

## Pancreatic enzyme secretion in response to test meals differing in the quality of dietary fat (olive and sunflowerseed oils) in human subjects

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The aim of the present study was to investigate in human subjects whether or not the ingestion of two liquid meals that differed only in their fatty acid composition (due to the addition of olive oil (group O) or sunflowerseed oil (group S) as the source of dietary fat) would lead to differences in the pancreatic enzyme activities secreted into the duodenum. The experiments were performed in eighteen cholecystectomized subjects who, during the 30 d period immediately before surgery, modified their habitual diets in such a way that their fat composition would reflect, as far as possible, that of the experimental meals. Lipase (EC 3.1.1.3), colipase, amylase (EC 3.2.1.1), chymotrypsin (EC 3.4.21.1) and trypsin (EC 3.4.21.4) activities were measured in duodenal contents aspirated before and after the ingestion of the test meals. The plasma levels of secretin and cholecystokinin (CCK) were also examined. Duodenal enzyme activities were similar in resting conditions. No significant differences were revealed in postprandial enzyme activities, except for lipase activity, which was higher in group O, probably in relation to the greater plasma CCK concentrations observed in this group. In the absence of enzyme output data, we should not exclude the possibility that the type of dietary fat will affect human pancreatic enzyme secretion to a greater extent than is evident from the present study, for instance through a flow-mediated effect, as we previously observed in dogs.

### Dietary fat: Exocrine pancreatic secretion

Many authors have investigated in animals the adaptation of the exocrine pancreas to the type of food available. The results of these studies demonstrate clearly that tissue proteolytic, amylolytic and lipolytic enzyme contents change in proportion to the amount of their respective nutritional substrates (Deschodt-Lanckman *et al.* 1971; Snook, 1971; Sabb *et al.* 1986; Wicker & Puigserver, 1987; Hara *et al.* 1995).

Concerning dietary fat, in contrast to the consensus of the studies about its amount in the diet, considerable controversy exists over the effects of the type of this nutrient, i.e. degree of saturation or major chain-length, on adaptation of pancreatic enzymes, especially lipase (EC 3.1.1.3) (Deschodt-Lanckman *et al.* 1971; Saraux *et al.* 1982; Sabb *et al.* 1986; Simoes-Nunes, 1986; Ricketts & Brannon, 1994). On the other hand, there have been very few studies to examine the effect of diets of different composition on the human exocrine pancreas, and those available have focused on the influence of quantity (not quality) of the three major nutritional components in the diet (Emde *et al.* 1985; Boivin *et al.* 1990).

We previously investigated (Ballesta *et al.* 1990), in conscious dogs, the long-term adaptation of exocrine pancreatic secretion to two diets that differed only in the type of fat included (olive or sunflowerseed oil). After comparing the enzyme composition of pancreatic juice in the basal period and in response to food, some differences became apparent between the experimental groups.

The aim of the present study was to investigate in human subjects whether or not the ingestion of two liquid meals (with a defined composition, isoenergetic and isonitrogenous) that differed only in their fatty acid profile (due to the addition of olive or sunflowerseed oil as the only source of dietary fat) would lead to differences in the pancreatic enzyme activities secreted into the duodenum. In addition, during the 30 d before the experiments, the subjects from both groups were asked to modify their habitual diets in such a way that their fatty acid composition would reflect, as far as possible, that of the experimental diets.

## MATERIALS AND METHODS

### *Subjects*

Patients with gallstones in the gall-bladder, showing current clinical signs or symptoms and awaiting surgery (cholecystectomy), were studied. The exclusion criteria were: (a) asymptomatic cholelithiasis cases; (b) presence of gallstones in the bile ducts (choledocolithiasis); (c) history of systemic (atherosclerosis, diabetes), pancreatic (acute or chronic pancreatitis) or gastrointestinal disease of any other aetiology (gastric or duodenal ulcer); (d) chronic consumption of drugs, especially antacids or histamine H<sub>2</sub>-receptor antagonists. These subjects were chosen because of the large number available, the possibility of strictly controlling the participants according to experimental protocol, and, finally, the applicability of this ailment to our research. The experimental protocol was approved by the local ethical committee, and all subjects gave written consent after being fully informed of the nature and procedures of the study. The patients were divided into two experimental groups, the olive oil group (group O) and the sunflowerseed oil group (group S), according to their dietetic habits, particularly the type of dietary fat habitually consumed before the study (information gained from a dietary history interview at the beginning of the study). Each group contained nine patients, with a mean age of 54.4 (SE 4.04) and 41.7 (SE 3.85) years for group O and group S respectively.

