Adaptation of the lipase-colipase system to dietary lipid content in pig pancreatic tissue

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Summary. This report studies the evolution of some enzymatic activities, i. e. mainly of the lipase-colipase system, in pig pancreatic tissue in response to a change in dietary lipid content. Twenty pigs were divided into two lots of ten each. One lot was fed a diet containing 5 p. 100 of peanut oil and the other a diet containing 25 p. 100 of peanut oil. Lipase and colipase activities in the tissue increased when dietary lipids were augmented. However, colipase increased less (+ 37.7 p. 100) than lipase (+ 82.6 p. 100). The choice of a technique for studying the evolution of colipase activity is discussed.

Pancreatic lipase mediates dietary triglyceride hydrolysis and the process of this enzymatic action requires the presence of two other partners : bile salts and pancreatic colipase. Pancreatic lipase is inactivated by bile salts and the function of colipase seems to be to reactivate it (Borgstrom and Erlanson, 1973 ; Maylie *et al.*, 1973). Lipase and colipase levels are probably related and an examination of colipase variation in relation to modification of dietary lipid content is instructive. Two studies have been done in rat to clarify this question. Totally different results were obtained since Girard-Globa and Simond-Cote (1977), measuring lipase colipase saturation, showed that colipase adapted to dietary lipid content, whereas Vandermeers-Piret *et al.* (1977), determining colipasic activity in tissue, concluded that there was no adaptation.

This report attempts to determine if there is a possible modification of tissue colipase content in pig in response to a variation in dietary lipid content.

Material and methods.

Animals. — We used 20 pigs (10 intact males + 10 females) having a mean live weight of 23 ± 2 kg at the beginning of the experiment.

Diet. — Two semi-purified type diets were employed containing 23 p. 100 of Norwegian fish meal as the only source or protein. Diet A contained 25 p. 100 of

peanut oil and 31.5 p. 100 of cornstarch ; diet B contained 5 p. 100 of peanut oil and 51.5 p. 100 of cornstarch. We also added into each diet 12 p. 100 of purified wood cellulose, 5 p. 100 of vermiculite, 2.5 p. 100 of a mineral mixture (p. 100 of diet : bicalcic phosphate : 1.0 ; ground chalk : 0.5 ; sea salt : 0.3 ; potassium chloride : 0.4 ; magnesium carbonate : 0.2 ; trace element mixture : 0.1) and 1 p. 100 of a vitamin mixture (g/100 kg of diet : vitamin A (50 000 IU/g) : 10 ; vitamin D_3 (100 000 IU/g) : 1 ; vitamin E : 2.2 ; vitamin K : 0.44 ; thiamin : 0.22 ; riboflavin : 0.6 ; niacin : 3.6 ; calcium pantothenate : 2.2 ; pyridoxin : 0.3 ; folic acid : 0.2 ; Inositol : 20 ; para-amino-benzoic acid : 2 ; biotin : 0.02 ; vitamin B_{12} (100 μg/g) : 30 ; choline (25 p. 100) : 400). The mean results of diet analyses and diet energy value are shown in table 1.

TABLE 1

Mean analytical results and energy values of the diets

	Diet A	Diet B
Dry matter (p. 100)	93.5	91.2
Nitrogenous matter (p. 100 DM) ..	16.6	16.6
Organic matter (p. 100 DM)	89.1	89.5
Crude energy (kcal/kg DM)	5 378	4 302

Experimental procedure. — During the first preexperimental week, all animals were fed diet B containing 5 p. 100 of peanut oil ; they were then divided into two homogeneous lots according to sex and weight. One lot (lot A : 5 males + 5 females ; 25.3 ± 2.6 kg mean live weight) was fed diet A containing 25 p. 100 of peanut oil. The second group (lot B : 5 males + 5 females ; 25.5 ± 2.5 kg mean live weight) continued to receive diet B containing 5 p. 100 of peanut oil. All subjects were given the same quantity of feed per day and the intake level was increased in relation to live weight as follows : 1.3 kg/day between 24 and 28 kg live weight, 1.5 kg/day between 28 and 32 kg live weight and 1.65 kg/day between 32 and 50 kg live weight. The feed was distributed in 2 meals per day and the animals were weighed once a week. At the end of 27 days of experimentation, the pigs were slaughtered after a 22-hour fast.

