Plasma peptide YY and pancreatic polypeptide in dogs after long-term adaptation to dietary fats of different degrees of saturation: Olive and sunflower oil

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Mongrel dogs from weaning to 6 months of age were fed on one of two diets that differed only in the type of fat content (virgin olive oil or sunflower seed oil) to compare plasma levels of peptide YY (PYY) and pancreatic polypeptide (PP) in the basal period and in response to food. Under resting conditions, blood concentrations of both peptides, measured by specific radioimmunoassays, were significantly higher in the olive oil group. Food intake was not followed by any marked or significant changes in PYY or PP circulating levels, although some rises were observed. On the other hand, plasma PYY reached significantly greater values throughout the postprandial period in the dogs fed on the diet containing olive oil, whereas no differences were recorded between the groups as far as PP is concerned. Our results demonstrate that long-term intake of diets enriched in monounsaturated fatty acids (olive oil) produces significantly higher basal levels of PYY and PP, as well as significantly higher PYY levels in response to food compared with diets containing sunflower oil. The differences, traceable to the composition of the two types of dietary fat supplied, explain the attenuated pancreatic secretory activity observed by us previously in this animal species. This mechanism may be responsible, at least in part, for the adaptation of pancreatic secretion to the quality of dietary fat. (J. Nutr. Biochem. 8:502–507, 1997) © Elsevier Science Inc. 1997

Introduction

Adaptive changes in functional parameters of the digestive tract in response to an altered composition of the nutritional substrates in the diet have been repeatedly described since the reports of Pavlov in the early 1900s. These changes would optimize digestion and utilization of dietary components. As far as pancreatic secretion is concerned, this phenomenon has been studied in several species,[1–3] and, reportedly, in response to a high-fat diet, pancreatic lipase synthesis and content increase.[3] The quality of dietary fat

also affects this adaptation,[1,4–6] although conflicting results appear in the literature.

Most studies on pancreatic adaptation have focused on specific changes in the synthesis, content and secretion of the major pancreatic hydrolases in relation to the dietary content of their respective substrates, in an attempt to identify the exact mediators of such adaptation.[7] However, little is known about pancreatic responses to exogenous or endogenous stimulation after long-term intake of a nutritionally defined diet. The presence of fat in different segments of the intestine is known to affect not only hydrolase levels in tissue and pancreatic juice, but also the overall pattern of pancreatic response to food.[8] We have previously investigated in dogs[9] the long-term adaptation of exocrine pancreatic secretion to high levels of two types of fat that differed in fatty-acid unsaturation (sunflower oil and virgin olive oil). The effects of these two diets were

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analyzed in terms of response to food and both enzyme and electrolyte content in the juice secreted. The results showed that the two groups responded quite differently to the presence of food in the digestive tract, perhaps because of the existence of different profiles of the gastrointestinal hormones that control pancreatic secretion. However, no study was made concerning the changes in the plasma levels of these hormones.

The dual role of dietary fat, especially its digestive products, in releasing cholecystokinin (CCK) and secretin as major hormonal stimuli for exocrine pancreatic secretion is well known,[10–12] but both the magnitude and time course of the pancreatic response to feeding are also determined by pancreatic polypeptide (PP) and peptide YY (PYY), two inhibitory peptides[13–17] that are released into the plasma after the ingestion of a mixed meal.[15,18] In addition, fatty acids are one of the most potent stimuli of this release.[19,20] Several authors have questioned whether this response is influenced by the type of fatty acid, subsequently demonstrating the great effectiveness of oleic acid.[21,22] However, no study to date has shown that plasma levels of PYY and PP can be altered by the degree of unsaturation of the major fatty acid present in a diet consumed for a long period and thereby modulate exocrine pancreatic secretion. This mechanism may account for the adaptation of the pancreatic secretory response to dietary fat.

Thus, the aim of the present study is to examine the effect that long-term adaptation to diets differing in fatty-acid profiles (degree of unsaturation) exerts on plasma levels of PYY and PP, both in the fasting and digestive states.

Methods and material

Animals

Eight healthy mongrel dogs, weaned at 15 to 20 days old (provided by the Laboratory Animals Service of the University of Granada) were divided randomly into two experimental groups (four animals each): olive oil group (Group O) and sunflower seed oil group (Group S). Both groups were submitted to a 6-month dietary adaptation period, one group receiving olive oil (Group O) as the fat source, and the other group sunflower oil (Group S). During this period, the dogs were maintained in individual cages, with free access to water. Each day, the animals were allowed 1 hr of unlimited feeding, on a fluxuating schedule (from 09.30 to 12.00 hr) to avoid conditioning to a specific feeding time. Body weight evolution was similar in both dietary groups, and all animals seemed healthy throughout the study.