### *Experimental protocol and diets*

During the 30 d period immediately before surgery, the subjects from both groups were asked to consume their habitual diets (at home), except for the two following points: (a) the only source of dietary fat used to prepare their meals had to be olive oil (group O) or sunflowerseed oil (group S); (b) they had to avoid eating food items high in saturated fat (butter, all types of sausage, etc.). Four 7 d dietary records were completed to establish the energy intake and the composition of the diets, the subjects recording all foods and beverages ingested each day. Careful instruction was given not only regarding the methods for recording amounts of food and drinks but also regarding the need to record all additions to foods as well as cooking methods. These records were collected at every visit to the hospital, the data quantified and the energy and nutrient intakes evaluated by the computer program *Nutrition and Health* (Asde Alimentacion, Valencia, Spain), which we developed in the Institute of Nutrition of the University of Granada. The database used was *Spanish Food Composition Tables*, which we published (Mataix *et al.* 1995). The results were validated by using the package *Nutritionist IV* (First Data Bank, San Bruno, CA, USA),

Table 1. Calculated daily energy and nutrient intakes during the 30 d period before surgery for groups of subjects consuming sunflowerseed oil (group S) or olive oil (group O)

(Values are means and standard deviations for four 7 d dietary records per subject and nine subjects per group)

	Group S		Group O	
	Mean	SD	Mean	SD
Energy (kJ)	6209.4	1245.2	6786.2	640.8
Protein (% energy)	18.2	2.1	18.6	1.7
Carbohydrate (% energy)	38.8	3.9	39.4	2.5
Fat (% energy)	42.6	3.3	41.6	2.5
Monounsaturated fat (g)	26.2	2.9	40.1	2.6
Polyunsaturated fat (g)	19.8	2.5	8.3	0.8
Saturated fat (g)	18.8	3.2	20.6	3.8

after entering the composition of the necessary food items into the database, also taken from the aforementioned food composition tables. As shown in Table 1, the two dietary groups differed primarily in relation to polyunsaturated- and monounsaturated-fat intake, whereas the composition of the remaining part of the adaptation diet was similar. Thus, although this preparation period cannot be strictly considered as an adaptation period to the diet, as in the animal studies, these previous diets do reflect, to a great extent, the fat composition of the meals tested during the experimental period, which was our intention.

The experiments were performed 48 h after surgery, once it had been confirmed that the subjects had recovered normality of digestive function and could take the meals. These (pH 6.33; 294 mOsmol/l) contained 4.18 MJ/l, and were composed of 17 % energy as protein, 30 % as fat, and 53 % as carbohydrates, together with vitamins and minerals. They were prepared by adequately mixing the separate components according to protein (lactalbumin), carbohydrate (maltodextrins) and vitamin–mineral mixture modules (Eda modular, Iby Nutrition, Madrid, Spain). Olive oil was added to the meal given to group O and sunflowerseed oil to the meal given to group S. The fatty acid composition of the liquid meals was determined. After direct transesterification according to the method of Lepage & Roy (1986), methylated esters were analysed by GLC using a Hewlett Packard chromatograph (Model 3396, Hewlett Packard, Palo Alto, CA, USA) equipped with an automatic injector (Hewlett Packard, Model 7673) and a 60 m silica column (i.e. 0.32 mm; particle size 0.20 µm; SP<sup>TM</sup>-2330, Supelco, Inc., Bellefonte, PA, USA). The two liquid meals were isoenergetic and isonitrogenous, thus differing only in their fatty acid composition (Table 2).

Each subject was studied on two consecutive days and after at least 8 h fasting. The participants were intubated with a radio-opaque two-lumen nasoduodenal tube, enabling separate aspiration of gastric and duodenal contents. The first aspiration site of the tube was situated in the fundus. The duodenal contents were collected at the distal aspiration site, placed in the third or fourth duodenal segment. Adequate positioning of the tube was checked frequently during the investigation by radiological control.

