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Release of secretin and somatostatin after test meals with different fatty-acid composition in cholecystectomized humans

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The release of secretin and somatostatin by liquid meals with different fatty-acid composition was investigated in humans. For this purpose, plasma secretin and somatostatin concentrations were measured in two groups of cholecystectomized subjects, before and after the ingestion of a meal that contained sunflower oil (Group A) or equicaloric amounts of sunflower oil, olive oil and cream (Group B) as the fat source. The changes in pH values, bicarbonate concentration, and amylase activity in duodenal contents were also studied. Significant release of secretin and somatostatin by food was observed only in Group B. In addition, the plasma levels of both hormones were significantly higher in Group B than in Group A during the postprandial period. Duodenal bicarbonate concentration and amylase activity were consistently lower in the Group B subjects. Our findings indicate that the ingestion of liquid mixed meals that only differ in their fatty-acid profile evokes different secretin and somatostatin release patterns, which, in turn, affects the pancreatic response to food. The existence of an autoregulatory system including secretin and somatostatin is also supported. (J. Nutr. Biochem. 9:186–192, 1998) © *Elsevier Science Inc. 1998*

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Introduction

The control of exocrine pancreatic secretion involves the action of neural, paracrine, and endocrine factors. Among the latter, cholecystokinin (CCK) and secretin are assumed to exert the most important stimulatory effects, owing to their biological potency. Other stimulants include gastrin, neurotensin, motilin, and insulin. On the other hand, a number of peptides have been proposed as physiological inhibitors of pancreatic secretion: somatostatin, pancreatic polypeptide (PP), peptide YY (PYY), and glucagon.[1]

The release of secretin from the upper small intestine is governed by a number of factors. Previously, a low luminal pH in the proximal duodenum had been the only well-established physiological stimulus for the release of secretin.[2] However, with the advent of sensitive and specific radioimmunoassays, it has now been demonstrated that fat or fatty acids release secretin in humans.[3,4] Concerning its biological actions, this hormone is thought to be the major mediator of pancreatic volume and bicarbonate secretion.[5,6] although it has been established[7] that this action can be potentiated by CCK.

Another hormonal factor involved in the regulation of exocrine pancreatic secretion is somatostatin. This hormone

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has been shown to be a potent inhibitor of the pancreatic enzyme[8] and bicarbonate secretion.[9,10] In addition, plasma somatostatin-like immunoreactivity increases, both in humans and dogs, in response to a mixed meal[11,12] and after intragastric or intraduodenal administration of defined nutrients, including fat.[11,13,14]

The changes in the composition of a mixed meal alter the delivery of nutrients to the duodenum and, thus, the stimulation of the digestive functions. The presence of fat in different segments of the intestine is known to affect the secretion of pancreatic fluid, bicarbonate, and protein.[15,16] Because dietary fat accounts for a great percentage (about 40%) of the energy provided by food intake, we thought it was clearly of interest to investigate the effects of the ingestion of two meals, that differed only in their fatty-acid composition, on the release pattern of the above mentioned peptide hormones. The changes in the pH values, bicarbonate concentration, and amylase activity in duodenal contents were also evaluated. Finally, the liquid test meals studied were similar to some feeding solutions commercially available, so the information obtained may be of use in deciding the most appropriate nutritional therapy for patients recovering from different gastrointestinal illnesses.

Methods and materials

Subjects

Nineteen female volunteers who had undergone a cholecystectomy for gallstones in the gallbladder participated in the study. The main reason for choosing these subjects was the high number of patients having a cholecystectomy procedure at our hospital, this allowing us more easily to find among them those fitting the eligibility criteria for the study. The fact that all the participants were women is associated with the higher frequency of cholelithiasis in this sex as compared to men (relation 3/1), at least in our geographic area. The following exclusion criteria were considered: a) Previous pancreatic, gastric, or biliary tract surgery; b) Choledocholithiasis cases; c) History of systemic (atherosclerosis, diabetes), pancreatic (acute or chronic pancreatitis), or gastrointestinal disease of any other etiology (gastric or duodenal ulcer); d) Chronic consumption of drugs, especially antacids or histamine H2-receptor antagonists. The experimental protocol was approved by the local ethical committee and all subjects gave a written consent after being fully informed of the nature and procedures of the study.