Diets

The diets were formulated using a commercial dog chow (Extra®, Gabrina Purina), prepared fat free especially for the present study. Sunflower oil (Koypesol®) was added to the chow given to Group S, and virgin olive oil (Carbonell®) was added to the chow given to Group O. The diets were prepared daily, to avoid peroxidation. The two diets were nearly isoenergetic and isonitrogenous, thus differing only in their fatty-acid composition (*Tables 1* and *2*).

Time-course of the experiments

The experiments were conducted after the dogs were adapted to the diets for 6 months. The animals were deprived of food, but not water, for 24 hr before each test, and at least 48 hr elapsed between

Table 1 Composition of the experimental diets

	Sunflower Oil Diet		Olive Oil Diet	
	g/kg	Energy (%)	g/kg	Energy (%)
Protein	218.0	18.8	222.0	19.6
Fat	187.8	36.5	170.0	33.7
Carbohydrate	517.2	44.7	531.0	46.7
Ash	77.0	—	77.0	—

The dog chow (Extra, Gabrina Purina), which was the basal diet for the above experimental diets, contained: (g/kg) sodium chloride 12, calcium 15, phosphorus 9, choline 2 and (mg/kg) iodine 1.3, magnesium 80.0, copper 30.0, iron 400.0, zinc 140.0, cobalt 1.0, retinol equivalent 6.0, vitamin E 13.4, thiamin 10.0, riboflavin 8.0, pyridoxine 10.0, cyanocobalamin equivalent 0.3, menadione 1.0, cholecalciferol equivalent 0.05, nicotinic acid 80.0, folic acid 5.0, and pantothenic acid 25.0.

two consecutive tests. All the experiments began at the same time of the day.

On each experimental day, the dogs were fed 500 g of their assigned diet, the animals eating all this amount at once (in a very few minutes). Peripheral blood samples were collected 60 min before eating as well as 60, 120, 180, 240, and 300 min postprandially. These samples were taken in heparinized tubes containing aprotinin (Sigma Chemicals Co., St. Louis, MO, USA) to obtain a concentration of 360 Kallikrein Inactivator Units/mL blood. The tubes were placed on ice immediately and, at the end of each experiment, plasma was separated by a refrigerated (4°C) centrifugation at 3,000 rpm for 15 min. Plasma samples were stored as aliquots at −80°C until radioimmunoassay of PP or PYY.

Analytical methods

Immunoreactive PYY was measured by a dextran-coated charcoal radioimmunoassay. The labeled peptide (synthetic, iodinated by the chloramine-T method and purified by high-performance liquid chromatography—HPLC—) and the antiserum (raised in rabbits against porcine-sequence PYY) were purchased from Eurodiagnostica (Malmö, Sweden). The radioimmunoassay procedure was based in that described by Ekman et al.[23] Briefly, assays were set up in polystyrene tubes (11 × 55 mm). One hundred μL of either plasma sample or standard PYY (synthetic porcine, 98% HPLC purity, purchased from Sigma and prepared to obtain a concentration varying from 18.5 to 1,180 pmol/L) in sodium phosphate buffer, 0.05 mol/L and pH 7.50, containing human serum albumin (2.5 g/L), disodium ethylenediaminetetraacetate (2.5 g/L), sodium azide (0.5 g/L), and 500 Kallikrein Inactivator Units aprotinin/ mL), were incubated with 200 μL antiserum (final dilution 1:5,000) for 24 hr at 4°C. Then, 200 μL of iodinated PYY (about 10,000 cpm) were added and the incubation continued for another 24 hr. Antibody-bound tracer was separated from free tracer by the addition of 500 μL of charcoal (5 g/L) in the assay buffer without

Table 2 Fatty-acid composition of the experimental diets (g/100 g total fatty acids)

Fatty Acid	Sunflower Oil Diet	Olive Oil Diet
Palmitic (16:0)	11.32	10.25
Palmitoleic (16:1 n − 7)	1.91	0.77
Oleic (18:1 n − 9)	36.37	69.44
Linoleic (18:2 n − 6)	38.66	10.05
Octadecatrienoic (18:3 n − 6)	0.07	0.34
Araquidonic (20:4 n − 6)	0.11	0.02

aprotinin, containing Dextran T70 (0.5 g/L). After 25 min at 4°C, the tubes were centrifuged at 1,700 *g* for 15 min at 4°C. The radioactivity of the supernatants was counted to calculate the percentage of PYY bound to the antibody in each assay tube. The detection limit of the radioimmunoassay was calculated from the quantity of standard PYY required to produce a 2 SD decrease in the initial binding of the tracer to the antiserum. The assay could detect 3 pmol/L. The intra- and interassay variations were 2.6% and 6.3%, respectively. The antiserum registered a cross-reactivity lower than 0.01% with human, bovine and aviar PP, porcine neuropeptide Y, human vasoactive intestinal peptide, peptide histidine-methionine, and porcine secretin.