Assays. — Immediately after slaughter, the intact pancreas was taken, weighed and ground at 0 °C in distilled water (6.5 ml/g fresh pancreas). Total proteins and the enzymatic activities of chymotrypsin, trypsin, amylase and lipase were determined in homogenates of pancreatic tissue using the techniques previously described in studies on pancreatic juice (Corring and Saucier, 1972 ; Corring, 1974). Lipase activity was first determined at pH 9.0 on trioleate substrate in presence of purified bile salts from cattle bile (6 mM final concentration) ; this activity, called residual, represents colipase-dependent activity in the tissue. The same reaction was carried out after lipase saturation by colipase prepared elsewhere (from fresh pig pancreas, precipitated at pH 2, supernatant centrifuged and freeze-dried). This activity, called potential, is used to estimate the degree of residual lipase saturation. Our results relating to lipase activity only concern potential activity.

Colipase activity has also been determined after inactivation of the lipase at acid pH (Rathelot *et al.*, 1975). The assay technique for colipase activity is as follows : after acidification at pH 2.0 of 1 ml of the pancreatic homogenate, the substrate emulsion and bile salts were added. The pH was adjusted to 9.0 with 5 N and 25 µl of colipase-free pig lipase was added. Colipase activity was expressed by the activity of the lipase added, as determined by titrimetry. The efficiency of this technique has been proven in trials to determine the activity of different amounts of isolated colipase after lipase inactivation.

Results.

Animal growth, pancreatic weight and total protein content of pancreatic tissue. — For the same intake during the whole experimental period (1.4 kg/day/animal), the growth of pigs fed the 25 p. 100 peanut oil diet was higher than that of animals receiving the diet containing 5 p. 100 of peanut oil. Mean daily gain was 685 g and 592 g, respectively (P < 0.01).

Relative weight of the pancreas (per kg of live weight) and total protein content of pancreatic tissue were the same for both lots of animals (table 2).

TABLE 2

Effect of dietary peanut oil content on ponderal evolution and specific enzyme activities of pig pancreatic tissue

	Lot A [1]	Lot B [2]	Statistical significance
Fresh pancreas (g)/live weight (kg)......	1.56 ± 0.17 [3]	1.68 ± 0.15	NS
Total proteins (mg)/fresh pancreas (g) ..	146.3 ± 26.3	162.2 ± 9.3	NS
Lipase [4]	67.7 ± 6.9	37.0 ± 10.2	P < 0.001
Amylase [5]	1 913 ± 432	3 030 ± 581	P < 0.001
Chymotrypsin [6].....................	6.56 ± 0.8	7.13 ± 0.6	NS
Trypsin [7]	1.46 ± 0.34	1.91 ± 0.19	P < 0.01
Colipase	119.9 ± 15.4	87.2 ± 21.2	P < 0.005
Colipase/potential lipase	1.77 ± 0.18	2.39 ± 0.21	P < 0.001
Residual lipase/potential lipase (p. 100) .	91.2 ± 15.9	93.4 ± 32.3	NS

[1] Lot fed diet A.
[2] Lot fed diet B.
[3] Mean ± standard deviation.
[4] µmoles free fatty acids/min/mg proteins.
[5] Units of amylasic activity after hydrolysis of 1.0 mg of soluble Merck starch for 30 min. at 37 °C, expressed/mg of proteins.
[6] µmoles of ATEE hydrolyzed/min/mg of proteins.
[7] µmoles of BAEE hydrolyzed/min/mg proteins.

Enzymatic activities. — The specific enzyme activities (per mg of total pancreatic tissue proteins) are shown in table 2. Specific potential lipase activity was significantly higher in the pancreatic tissue of lot A animals (+ 82.6 p. 100), while that of amylase was significantly higher in the pancreas of lot B subjects (+ 58.4 p. 100). While specific chymotrypsin activity was not significantly different in the two lots, there was a signi-

ficant difference between the values of specific trypsin activity, which was higher in lot B animals (+ 30.5 p. 100). Finally, specific colipase activity was significantly higher in the pancreas of pigs fed the lipid-rich diet (+ 37. 7 p. 100).

Discussion.

