and gastric juice mixture, the level of lipolysis increased rapidly, reaching $52.9\pm2.1\%$ at $t=90$ min (cf. Table 2 and Fig. 2). By measuring the residual lipase activity on tributyrin, we checked that HGL and HPL remained stable and active (>90%) during these experiments, for at least 90 and 60 min, respectively.

In Vitro Lipolysis of the Test Meal by Purified HGL and HPL

When purified human digestive lipases were used, the gastric and duodenal lipolysis levels reached $2.8\pm0.4\%$ ($t=29$ min) and $45.5\pm0.7\%$ ($t=90$ min), respectively (see Table 2 and Fig. 2). These levels of lipolysis did not differ significantly from those obtained with human gastric and pancreatic juices, and HGL and HPL remained stable and active (>90%) during these experiments, for at least 90 and 60 min, respectively.

In Vitro Lipolysis of the Test Meal by RGE and PPE

When RGE and PPE were used, the levels of gastric and duodenal lipolysis reached $5.6\pm0.4\%$ ($t=29$ min) and $51\pm3.5\%$ ($t=90$ min), respectively (see Table 2 and Fig. 2). Gastric lipolysis of the test meal triacylglycerols by RGE was slightly higher than that measured with human enzymes, but the final level of duodenal lipolysis recorded at $t=90$ min was nearly identical to that measured with human digestive

juices. We checked that RGL and PPL remained stable and active (>90%) during these experiments, for at least 90 and 60 min, respectively. The specific activity of RGL on test meal TAG appears to be higher than that of HGL. This was not expected from the previous in vitro characterization of RGL and HGL using intralipid emulsions [23, 31, 32], but it has been shown later that the specific activities of digestive lipases measured under optimized conditions with fine emulsions are not predictive of the specific activities of the same lipases on meal TAG [10].

Evolution of Lipolysis Products and Residual TAGs Upon Test Meal Lipolysis

The changes in the lipolysis products and residual TAGs recorded upon incubating the test meal with either human gastric and pancreatic juices, or human purified enzymes (HGL + HPL + colipase), or RGE + PPE are presented in Fig. 3A–C, respectively. The relative proportions of molecular species containing glycerol (TAG, DAG, MAG, and calculated free glycerol) were expressed as percentages of the total number of glycerol moles (glycerol mole percent) and plotted as a function of the level of lipolysis level expressed as FFA% versus total fatty acids initially present in the TAGs (L1%). This method of presentation does not make it possible to distinguish the gastric and the duodenal phases of lipolysis, but it gives an overall picture of the regioselective hydrolysis of TAG that can be associated with a specific lipase or a set of lipases [28, 29, 34]. This representation is useful to predict the potential role of these lipases in TAG hydrolysis. The amounts of TAG, DAG, and MAG have been determined by performing lipid extraction and TLC-FID analysis, whereas the amounts of free glycerol were calculated by analyzing the other molecular species present, including the free fatty acids. These analyses and calculations consistently showed that the meal TAGs were sequentially transformed into DAGs, MAGs, and glycerol and that the three TAG ester bonds were therefore hydrolyzed by the combination of enzymes present during the reaction. The TAG levels decreased continuously as a function of the rate of hydrolysis, while DAGs and MAGs accumulated transiently and the glycerol levels increased (Fig. 3). The maximum DAG and MAG levels were reached consecutively, as expected in view of the sequential pattern of hydrolysis of

Table 2 Lipolysis levels of test meal triglycerides after the gastric and the duodenal steps in the in vitro process of gastrointestinal lipolysis by various combinations of enzymes

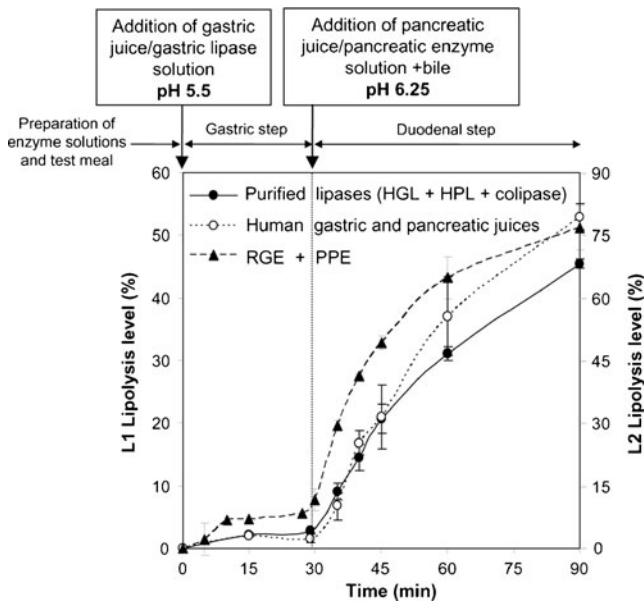| Source of lipases	Gastric lipolysis% ($t=29$ min)	Duodenal lipolysis% ($t=90$ min)
Human gastric and pancreatic juices	1.6 ± 0.7	52.9 ± 2.1
HGL + HPL + colipase	2.8 ± 0.4	45.5 ± 0.7
RGE + PPE	5.6 ± 0.4	51.2 ± 3.5

