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Effects of a Western-style diet high in cholesterol and saturated fat on the rabbit exocrine pancreas

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Abstract: Despite the well-known cardiovascular effects of an atherogenic diet, there are no data relating to its effect on the exocrine pancreas. Therefore, the aim of this study was to investigate the consequences of this eating pattern on the exocrine pancreas. Twelve New Zealand rabbits were randomised to one of two dietary groups, control group (C) and hypercholesterolemic (HCHOL), that were fed for 50 days with a standard chow or a diet containing 95.7% standard chow, 3% lard, and 1.3% cholesterol, respectively. Pancreatic enzyme activity, cholesterol, and membrane fatty acids were determined by established methods. The activity of pancreatic lipase (6.46 ± 0.948 vs. 1.40 ± 0.460; $P < 0.05$), colipase (8.93 ± 2.138 vs. 2.36 ± 0.512; $P < 0.05$), and chymotrypsin (18.54 ± 3.125 vs. 9.69 ± 1.363; $P < 0.05$) were greater in group HCHOL than in group C. The HCHOL diet increased monounsaturated fatty acids and decreased saturated fatty acids in pancreatic plasma membranes compared with the standard chow. These results suggest a homeostatic adaptation of the pancreas to an atherogenic diet and a greater resistance to the development of lesions than exists in other organs. Further studies are needed, given the lack of research on this issue.

Key words: Western diet, exocrine pancreas, membrane fatty acids, cholesterol, saturated fat, rabbit

1. Introduction

Recent trends in the world food economy as a result of industrialisation, economic development, and market globalisation have contributed to rapid changes in lifestyle and dietary patterns, for example, increased consumption of energy-dense diets high in lipids, particularly saturated fat and cholesterol (WHO, 2003). Because of these changes, chronic noncommunicable diseases are becoming significant causes of death in both developing and developed countries (WHO, 2003). A well-documented case is cardiovascular disease; coronary artery disease risk is strongly related to increased low density lipoprotein (LDL) cholesterol levels (Fernández et al., 2008; Smith et al., 2014). A number of food components can raise LDL cholesterol, including saturated fatty acids (SFA), trans-unsaturated fatty acids, and, to a lesser extent, cholesterol (Krauss et al., 2001). The World Health Organization (WHO) currently attributes one-third of all global deaths (15.3 million) to cardiovascular diseases (WHO, 2003), which probably explains why most studies about Western diet and the influence of dietary fats on human health have focused on these particular pathological processes in detriment of other systems like the gastrointestinal system.

It is well known that the exocrine pancreas adapts to dietary constituents. Studies by our group (Ballesta et al., 1990; López-Palomo et al., 1997; Díaz et al., 2003; Martínez et al., 2004) and other authors (Snook et al., 1971; Sabb et al., 1986; Flores et al., 1988; Wicker et al., 1990; Okada et al., 1993) have provided evidence for a clear influence of amount and type of dietary fat upon pancreatic enzyme content or secretion in different species. The mode of action of dietary fat is probably multifactorial. A first option involves an effect on hormonal mediators. Pancreatic enzyme synthesis and secretion is regulated by a complex integration of stimulatory and inhibitory hormones whose plasma levels are modified by dietary fat (Serrano et al., 1997; Yago et al., 1997). A second, more direct mechanism has been subsequently suggested by our results showing

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that chronic intake of diets containing different fats can change the responsiveness of the pancreas to pancreatic secretagogues such as cholecystokinin or acetylcholine, in association with profound modification of pancreatic membrane lipid composition (Yago et al., 2004, 2006).

A large increase in dietary fat intake between 1968 and 1998 has been observed all over the world. Intake largely exceeds the level of 30% of total calories, with SFA providing more than 10% of total energy in many regions of North America and Europe (WHO, 2003). Fast-food consumption, a growing component of the world "global" diet, is strongly and positively associated with intake of saturated fat and cholesterol (Paeratakul et al., 2003; Song et al., 2005). For these reasons, and because no one has examined this topic before, it was pertinent to determine the effects a diet high in saturated fat and cholesterol, the hallmark of a Western diet, on the exocrine pancreas.

To achieve our objectives, twelve New Zealand rabbits were fed on a diet rich in lard (3%) and cholesterol (1.3%). This is a widely used model for atherosclerosis (Quiles et al., 2002), but there are no data on changes in the pancreas of this animal model. After feeding rabbits for over 50 days with the experimental or commercial chow diet, we examined protein and enzyme contents of the pancreas. Furthermore, given that both cholesterol and fatty acids are major determinants of membrane and cell function, the composition of pancreatic cellular and subcellular membranes was also determined.

2. Materials and methods

2.1. Materials

Unless otherwise stated, chemicals and solvents of the highest quality available were all purchased from Sigma (St Louis, MO, USA), Merck (Darmstadt, Germany), and Panreac Química S.A. (Barcelona, Spain).