The volunteers were randomly divided into two experimental groups, named A and B, containing nine (41.7 ± years (SEM 3.85), 73.2 kg (SEM 3.12)) and ten (46.7 years (SEM 2.89), 67.2 kg (SEM 2.30)) subjects, respectively.

Test meals

The liquid test meals (pH 6.33; 294 mosmol/L) contained 4.18 MJ/L and were composed of 17% of energy as protein, 30% as fat, 53% as carbohydrates, vitamins, and minerals. They were prepared by adequately mixing the separate components, according to protein (lactalbumin), carbohydrate (maltodextrins), and vitamin-mineral mixture modules (Edda modular, Ibys Nutrición, Madrid, Spain). As far as the fat source is concerned, sunflower oil was used in the meal given to Group A, and a mixture of equicaloric amounts of sunflower oil, olive oil, and cream, was used in the meal given to Group B. The fatty-acid composition of the liquid meals was determined. After direct transesterification according to Lepage and Roy,[17] methylated esters were analyzed by gas-liquid chromatography 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.d.: 0.32 mm; particle size: 0.20 μm; SP™-2330, Supelco, Inc., Bellefonte, PA, USA). The two liquid meals were isoenergetic and isonitrogenous, thus differing only in their fatty-acid composition (*Table 1*).

Table 1 Fatty acid composition of the liquid test meals

	Group A	Group B
Caprilic (C8:0)	0.19 ± 0.04	0.24 ± 0.07
Capric (C10:0)	0.56 ± 0.08*	0.93 ± 0.08
Lauric (C12:0)	0.73 ± 0.10*	1.26 ± 0.09
Oleic (C18:1 n − 9)	29.03 ± 0.66*	42.04 ± 2.00
Linoleic (C18:2 n − 6)	42.16 ± 1.70*	17.59 ± 1.40
Saturated	25.93 ± 1.93*	36.23 ± 1.50
Saturated (medium chain)	1.48 ± 0.21*	2.43 ± 0.23
Monounsaturated	29.67 ± 0.66*	43.37 ± 2.00
Polyunsaturated	44.62 ± 1.55*	20.77 ± 1.30

Expressed as g/100 g total fatty acids. Mean ± SEM ($n = 6$).
*Significant differences ($P < 0.05$) between both diets.

Experimental design

The experimental period began 48 hr after surgery, once confirmed that the subjects had recovered the normality of the digestive function and that the meals could be well tolerated. None of the participants were receiving at the time of experimentation any medication known to influence gastrointestinal secretions (or motility) or postprandial hormonal responses. Each participant was studied on 2 consecutive days and after at least 8 hr of fasting. All the experiments started at 0900 hr. 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 frequently checked 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 corresponding liquid test meal (200 mL ingested over 30 min). The complete feeding and sampling procedure was repeated on the second experimental day, each individual receiving the some meal than the day before.

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 aliquots at −80°C until radioimmunoassay of secretin and somatostatin. Two-mL duodenal samples were slow and manually collected by aspiration with an adequately adapted syringe, avoiding the entry of air. An aliquot was kept from each sample for the immediate determination of pH and bicarbonate concentration, and the remainder was frozen at −80°C for the later analysis of amylase activity.

Analytical methods

Plasma levels of secretin were measured by means of 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 vasoactive intestinal peptide