For the same intake, the diet containing 25 p. 100 peanut oil induced better animal growth. This confirms the results of Henry and Rérat (1964) and of Henry (1974) who showed that the feed conversion ratio (kg feed/kg gain) in pig decreased with a diet progressively enriched with peanut oil. Ponderal pancreatic development and total proteins in the pancreatic tissue are also stimulated by a lipid-rich diet.

As concerns enzymatic activities, the diet rich in peanut oil caused a rise in lipase level, while the diet containing 5 p. 100 of peanut oil, richer in starch, resulted in increased specific amylase activity. These data confirm studies on long-term adaptation of the exocrine pancreas to diet composition (Desnuelle et al., 1962 ; Bucko and Kopec, 1968 ; Behrman and Kare, 1969 ; Corring, 1975). Specific chrymotrypsin activity in the pancreas was the same in both lots ; this may be explained by the fact that both diets were isoproteic. It confirms the results obtained on pig pancreatic juice (Corring, 1975). On the other hand, the increased trypsin level, observed in the pancreas of animals fed the diet containing 5 p. 100 peanut oil and 51.5 p. 100 starch, as compared to that in lot A, is unexplainable. This is particularly striking as in a previous study (Corring and Saucier, 1972), trypsin seemed less sensitive than chymo-trypsin to any changes in diet composition.

As related to the diet given, variations of specific colipase activity show adaptation of lipase cofactor to the lipid level of the diet. However, it should be noted that the diet containing 25 p. 100 of peanut oil caused a greater increase of specific lipase activity than of colipase activity (82.6 p. 100 vs. 37.7 p. 100). This dissimilar evolution appeared in the colipase/potential lipase ratio which decreased from 2.39 to 1.77 when the dietary peanut oil content ranged from 5 to 25 p. 100. At least in man, lipase and colipase (Figarella, Negri and Sarles, 1972) are independently synthesized ; this would explain the dissimilar evolution of the activies in the tissue. What would be the nutritional significance of colipase adaptation as compared to that of lipase ? Could there be a partial adaptation ? In the pancreas of both lots of pigs, residual lipase can be considered as saturated by the colipase in the tissue (93.4 p. 100 in animals fed the diet containing 5 p. 100 of peanut oil and 91.2 p. 100 in those given the diet containing 25 p. 100 of peanut oil). It could be that after the dietary lipid content is augmented, the increase of colipase activity, although less than that of lipase activity, is sufficient to maintain an almost total saturation of the lipolytic enzyme (91.2 p. 100).

In rat, Vandermeers-Piret et al. (1977) could not show colipase adaptation to dietary lipid content, while Girard-Globa and Simond-Cote (1977) demonstrated that there was adaptation in the same species. A difference in the type of dietary lipid used may be the cause of these contradictory results. However, it would appear more likely that these differences are due to the technique used in studying colipase evolution in rat tissue. Vandermeers-Piret et al. (1977) determined tissue activity,

while Girard-Globa and Simond-Cote (1977) studied the evolution of the degree of residual lipase saturation. If we had only studied the degree of saturation, we would have concluded that there is no pancreatic colipase adaptation in pig. Perhaps rat lipase is more responsive to bile salt inhibition than pig lipase, or there may be a species difference in the relative quantities of colipase and potential lipase (Léger and Charles, 1979). Nevertheless, it seems that studying only the evolution of the degree of residual lipase saturation is insufficient ; it is preferable to systematically determine colipase activity in the tissue or pancreatic juice in standardized conditions if the work on colipase physiology is to advance.

Résumé. Le but de l'étude rapportée a été de suivre dans le tissu pancréatique du Porc l'évolution de certaines activités enzymatiques et principalemert du système lipase-colipase, en réponse à une modification de la teneur en lipides du régime.

Deux lots de 10 porcs ont reçu l'un un régime renfermant 5 p. 100 d'huile d'arachide. l'autre un régime renfermant 25 p. 100 d'huile d'arachide. Les activités tissulaires lipasique et colipasique augmentent lorsque le régime est enrichi en lipide. Cependant, l'augmentation est moins importante pour la colipase (+ 37,7 p. 100) que pour la lipase (+ 82,6 p. 100). Le choix de la technique d'étude de l'évolution de l'activité de la colipase est discuté.

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