Peripheral-vein blood and duodenal samples were taken before, and at 30, 60, 120 and 180 min after beginning the slow ingestion of the liquid test meal (200 ml ingested over 30 min). The complete feeding and sampling procedure was repeated on the second experimental day. Blood samples were collected in heparinized tubes containing aprotinin (Sigma Chemicals, St Louis, MO, USA) to obtain a concentration of 360 000 kallikrein inactivator units/l blood. The tubes were placed immediately on ice and, at the end of each experiment, plasma was separated by a refrigerated centrifugation and stored as portions at

Table 2. *Fatty acid composition of the liquid test meals (g/100 g total fatty acids)*  
(Mean values with their standard errors for six determinations)

	Group S		Group O	
	Mean	SE	Mean	SE
Oleic (18:1 <i>n</i> -9)	29.03*	0.66	61.89	2.00
Linoleic (18:2 <i>n</i> -6)	42.16*	1.70	5.06	0.12
Monounsaturated	29.67*	0.66	63.08	1.95
Polyunsaturated	44.62*	1.55	8.19	0.14
Saturated	25.93	1.93	29.03	1.86
U:S	2.99	0.30	2.51	0.23

U:S, unsaturated fat:saturated fat ratio.

\*Mean values were significantly different from those for group O,  $P < 0.05$ .

–80° until radioimmunoassay of secretin and cholecystokinin (CCK). Duodenal samples (2 ml) were slowly and manually collected by aspiration with an adequately adapted syringe, avoiding the entry of air. The samples were split into portions, and stored at –80° until enzymic assays.

#### *Analytical methods*

**Secretin assay.** Plasma levels of secretin were measured by a double-antibody radioimmunoassay, using a Daiichi secretin immunoassay kit (Tokyo, Japan). The secretin antibody had been raised in rabbits against synthetic porcine secretin and showed a cross-reactivity lower than 0.005 % with other gastrointestinal hormones, such as vasoactive intestinal peptide (VIP), glucagon, motilin and gastrin. Synthetic porcine secretin was used as standard. The minimum plasma secretin concentration detected by this assay was 16 pmol/l. The intra-assay and inter-assay CV were 2.9 and 8.1 % respectively.

**Cholecystokinin assay.** A commercially available radioimmunoassay kit (Peninsula Laboratories GmbH, Belmont, CA, USA) with 80 % specificity for the sulfated CCK(26-33) octapeptide was used to determine the plasma concentration of this hormone by a double-antibody procedure. CCK(26-33) was used as standard. The sensitivity of the assay was 10.0 pmol/l, and the CV within and between assays were 4.3 and 5.6 % respectively. The antiserum, generated in rabbits against synthetic CCK(26-33) octapeptide showed a cross-reaction equal to 63 %, 14 % and 100 % with CCK(27-33), CCK(30-33) and porcine CCK-33 respectively, and did not cross-react (<0.1 %) with VIP or human pancreatic polypeptide (PP). The cross-reactivity with gastrin, assessed by Gomez Cerezo *et al.* (1991) was 4.2 %.

**Duodenal contents.** Lipase and colipase activities were determined by a titrimetric method (Erlanson-Albertsson *et al.* 1987) at pH 7.50, using tributyrin as the substrate. Briefly, when lipase activity was assayed, 100 µl samples (previously diluted with assay buffer 1:10, v/v) were added to 15 ml buffer (2 mM-Tris maleate, 150 mM-NaCl, 1 mM-CaCl<sub>2</sub>, 4 mM-sodium taurodeoxycholate) plus 0.5 ml tributyrin, and 10 µg pure colipase (from porcine pancreas, purchased from Sigma Chemicals, catalogue no. C3028). In the colipase assay, the same volumes of substrate and buffer were used. In this case, however, 40 U pure lipase (from porcine pancreas, purchased from Sigma Chemicals, catalogue no. L0382) was added simultaneously (to overcome a previous lipolysis phenomenon due to the lipase preparation *per se*) with the sample (100 µl diluted with buffer 1:10, v/v). Lipase