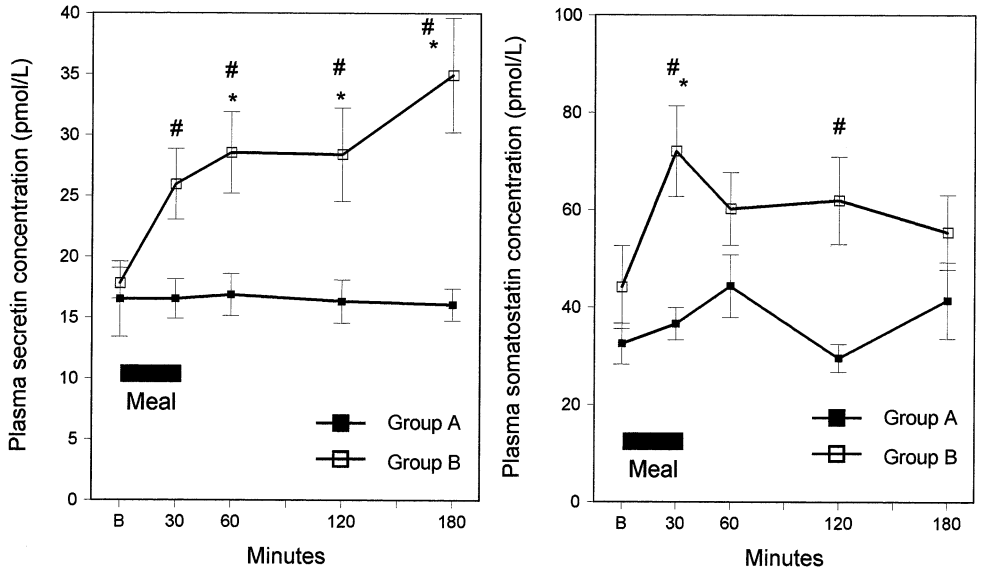

Figure 1 Plasma secretin and somatostatin concentrations in cholecystectomized subjects before (B) and after the oral administration of liquid meals containing sunflower oil (Group A) or equicaloric amounts of sunflower oil, olive oil, and cream (Group B), as the fat source. The meal was ingested within 30 min (dark bar). Each value represents the mean ± SEM of two experiments on nine (Group A) or ten (Group B) subjects per group. (*) Mean values for each dietary group were significantly ($P < 0.05$) different from the fasting ones. (#) Mean values for the two dietary groups were significantly ($P < 0.05$) different at the same time points.

(VIP), glucagon, motilin, and gastrin. Synthetic porcine secretin was used as standard. The minimal plasma secretin concentration detected by this assay was 10 pmol/L. The intra-assay and interassay coefficients of variability were 2.9% and 8.1%, respectively.

For somatostatin assay, plasma specimens (250 μL) were extracted with 0.5 mL of the mixture acetic acid (6 mol/L):ethanol (96%), 5:95 (vol:vol), according to Peeters et al.[18] The extraction recovery was 80.2%. Next, a commercial radioimmunoassay kit (Peninsula Laboratories GMBH, Belmont, CA, USA) with 100% specificity for somatostatin-14, somatostatin-28, and somatostatin-25, was used to determine the plasma concentration of this hormone by a double-antibody procedure. The antiserum, generated in rabbits against synthetic somatostatin, did not cross-react with VIP, substance P nor porcine neuropeptide Y (NPY), showing a cross-reaction equal to 0.002% with porcine pro-somatostatin 1-32. The sensitivity was 12 pmol/L and the coefficients of variability within and between assays were 5% and 15%, respectively.

The pH of the duodenal aspirates was measured using a pHmeter (Model micro pH 2002, Crisson Instruments S.A., Barcelona, Spain) calibrated at pH 4.00 and 7.00. Bicarbonate concentration was determined by adding 0.5 mL hydrochloric acid (0.01 mol/L) to a 0.5-mL sample, gently boiling, cooling down, and backtitrating the excess acid with sodium hydroxide (0.005 mol/L) to pH 7.00. Amylase activity was measured by hydrolysis of starch substrate and determination of the amount of maltose released, in accordance with the technique described by Hickson.[19] The results were expressed in units of activity as defined by the latter.

Statistical treatment

Two studies were carried out on consecutive days for each subject, and the overall mean of the studies was used to calculate the group mean and the standard error of the mean (SEM). For statistical comparisons within the groups (as compared with fasting values), analysis of variance was made, using the Duncan test. Differences between the two dietary groups at the same points in time were tested for significance by using the unpaired Student's *t* test. A *P* value < 0.05 was considered statistically significant.

Results

Plasma hormone concentrations

Fasting plasma secretin concentration was quite similar in both experimental groups, ranging from 16.52 ± 3.09 pmol/L (Group A) to 17.81 ± 1.27 pmol/L (Group B). A prompt increase in plasma secretin concentration occurred in Group B after the oral administration of the liquid test meal (*Figure 1*). The increase was statistically significant, compared with the fasting value, within 60 min after the ingestion was begun, and the levels remained elevated significantly until the end of the study. In contrast, mean plasma secretin concentration did not change in Group A after food ingestion. Consequently, at any point during the postprandial period, plasma secretin concentration was significantly higher in Group B than in Group A.

No significant difference was registered between Group A and Group B concerning the plasma somatostatin levels in the fasting state. As shown in *Figure 1*, the liquid meal evoked in Group B a significant increase 30 min after beginning its ingestion, whereas only a slight rise was found in Group A. It is noteworthy that somatostatin levels were consistently higher in Group B during the digestive period, the differences becoming significant as compared to Group A at 30 and 120 min postprandially.