For assay purposes, antiserum against pure bovine PP (Euro-diagnostica) was used in a final dilution of 1:20,000. The labeled peptide (synthetic human PP, iodinated by the chloramine-T method and purified by HPLC), was also purchased from Eurodiagnostica. Highly purified (99% HPLC purity) synthetic human PP (Sigma Chemicals) was used as standard after serial dilution (from 15 to 480 pmol/L) in assay buffer. This was a sodium barbital buffer, 0.02 mol/L and pH 8.60, containing bovine serum albumin (2 g/L), and sodium azide (0.5 g/L). The following reagents were added to a polystyrene tube (11 × 55 mm): 100 μL plasma specimens and standards, 500 μL diluted PP antiserum, and either 100 μL of assay buffer (unknown) or 100 μL of PP-free plasma (standard), the latter obtained by processing fresh plasma through a Sep-Pak C18 cartridge (Millipore Corporation, Mildford, MA, USA). After incubation for 72 hr at 4°C, 100 μL of labeled PP (about 5,000 cpm) were added and the tubes incubated for an additional 24 hr. The free and bound peptides were separated by the addition of 50 μL of diluted normal rabbit serum (Eurodiagnostica) and 500 μL of goat antirabbit-IgG antiserum (Eurodiagnostica), previously diluted with assay buffer containing polyethyleneglycol 6,000 (75 g/L). The mixture was incubated at 20° to 25°C for 45 min and then centrifuged at 1,700 *g* for 15 min at 4°C. Finally, the pellet radioactivity was counted. The assay had a detection limit of 3 pmol/L, calculated as mentioned for the PYY assay. The antiserum chosen was free (0.03%) of cross-reactivity with tetragastrin, human gastrin-17 and -34, porcine gastric inhibitory peptide, CCK-39, secretin, pancreatic glucagon, insulin and adrenocorticotrophic hormone 1-39, showing a cross reaction equal to 120% with bovine PP. The intra- and interassay coefficients of variability were 5.6% and 5.7%, respectively.

Statistical analysis

Four studies were performed on separate days for each dog and the overall mean of the studies was used to calculate the group mean and the SEM. For statistical comparisons within the groups (above resting values), analysis of variance was made (Oneway Procedure, SPSS/PC v. 6.1, Chicago, Illinois), using Duncan's multiple-comparison test. Although the results reported in the current study correspond with the above procedure, analysis by repeated measures ANOVA with time and dietary fat as independent variables (ANOVA Repeated Measures, SPSS/PC v. 6.1) yielded similar results. The differences between the two dietary groups at the same time points were tested for significance by Student's *t* test (T-Test Groups Procedure, SPSS/PC v. 6.1). *P* < 0.05 was considered statistically significant.

Results

Plasma PYY

Basal plasma PYY levels were significantly (*P* < 0.05) higher in Group O dogs compared to Group S. Mean
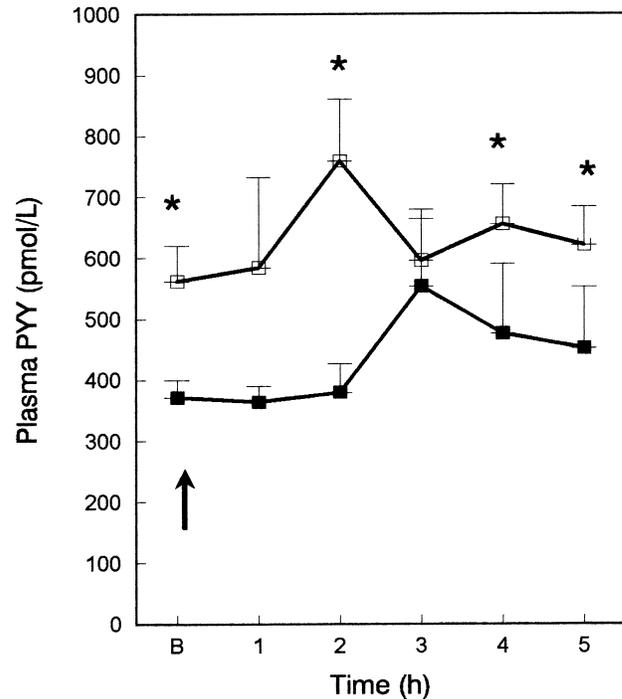
Figure 1 Time course of plasma PYY concentrations under resting conditions (B) and after food intake (↑) in dogs fed on diets containing sunflower oil (■) and olive oil (□). Values are means, with their standard errors represented by vertical bars for four experiments/dog. Statistical significant differences between groups: **P* < 0.05.