and colipase activities were expressed as units ( $\mu\text{mol}$  fatty acid liberated per min at  $27^\circ$ ). Amylase (EC 3.2.1.1) activity was measured by hydrolysis of starch substrate and determination of the amount of maltose released, in accordance with the technique described by Hickson (1970). The results were expressed in units of activity as defined by Hickson (1970). Chymotryptic (EC 3.4.21.1) and tryptic (EC 3.4.21.4) activities were estimated by a modification of the method of Reboud *et al.* (1962), since preliminary experiments showed that activation was not necessary in our duodenal samples. For both enzymes, the activity was measured by titration at  $27^\circ$  and pH 7.90 (Titrator CRISSON, model micro TT 2050, Barcelona, Spain). Acetyl-L-tyrosine ethyl ester and N-benzoyl-L-arginine ethyl ester were used as the substrates in chymotrypsin and trypsin assays respectively. Activities are reported as units, i.e.  $\mu\text{mol}$  substrate hydrolysed per min at  $27^\circ$ . Finally, all enzymic activities were related to 1 ml duodenal sample.

### *Statistical evaluation*

Two studies were carried out on consecutive days for each patient, and the overall mean of the studies was used to calculate the group mean (nine patients per group) and the standard error of the mean. For statistical comparisons within the groups (changes from baseline), repeated-measures ANOVA was made (ANOVA Procedure, SPSS/PC version 6.1, Chicago, IL, USA). The differences between the two dietary groups at the same points in time, as well as between the two liquid meals (fatty acid composition) were tested for significance by Students' *t* test (T-Test Groups Procedure, SPSS/PC version 6.1). A *P* value  $< 0.05$  was considered statistically significant.

## RESULTS

### *Resting conditions*

Plasma secretin and CCK concentrations were similar in both experimental groups (Fig. 1). In the case of colipase and all the enzymes tested, there were no significant differences between the two dietary groups in relation to their duodenal activities (Figs. 2, 3 and 4).

### *Response to food*

The presence of food in the digestive tract did not result in significant changes in plasma CCK concentration in either group (Fig. 1), although a rather pronounced increase was observed in group O 30 min after starting the meal. Nevertheless, plasma CCK levels in group O remained significantly ( $P < 0.05$ ) higher than those in group S throughout the 30–120 min postprandial period.

Plasma secretin levels hardly changed in group O or group S for the duration of the experiment. No significant differences were revealed between the two groups in secretin concentration during the postprandial period (Fig. 1).

The presence of food in the digestive tract induced, in the two groups, an increase in the colipase activity of the duodenal contents, peaking 30 min after starting the meal and remaining significantly elevated until the first hour. During the last postprandial hours, colipase activity returned to values close to the resting ones (Fig. 2). Lipase activity followed a very similar pattern to that of colipase, although an acute decrease was observed in group S after the first 30 min, and values remained low until the end of the study. As a