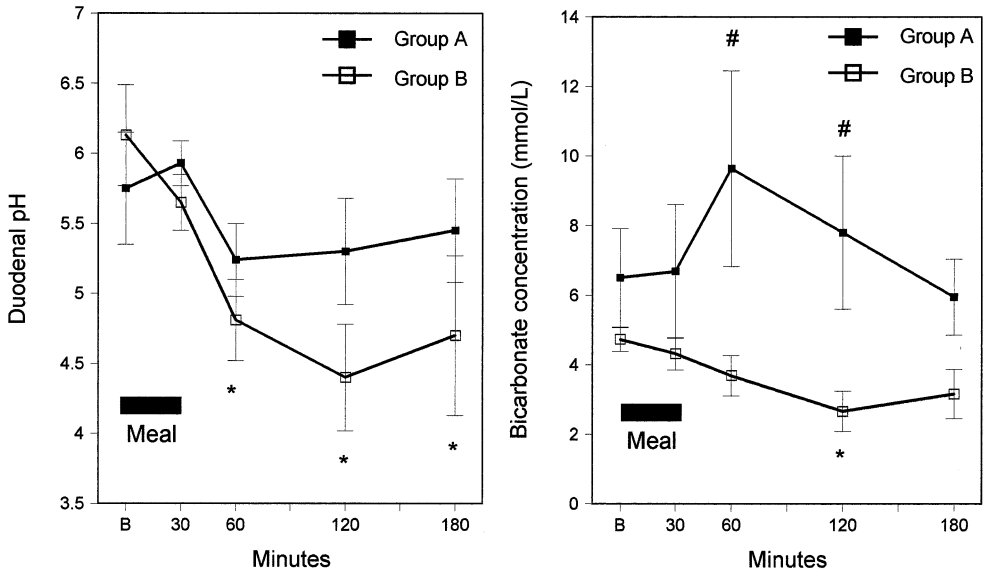

Figure 2 Duodenal pH and bicarbonate concentration in cholecystectomized subjects before (B) and after the oral administration of liquid meals containing sunflower oil (Group A) or equicaloric amounts of sunflower oil, olive oil, and cream (Group B), as the fat source. The meal was ingested within 30 min (dark bar). Each value represents the mean ± SEM of two experiments on nine (Group A) or ten (Group B) subjects per group. (*) Mean values for each dietary group were significantly ($P < 0.05$) different from the fasting ones. (#) Mean values for the two dietary groups were significantly ($P < 0.05$) different at the same time points.

Duodenal pH and bicarbonate concentration

Fasting duodenal pH values were similar in both groups: 5.75 ± 0.40, Group A; 6.13 ± 0.36, Group B. The ingestion of the liquid meal with a pH of 6.33 induced in Group B a prominent decrease in intraduodenal pH, that reached statistical significant, as compared to the fasting value, from the first postprandial hour onwards (*Figure* 2). A minimum significance (4.40 ± 0.38) was found 120 min after beginning the meal. In contrast, duodenal pH hardly modified in the Group A subjects for the duration of the experiment. Despite of these different patterns, no significant differences were revealed between both groups.

After food ingestion, duodenal bicarbonate concentration in Group B decreased gradually to 2.66 ± 0.58 mmol/L (at 120 min), representing a statistically significant decrease under the mean fasting concentration of 4.73 ± 0.34 mmol/L (*Figure* 2). In Group A, the concentration of this anion in duodenal content before the administration of the test meal was equal to 6.50 ± 1.41 mmol/L. The presence of food in the digestive tract evoked in this latter group a modest, though not significant, increase at the first postprandial hour, that was followed by a progressive decrease to approach the fasting values at the end of the 3-hr study. Mean bicarbonate concentration was significantly higher in Group A than in Group B at 60 and 120 min after starting the meal.

Duodenal amylase activity

In the fasting state, duodenal amylase activity was 2.611 ± 0.671 U/mL in Group A and 3.176 ± 0.743 U/mL in Group B. There were no significant changes in Group A in response to food, although a slight decrease was noted during the 30 to 60 min period, thereafter followed by a plateau. Food ingestion induced in the Group B subjects a decrease in amylase activity, that reached statistical significance at 60 and 120 min after the meal was started (*Figure* 3).