(±SEM) plasma levels of PYY were 561.5 ± 58.8 pmol/L and 371.2 ± 28.3 pmol/L in Groups O and S, respectively.

The PYY concentration did not change significantly in response to food ingestion in either group. This parameter, however, showed transient rises at 120 and 180 min postprandially in Group O and Group S, respectively, decreasing subsequently to approach resting values (*Figure 1*).

Nevertheless, plasma PYY levels in Group O of animals remained higher than in Group S throughout the postprandial period, reaching statistical significance (*P* < 0.05) 120, 240, and 300 min after food intake (*Figure 1*).

Plasma PP

Under resting conditions, the mean (±SEM) plasma PP concentration in Group O (172.9 ± 24.7 pmol/L) was significantly (*P* < 0.05) higher than in Group S (109.0 ± 15.9 pmol/L).

No significant change in PP levels was recorded in either of the experimental groups after food intake and there were no significant differences between the groups during the postprandial period (*Figure 2*). However, certain trends emerged, such as a rising plasma PP in Group S during the 0–120 min postprandial period, followed by a decline 180 min after feeding and, thereafter a new rise, reaching a maximum at 300 min postprandially. In Group O, the changes were less marked, with postprandial values quite similar to those of resting conditions, except for a slight increase at the third postprandial hour (*Figure 2*).
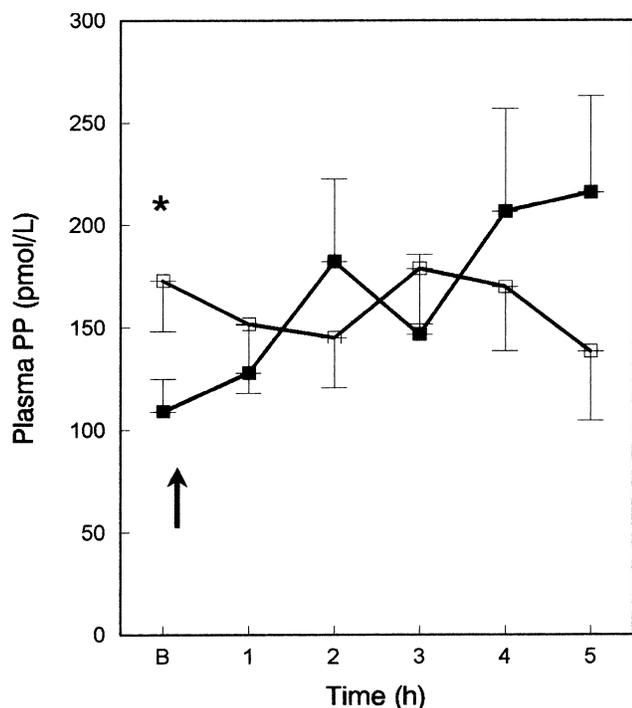
Figure 2 Time course of plasma PP concentrations under resting conditions (B) and after food intake (↑) in dogs fed on diets containing sunflower oil (■) and olive oil (□). Values are means, with their standard errors represented by vertical bars for four experiments/dog. Statistical significant differences between groups: *$P < 0.05$.

Discussion

Peptide YY

Regardless of the experimental group, basal plasma PYY values (*Figure 1*) exceeded those reported by other authors[18,24] for dogs in the fasting state. The differences may be related, at least in part, to the characteristics of the PYY assay employed, since we have also found higher human plasma PYY concentrations[25] than in the literature, as measured by this PYY radioimmunoassay. Nevertheless, we cannot rule out the possibility of some adaptation of the circulating levels of this peptide to the quantity of dietary fat. In fact, both diets in the present study contained 170 to 190 g of fat/kg, in contrast with most commercial dog chows, which usually contain from 30 to 70 g of fat/kg. No study to date has focused on basal plasma PYY concentrations in chronic experiments, such as long-term ingestion of a high-fat diet. However, highly elevated (10-fold) PYY levels during fasting are observed in patients with gastrointestinal diseases, particularly those associated with steatorrhea due to the presence of malabsorbed fat in the distal gut.[26] A similar situation may be occurring in this case. Thus, although the fat apparent digestibility coefficient (ADC) seems to increase in parallel with the dietary fat level,[27,28] it is possible that the amount of this nutrient escaping absorption (physiological malabsorption) and reaching the ileum can be significant, given its high content in the experimental diets.