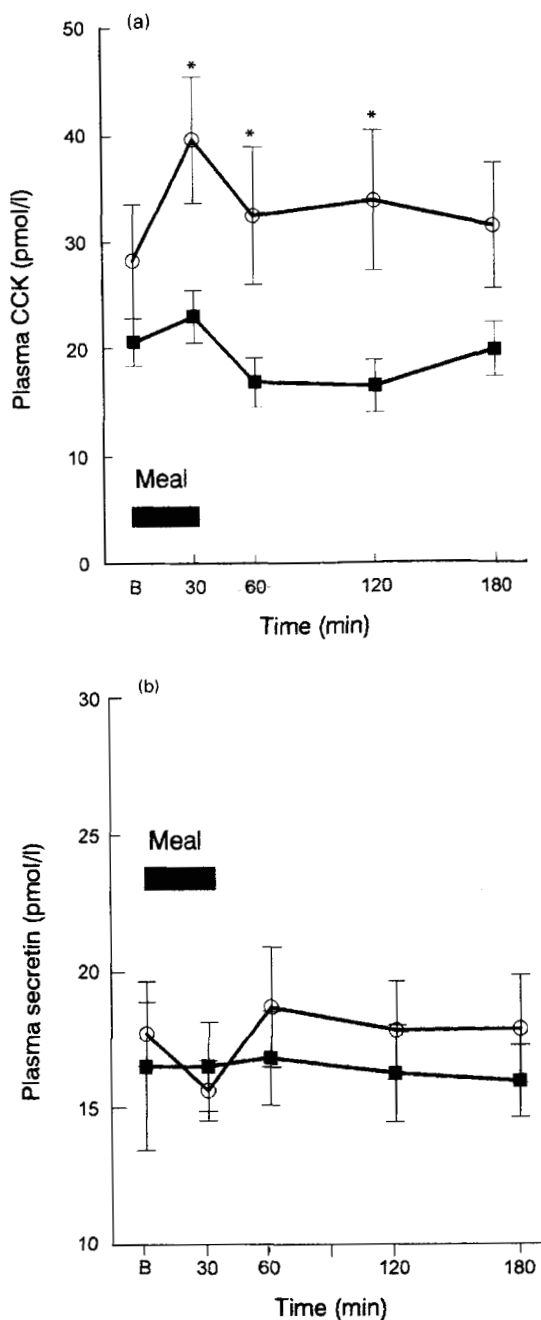


Fig. 1. Time-course of (a) plasma cholecystokinin (CCK) and (b) plasma secretin concentrations before (B) and after administering oleic or linoleic acid-enriched liquid meals to two groups of subjects who had been consuming olive (○) or sunflowerseed oil-enriched diets (■) during the 30 d period before the experiments. The meal was ingested within 30 min (dark bar). Values are means with their standard errors represented by vertical bars for two experiments per subject and nine subjects per group. \* Mean values for the two dietary groups were significantly different ( $P < 0.05$ ) at the same points in time.

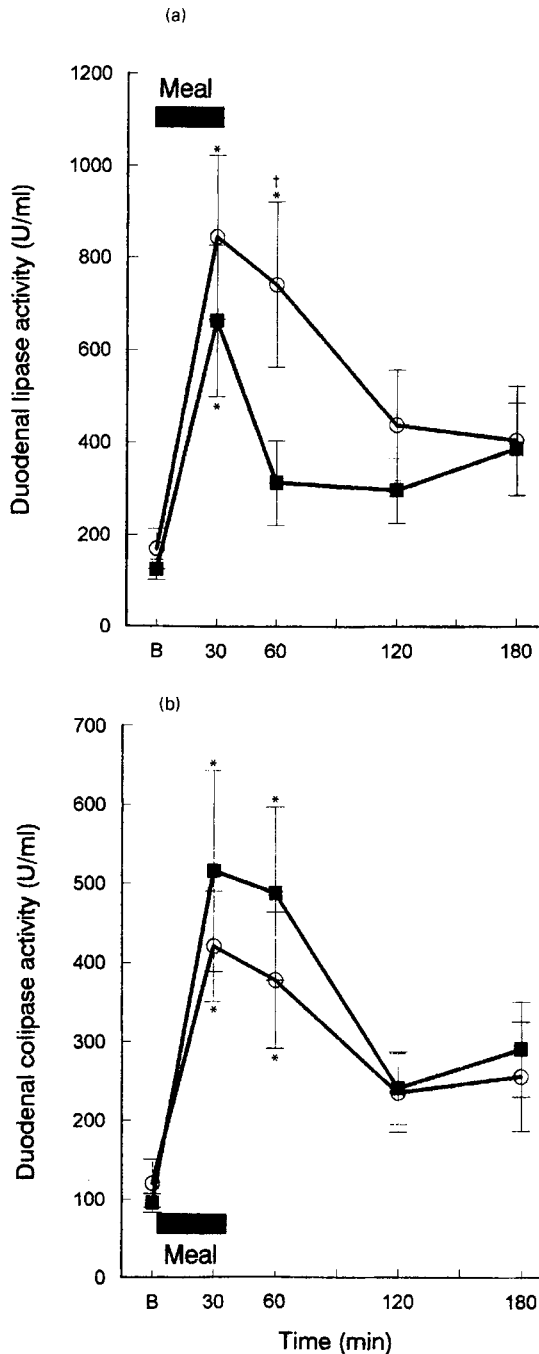


Fig. 2. Time-course of (a) duodenal lipase and (b) duodenal colipase activities before (B) and after administering oleic or linoleic acid-enriched liquid meals to two groups of subjects who had been consuming olive (○) or sunflowerseed oil-enriched diets (■) during the 30 d period before the experiments. The meal was ingested within 30 min (dark bar). Values are means, with their standard errors represented by vertical bars for two experiments per subject and nine subjects per group. \* Mean values for each dietary group were significantly different from the resting ones,  $P < 0.05$ . † Mean values for the two dietary groups were significantly different at the same points in time,  $P < 0.05$ .