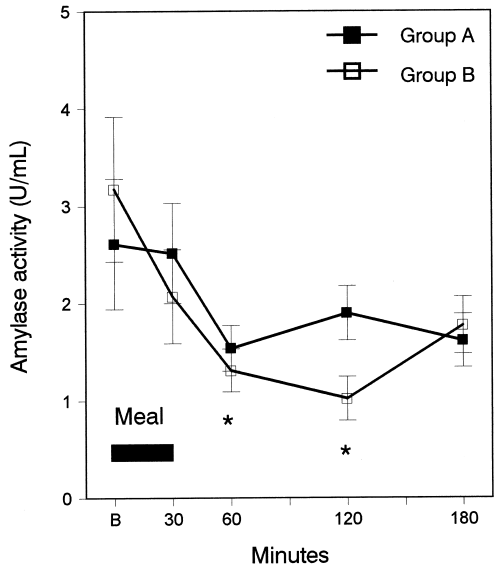

Figure 3 Duodenal amylase activity in cholecystectomized subjects before (B) and after the oral administration of liquid meals containing sunflower oil (Group A) or equicaloric amounts of sunflower oil, olive oil, and cream (Group B), as the fat source. The meal was ingested within 30 min (dark bar). Each value represents the mean ± SEM of two experiments on nine (Group A) or ten (Group B) subjects per group. (*) Mean values for each dietary group were significantly ($P < 0.05$) different from the fasting ones.

Discussion

Regardless of the experimental group, the plasma concentrations of secretin were slightly higher than those reported by other authors.[7] This may be related to the characteristics of the antiserum used, because several animal studies[20,21] where the same commercial RIA kit was used report values somehow greater than those found by other research groups in the same species.[22] After the ingestion of two liquid meals that differed only in their fatty-acid composition, a marked and significant increase in plasma secretin concentration was observed in Group B, whereas no significant changes were registered in Group A (*Figure* 1). This hormone is thought to be released primarily by intraduodenal hydrogen ions,[2] and it has been clearly stated that the pH threshold for its release is indeed 4.5 in the dog.[23] The information about this topic in humans is less clear, although a threshold between 4 and 5 is currently accepted. In our study, the ingestion of a liquid meal with a pH of 6.33 caused in Group B a prominent decrease in intraduodenal pH, that reached a minimum 120 min after beginning the meal, whereas pH values modified slightly in Group A, and remained above 5 for the duration of the experiment (*Figure* 2). This supports the existence of some influence of pH upon the plasma secretin concentrations observed in our study. However, we should consider two aspects: 1) There is a steep pH gradient along the duodenum, together with wide and rapid fluctuations in the upper part, such that values as low as 3 are recorded during the postprandial period.[24] As we measured the pH of luminal contents collected from the third or fourth duodenal segment, it is reasonable to think that pH values lower than the proposed threshold were reached at the most proximal centimeters of the duodenum in both Groups A and B. 2). When comparing pH values at any point during the postprandial period, no significant differences were revealed between our groups. It seems, therefore, that duodenal acidity cannot totally explain the different secretin release patterns found in our subjects. Furthermore, the above observations suggest that, apart from an acidic pH, other factors may play an important role in releasing this hormone during the postprandial period. Those factors may potentiate the secretin response to food via synergisms with the acid stimulus, and even alter the pH threshold for secretin release. Under our experimental conditions, the best candidates are fatty acids, since it has been known for a long time that they liberate secretin[16] and, what is more, the only difference between the meals given to our groups was related to their fatty-acid composition (*Table* 1). The ingestion of a liquid meal that contained 30% of energy as sunflower oil did not induce any changes in secretin concentration in the Group A subjects (*Figure* 1), which is in good agreement with the results from Rhodes et al.[4] during the intraduodenal infusion of a tube feeding solution with a pH of 6.4 in which corn oil provided 31.5% of its caloric value. Neutral fat *per se* does not increase the plasma level of secretin; this hormone is only released by fatty acids or by the digestive products of fat.[22,25] In the meal given to group A, sunflower oil was present in a non-emulsified form, which may have determined the degree of the S-cell stimulation by delaying luminal lipolysis and, then, reducing the amount of free fatty acids. Moreover, the subjects we chose for our study had undergone a cholecystectomy, and any modification in the intraduodenal bile salt concentration may have affected the emulsification and hydrolysis of dietary triacylglycerols as well as the emulsification of fatty acids, which, in the physiological range of pH, is important for the stimulation of secretin release.[26] In contrast, a rapid and significant increase in secretin levels occurred in Group B after food ingestion (*Figure* 1). We think that the marked differences in the secretin release pattern between Group A and B can only be explained by the significantly higher amount of medium-chain fatty acids contained in the meal administered to the latter group (*Table* 1), consequent to the addition of milk fat. It has been reported[27] that gastric lipase specific activity augments with diminishing the fatty-acid chain length. Also, the ester bonds in the *sn*-3 position of triacylglycerols, usually occupied by short- and medium-chain fatty acids, are hydrolyzed first.[28] As a consequence, a higher amount of free fatty acids may be emptied to the duodenum in the Group B subjects. Here, the liberated medium-chain fatty acids may, in turn, act as emulsifiers and favor the hydrolysis of long-chain triacylglycerols. Taken together, all these data indicate that an important amount of free fatty acids may be present in the duodenum of the Group B subjects, thus leading to a greater stimulation of secretin release.