The plasma PYY concentration showed no significant changes in response to food in either of the experimental groups (*Figure 1*), although there were transient, nonsignificant, rises at 120 and 180 min postprandially in Group O and S, respectively. It has been demonstrated that, after large meals[29] or under conditions allowing a great amount of unabsorbed nutrients to reach the distal intestine, PYY cells are directly stimulated. For this reason, we suggest the existence of a delayed response to food in our experimental groups, in good agreement with the results obtained by Taylor[18] and McFadden et al.,[24] in dogs. Moreover, a delayed gastric emptying produced by the high fat content of the diets may have played a role in the observed PYY response.

It is noteworthy that, in the current study, PYY concentrations were higher in Group O throughout the entire experimental period, the differences being significant under resting conditions and 2, 4, and 5 hr after feeding (*Figure 1*). These results help to explain those previously obtained in our laboratory[9] in dogs after long-term adaptation to two diets that differed only in the quality (degree of saturation) of dietary fat (olive and sunflower oil). We found that, in sunflower oil group, food intake was followed by a rise in the pancreatic flow rate as well as protein and electrolyte outputs, whereas there was a lack of response to food in olive oil group as determined by the same parameters. As a consequence, the production of total protein and bicarbonate was significantly lower in the latter group throughout the entire postprandial period. Plasma hormone concentrations were not measured, but we thought that these differences in the results were related to different profiles of those gastrointestinal factors controlling exocrine pancreatic secretion, with lower levels of stimulating factors (CCK, secretin) and/or greater levels of the inhibiting ones (such as PYY or PP) in the animals fed olive oil. Indeed, we have recently observed higher plasma PYY levels, in the fasting and postprandial situations, in human subjects previously submitted to a 30-day adaptation period to diets in which the major source of fat was olive oil, compared with the group of subjects given sunflower oil.[25] In the present study on dogs, the results support our previous hypothesis as far as the inhibitory component is concerned.

Pancreatic polypeptide

The characteristically food-mediated PP release consists of a rapid primary phase, with an early peak response, followed by a prolonged second phase, with PP levels elevated over basal for several hours.[13,29,30] We failed to show such a clear response. Thus, in our study, plasma PP did not change significantly in response to food in either experimental group. In previous studies, cephalic,[31] gastric,[32] and intestinal[19] phases of PP release have been described, the two former associated with the early increase in plasma PP concentration after food intake. During our experiments, the animals were given 500 g of the previously described diets and, thus, cephalic and gastric phases should be reflected in PP circulating levels, especially for the gastric phase, since Scarpello et al.,[19] have reported that this phase is of greater

importance when a solid meal is ingested, because of the greater gastric distension and/or the more prolonged contact of nutrient stimuli with the gastric mucosa. Under our experimental conditions, the lack of a prompt significant postprandial increase in PP levels is probably because the first blood sample was collected 1 hr after the meal ingestion (*Figure 2*), thereby masking the early peak response that many authors have observed 5 to 30 min after feeding.[13,19,29,30,33]

Finally, and despite that there were no differences between our experimental groups after the ingestion of the meal (*Figure 2*), the significantly higher PP values obtained in Group O under resting conditions suggest the occurrence of some adaptation of PP release to the quality of dietary fat.

In summary, our results confirm that long-term intake of diets enriched in monounsaturated fatty acids (olive oil) modifies circulating levels of PYY and PP, as shown by the higher blood concentrations of both peptides registered under resting conditions. In addition, regarding PYY, the differences observed during both the fasting and digestive states explain the attenuated pancreatic secretory activity observed by us in a previous study[9] on dogs fed diets containing olive oil as source of dietary fat. We suggest that the changes in plasma concentration of these inhibitory peptides, associated with the degree of saturation of the major fatty acid present in a diet consumed for a long period, may play an important role as the mechanism responsible for the adaptation of the pancreatic secretory response to the quality of dietary fat.

Acknowledgments

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