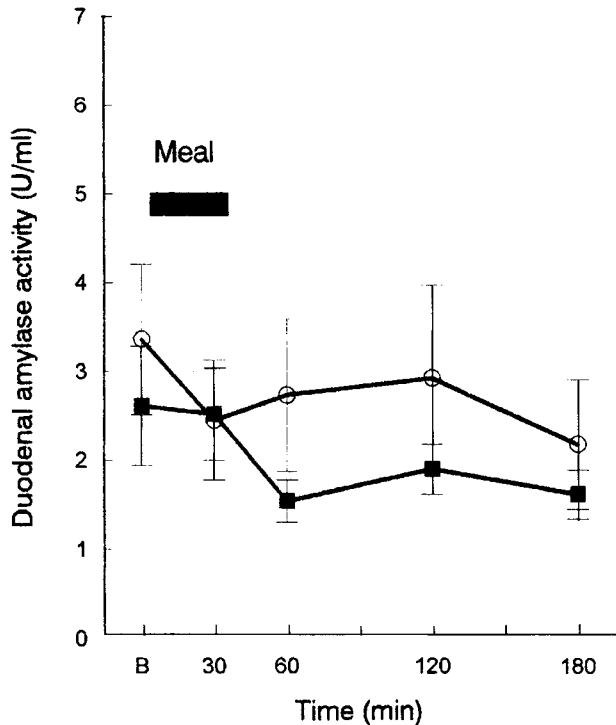


Fig. 3. Time-course of duodenal amylase activity before (B) and after administering oleic or linoleic acid-enriched liquid meals to two groups of subjects who had been consuming olive (○) or sunflowerseed oil-enriched diets (■) during the 30 d period before the experiments. The meal was ingested within 30 min (dark bar). Values are means, with their standard errors represented by vertical bars for two experiments per subject and nine subjects per group.

consequence, duodenal lipase activity was significantly higher in group O than in group S at 60 min after ingestion of the test-meal (Fig. 2).

As shown in Fig. 3, duodenal amylase activity showed no significant change in response to food in either of the groups, although modest decreases were observed 30 and 60 min after the beginning of the meal in groups O and S respectively, thereafter followed in both cases by a plateau. No significant difference between the two groups was found in postprandial amylase activity (Fig. 3).

Chymotrypsin activity rose in both groups in response to liquid food ingestion, the changes being significant at 30 and 60 min postprandially for group S, whereas in group O, significance was only achieved after 30 min. Nevertheless, this variable did not differ significantly when the two dietary groups were compared (Fig. 4).

A rapid increase in duodenal trypsin activity occurred in group O and group S after the administration of the liquid test meal (Fig. 4). The increase was statistically significant within 30 min after the ingestion was begun, remaining significantly elevated until the end of the study period. At any point in time during the postprandial period, trypsin activity achieved very similar values in both experimental groups (Fig. 4).

#### DISCUSSION

Plasma secretin levels were very similar in group O and group S, and no significant differences became apparent between them during rest or in response to food ingestion