Several studies have shown increases in plasma somatostatin concentration after a mixed meal[11,12] and intragastric or intraduodenal administration of defined nutrients, including fat.[11,13,14] In the present study, a somatostatin response to the presence of food in the digestive tract was observed only in Group B. Moreover, in these subjects somatostatin levels were consistently higher than in Group A throughout the entire postprandial period. It has been suggested that secretin may play a role in the secretion of gastric and pancreatic somatostatin during intraduodenal instillation of hydrochloric acid and fat in dogs.[13] In fact, secretin has demonstrated to release somatostatin from the isolated perfused rat stomach[29] as well as from the stomach and pancreas of anaesthetized dogs.[30] Thus, after taking into consideration the differences in the secretin release patterns between Group A and B (*Figure* 1), we suggest that both events may be in close relationship, supporting the existence of an interesting autoregulatory system whereupon a stimulatory signal, secretin, is also capable of inhibiting its own action by eliciting the release of an inhibitory or counter-regulatory factor, somatostatin.

It is noteworthy that, in Group A, food ingestion did not induce any significant change in duodenal bicarbonate concentration (*Figure* 2), which is in accordance with the absence of postprandial increases in the plasma secretin concentration (*Figure* 1). On the other hand, several authors have demonstrated that somatostatin and its analogues inhibit the pancreatic bicarbonate secretion stimulated by exogenous secretin,[9,10] as well as the existence of receptors for somatostatin on ductular cells.[31] Under the experimental conditions we used, it seems that somatostatin may have counteracted in the Group B subjects the stimulatory effects of secretin, though released in great amounts (*Figure* 1), on pancreatic bicarbonate secretion. Furthermore, if we take into account the fatty-acid profile of the liquid meals tested

(*Table* 1), our hypothesis is strongly supported by the findings from Ballesta et al.,[32] who observed that postprandial pancreatic bicarbonate output was marked and significantly higher in fistulated dogs fed a linoleic acid-enriched meal than in those animals given an oleic acid-enriched one.

Although there were no significant differences in duodenal amylase activity between the two experimental groups (*Figure* 3), a lack of changes was found in Group A after food ingestion, together with a significant decrease in Group B. The latter may be associated with the well-known potent inhibitory effect of somatostatin on the secretion of pancreatic enzymes, including that of amylase.[33] Our data do not allow us to know which is the underlying mechanism for the observed effect. In fact, both a direct (somatostatin receptors on acinar cells) and indirect (insulin-mediated) mechanism has been suggested by different authors[34–39] to explain the inhibitory effect of somatostatin on pancreatic enzyme secretion.

Despite of all the above considerations, caution should be used in interpreting the results of both the amylase and bicarbonate measurement, because we do not dispose of output data. Certainly, it may be suggested that the decrease in duodenal amylase activity and bicarbonate concentration in the subjects of group B is because of dilution of the intestinal contents by an increased fluid output from the gland induced, in turn, by the high secretin levels. However, it is well known the strong inhibitory effect of somatostatin and analogues on endogenously- and exogenously stimulated pancreatic fluid secretion,[40,41] so we rather think that such dilution effect does not exist, mainly because of the high plasma levels of somatostatin in those subjects.

In conclusion, our findings indicate that the ingestion of two liquid meals that differ only in their fatty-acid composition induces different secretin and somatostatin release patterns, which, in turn, affects the pancreatic response to food. Moreover, the results from the present study support the existence of an autoregulatory system including secretin and somatostatin.

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