Fig. 2 Changes with time in the lipolysis level of a solid/liquid test meal using a two-step in vitro lipolysis model. *Filled circle* consecutive addition of purified human enzymes (HGL + HPL + colipase); *open circle* consecutive addition of human gastric and pancreatic juices; *filled triangle* consecutive addition of crude animal enzymes (RGE + PPE). Values are means ± SD (*n*=3). See "Materials and Methods" section for the definitions of L1 and L2 lipolysis levels

TAGs. With all the combinations of enzymes tested, the maximum DAG and MAG levels were observed at similar amounts of lipolysis (30–40% with DAGs and 60–70% with MAGs, respectively), but these levels differed from one combination of enzyme to another. The process of DAG

accumulation reached maximum values of 21–22 glycerol mol % with human gastric and pancreatic juices (Fig. 3A), 46–47 glycerol mol% with HGL and HPL (Fig. 3B) and 32–33 glycerol mol% with RGE and PPE (Fig. 3C), respectively. In the case of MAGs, the maximum levels reached values of almost 50 glycerol mol% with human gastric and pancreatic juices (Fig. 3A), 30 glycerol mol% with HGL and HPL (Fig. 3B), and 30 glycerol mol% with RGE and PPE (Fig. 3C). It is worth noting that DAG reached lower levels and MAG higher levels in the case of human digestive juices than with the combinations of HGL and HPL, and RGE and PPE. This finding suggests that the rate of lipolysis of DAG was faster than that of MAG with human digestive juices, whereas the opposite was observed with purified human lipases and no difference was observed between DAG and MAG in this respect with RGE and PPE.

Conclusion

All in all, the results of these experiments show that all the combinations of enzymes tested here are able to generate the same lipolysis products (Fig. 3) and that the lipolysis levels reached during the duodenal step are generally similar (Fig. 2; Table 2). The level of gastric lipolysis reached with RGE is 3.5-fold higher than that measured with human gastric juice and 2-fold higher than that measured with purified HGL (Table 2). However, the range of gastric lipolysis levels recorded always corresponded to the levels of intragastric lipolysis recorded in vivo [6, 8, 10–12]. A combination of RGE and PPE can therefore be used to testing the lipolysis of various fats and emulsions in vitro.
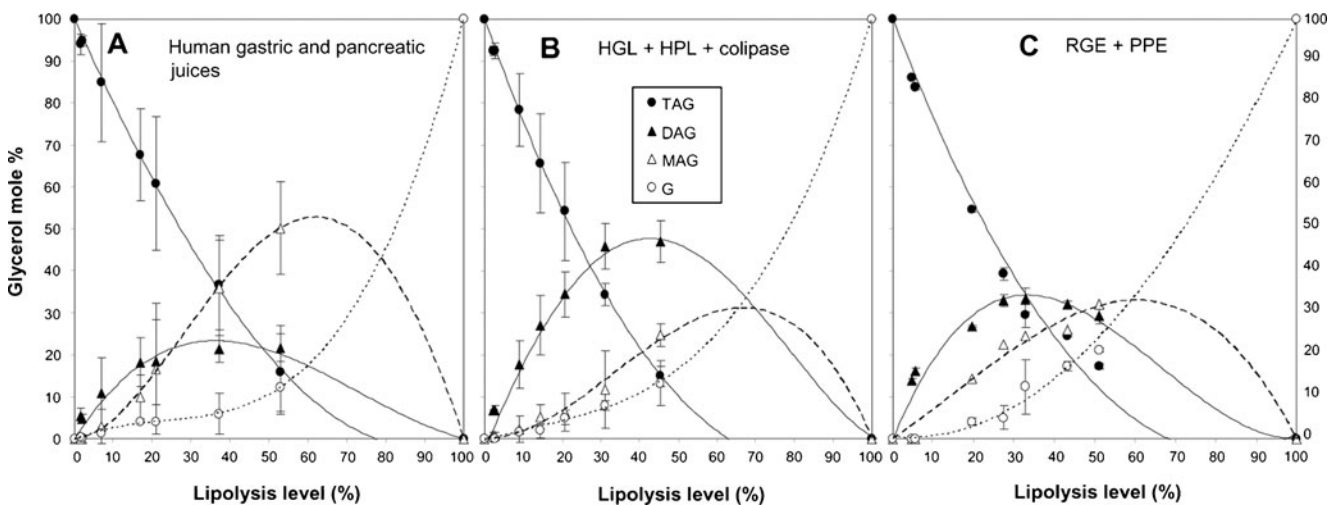

Fig. 3 Changes in the residual TAG and lipolysis products with the lipolysis level. **A** Purified human enzymes (HGL + HPL + colipase); **B** human gastric and pancreatic juices; **C** crude animal enzymes (RGE + PPE). Values are means ± SD (*n*=3). The *curves* describing these changes and their extrapolations above 50% lipolysis level were obtained by polynomial regression and drawn to show the general trend of what would happen at later times/higher lipolysis levels

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