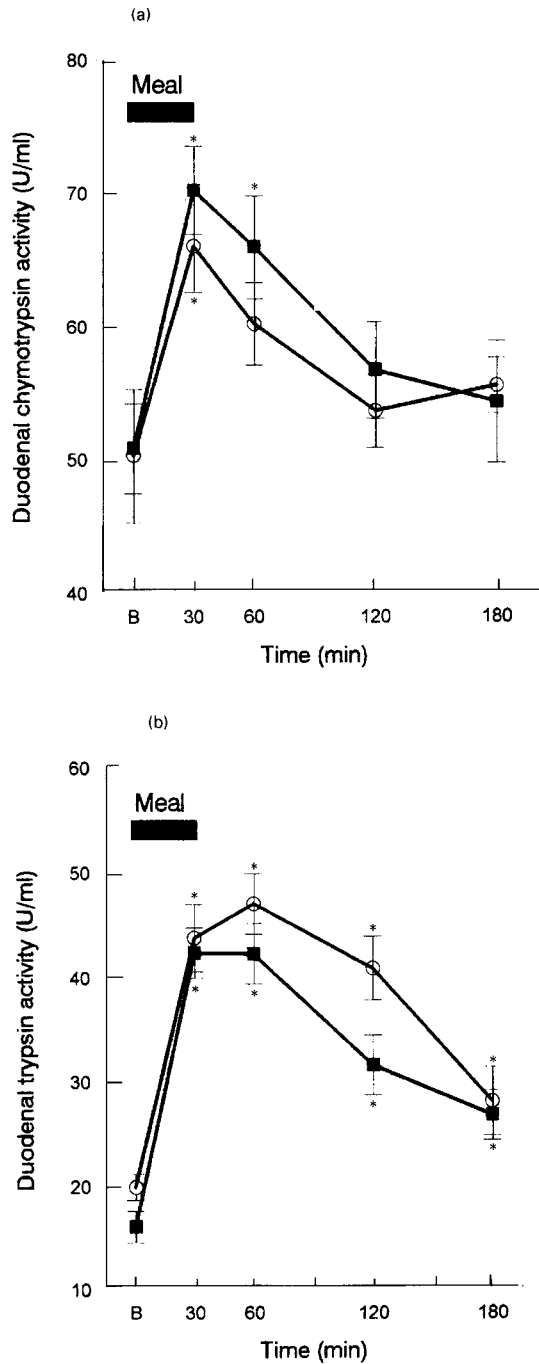


Fig. 4. Time-course of (a) duodenal chymotrypsin and (b) duodenal trypsin activities before (B) and after administering oleic or linoleic acid-enriched liquid meals to two groups of subjects who had been consuming olive (○) or sunflowerseed oil-enriched diets (■) during the 30 d period before the experiments. The meal was ingested within 30 min (dark bar). Values are means, with their standard errors represented by vertical bars for two experiments per subject and nine subjects per group. \* Mean values for each dietary group were significantly different from the resting ones,  $P < 0.05$ .

(Fig. 1). The significance of these results lies in the following points: (a) although a potentiation effect between secretin and CCK has been described (You *et al.* 1983), secretin is known to have the most important regulatory role in the secretion of pancreatic fluid (Singer, 1987); (b) CCK affects pancreatobiliary secretion mainly through the modulation of gall-bladder contraction and pancreatic enzyme-secretory response (Williams & Blevins, 1993); (c) in our experimental conditions, i.e., subjects lacking the gall-bladder, secretin should be considered the major mediator in the biliary output of water (Chey & Chang, 1989). Therefore, although we do not pretend that measuring plasma secretin can be an adequate surrogate for pancreatic flow, our results suggest that the flow might be similar in both experimental groups, so information concerning luminal activities (U/ml duodenal content) may be useful.

#### *Resting conditions*

Mean values of lipase, colipase, amylase, chymotrypsin and trypsin activity were similar in group O and group S (Figs. 2, 3 and 4). These results are only partially consistent with previous data obtained by us in dogs adapted for 8 months to diets containing the same two dietary fats, i.e. olive and sunflowerseed oils (Ballesta *et al.* 1990). In that study, on the basis of very similar resting pancreatic flows, we found that amylase activity in pancreatic juice was higher in the group of animals fed on the sunflowerseed-oil diet, whereas no significant differences were observed in lipase and chymotrypsin activities.

#### *Response to food*

The enzyme activities in duodenal contents after the ingestion of the liquid test meals showed similar trends in group O and group S (Figs. 2, 3 and 4).

Considerable controversy exists over whether the adaptation of lipase is affected by the type of dietary fat, i.e. its degree of saturation or its chain length. Deschodt-Lanckman *et al.* (1971) reported a twofold greater response of lipase to unsaturated dietary fats than to saturated fats, although both types of fat increased the pancreatic lipase content compared with a low-fat diet. In contrast, Saroux *et al.* (1982) found no difference in lipase adaptation to highly saturated or unsaturated fats. Rather, long-chain triacylglycerols (LCT) increased the lipase content more than medium-chain triacylglycerols (MCT). In dogs adapted to one of two diets that differed only in the type of fat content (olive and sunflowerseed oil), no differences were revealed between the groups concerning the activity of lipase in the pancreatic juice secreted after the ingestion of the olive- or sunflowerseed oil-containing meals (Ballesta *et al.* 1990). In the present study in human subjects, duodenal lipase activity was higher in group O than in group S, but significance was achieved only during the first postprandial hour. We think that this difference may be related to the higher levels of CCK observed in this group. The greater suppression of meal-stimulated lipase output by the CCK receptor antagonist loxiglumide in relation to other pancreatic enzymes, such as trypsin, observed in human acute experiments (Fried *et al.* 1991) seems to be in keeping with the former idea. On the other hand, the fact that the circulating levels of secretin, a gastrointestinal hormone that has been shown to stimulate the synthesis of pancreatic lipase (Rausch *et al.* 1986), were very similar in the two groups, both during rest and after food ingestion, could explain the observation that the differences in duodenal lipase activity achieved statistical significance only at a single point throughout the entire postprandial period.

Several articles report an adaptation of colipase to increasing dietary fat levels (Girard-Globa & Simond-Cote, 1977; Mourot & Corring, 1979; Saraux *et al.* 1982; Wicker & Puigserver, 1987), the response being less strong than that of lipase (Mourot & Corring, 1979; Wicker & Puigserver, 1987). To date, no studies have focused on the influence of the type of dietary fat on colipase content in the pancreatic gland or its secretion. In our experimental conditions, the activity of colipase in human duodenal contents was unaffected by the type of dietary fat provided. We also failed to find any significant differences concerning the luminal activity of amylase after the ingestion of oleic acid- or linoleic acid-enriched meals. Deschodt-Lanckman *et al.* (1971) demonstrated that in the pancreas of rats adapted to different high-fat diets, amylase activity achieved the minimum levels in those animals given the olive-oil diet. Again, amylase activity in the pancreatic juice secreted after food intake was consistently lower in dogs adapted to a diet containing olive oil, compared with those given sunflowerseed oil (Ballesta *et al.* 1990). No similar phenomenon could be confirmed in the present study.

We did not observe significant differences between group O and group S in relation to chymotrypsin or trypsin activities. In rats, adaptation to dietary fats with different degrees of saturation results in similar protease activities in pancreatic tissue (Ricketts & Brannon, 1994). In the study of Ballesta *et al.* (1990), chymotrypsin activity in pancreatic juice was higher throughout the postprandial period studied in the olive-oil-fed group of animals than in those fed with sunflowerseed oil. This was explained by the high oleic acid content of olive oil, as this fatty acid is a potent releaser of CCK (Konturek *et al.* 1986), a hormone known to increase the protease content in pancreas (Renaud *et al.* 1986). Our results in human subjects fit better with those of the rat study than with those from the dog studies. Nevertheless, in the present study, duodenal trypsin activity was slightly, though consistently, higher in group O, in agreement with the greater plasma CCK concentrations in these subjects. Why a similar influence on chymotrypsin secretion was not observed is not clear, although the demonstration of different mechanisms controlling pancreatic secretion of trypsin and chymotrypsin in humans, as evidenced by a recent study (Reseland *et al.* 1996), may be the underlying reason.

Taken together, our findings do not reveal any great influence of the quality of dietary fat on pancreatic enzyme activities in human duodenal contents, during rest or after the ingestion of the experimental meals. In adult human subjects, altering the quantity of carbohydrate, protein or fat in the diet for 10 (Emde *et al.* 1985) or 15 d (Boivin *et al.* 1990) does not induce changes in the ratios among the enzymes secreted. Since adaptative effects can be measured in rat experiments as early as 24 h after a change in the dietary regimen (Dagorn & Lahaie, 1981), one explanation for our results and those from the human studies mentioned earlier may be that longer periods of time, in relation to animals, are needed for the human pancreas to be affected by a modification in the dietary composition.

On the other hand, we should take into account that, in the present study, the 30 d period before surgery cannot be strictly considered as an adaptation period to the diet, at least with the meaning that this word receives in the animal studies, where the experimental subjects can be fed on chemically-defined, isoenergetic and isonitrogenous diets for long periods of time.

Finally, the difficulties in trying to obtain data on the enzyme outputs in human subjects obliges us to be careful about the interpretation of our results and, thus, we should not categorically exclude the possibility that the quality of dietary fat can affect human pancreatic enzyme secretion to a greater extent than is evident from the present study, for instance, through a flow-mediated effect, as we previously observed in dogs (Ballesta *et al.* 1990).

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