2.2. Animals, diets, and experimental design

Twelve male New Zealand rabbits (animal farm at the University of Granada) weighing 3.3–3.5 kg were randomly divided into two groups of six animals each and housed one per cage with an environmentally controlled atmosphere (22 °C) and a 12-h light/dark cycle. Drinking water was available ad libitum throughout the study, and food intake for each animal was standardised to 150 g/day. One group, which served as control (group C), was fed for 50 days with a standard chow diet (Panlab S.L., Barcelona, Spain) composed of 13.5% protein, 3% fat, 50% carbohydrates, 15.5% fibre, 7% minerals, and 11% water. The other group (high cholesterol and lard group, group HCHOL) was fed over the same period with a diet containing 95.7% Panlab standard chow, 3% lard, and 1.3% cholesterol (Abbott Laboratories S.A., Granada, Spain). Total fat content of the HCHOL diet was 7.2% (including added cholesterol). The fatty acid composition of the diets (Table 1) was determined by gas–liquid chromatography, as described later, for the membrane fractions. Diets were kept in darkness at 4 °C until used to avoid peroxidation. Rabbits were clinically examined and weighed weekly. The animals were handled according to the guidelines of the Spanish Society for Laboratory Animal Sciences and killed humanely. All procedures were approved by the ethical committee of the University of Granada.

2.3. Sample collection

At the end of the feeding period, overnight-fasted rabbits were anesthetised with sodium pentothal (16 mg/kg body weight) and exsanguinated from a cannulated carotid. All animals were sacrificed between 0900 and 1100 to avoid circadian effects. The pancreas was immediately excised and trimmed free of fat, connective tissue, and lymph nodes in cold saline solution (0.9% NaCl). The cleaned gland was

Table 1. Fatty acid composition and total fat content of experimental diets.

	C diet	HCHOL diet
Fatty acid		
14:0	0.62	1.04
16:0	20.27	22.71
18:0	6.43	9.28
16:1ω-7	0.86	1.35
18:1ω-9	28.08	30.04
18:2ω-6	34.49	28.45
18:3ω-3	2.64	2.25
20:4ω-6	0.15	0.14
20:5ω-3	0.16	0.14
22:6ω-3	0.33	0.32
SFA	29.21	35.18
MUFA	31.21	33.02
PUFA	39.58	31.80
ω-6 PUFA	36.20	28.74
ω-3 PUFA	3.38	2.68
Total fat content	3.0	7.2 [a]

C: control (standard rabbit chow); HCHOL: diet rich in cholesterol and lard. Fatty acid values are expressed as percentage of total fatty acids (mean values for four replicates). Total fat content is expressed as g/100 g diet. [a] This value includes added cholesterol (1.3 g). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

blotted, weighed, and divided into fragments. One of them was used for immediate isolation of membranes, and the rest was frozen in liquid nitrogen and stored at –80 °C for further determinations.

2.4. Membrane isolation, lipid extraction, and fatty acid analysis

Plasma membranes and total microsomal fractions were isolated from gland homogenates by differential and sucrose gradient centrifugation (Meldolesi et al., 1971). Lipid extraction and fatty acid methylation was done in a one-step reaction as described by Lepage and Roy (1986). A gas–liquid chromatography system, model HP 5890 series II (Hewlett Packard, Palo Alto, CA, USA) equipped with an automatic injector and a flame ionisation detector, was used to analyze fatty acids as methyl esters. Chromatography was performed using a capillary column impregnated with Sp(TM) 2330 FS (Supelco Inc., Bellefonte, Palo Alto, CA, USA) that was 60 m long, 32 mm in inner diameter, and 20 mm thick.

2.5. Assays in pancreatic homogenates

Gland fragments were homogenised (60 mg tissue/mL buffer) with an ice-chilled Teflon-glass homogeniser. For amylase, lipase, and colipase, the homogenisation buffer was the same as the one later used in the corresponding enzyme assay. Amylase: 20 mM sodium phosphate and 6.7 mM NaCl, pH 7.05. Lipase and colipase: 2 mM TRIS/maleate; 150 mM NaCl; 1 mM $CaCl_2$; and 4 mM sodium taurodeoxycholate, pH 7.50. For trypsin and chymotrypsin determinations, pancreatic fragments were homogenised in 200 mM potassium phosphate buffer, pH 7.60. Assay buffers for trypsin and chymotrypsin are described in the next paragraph.

Amylase activity was measured by hydrolysis of a starch substrate and further measurement of the amount of maltose released, according to the technique of Noelting and Bernfield (1948) as modified by Hickson (1970). Results were expressed in units of activity as defined by the latter (Hickson, 1970). Lipase and colipase activity was assayed with a micro TT 2050 titrator (CRISSON, Barcelona, Spain) using tributyrin dispersed in bile salt according to a titrimetric method of Erlanson-Albertsson et al. (1987). Activity was expressed as units (μmol fatty acid liberated per min at 27 °C). Trypsin activity was measured, after activation with enterokinase, by pH-stat titration using N-benzoyl-L-arginine ethyl ester as a substrate (Reboud et al., 1962). Trypsin assay buffer was 5 mM TRIS; 40 mM NaCl; and 20 mM $CaCl_2$, pH 7.90. Chymotrypsin activity was estimated, after activation with trypsin, by pH-stat titration using acetyl-L-tyrosine ethyl ester as a substrate (Reboud et al., 1962). Chymotrypsin assay buffer was 5 mM TRIS and 40 mM NaCl, pH 7.90. For both enzymes, trypsin and chymotrypsin, values are reported as units of activity (μmol substrate hydrolysed per min at 27 °C).

Protein concentration in pancreatic homogenates was assayed according to Lowry et al. (1951). Results are expressed as mg/g pancreas.

Cholesterol levels in pancreatic tissue were measured by enzymatic colorimetric assay using a commercial kit (Boehringer Mannheim, Munich, Germany). The results are expressed as mg/g pancreas.

2.6. Calculations and statistical analysis

The unsaturation index (UI) was calculated according to the formula: UI = (∑ (fatty acid content × number of double bonds))/total content of saturated fatty acids (SFA). Unless otherwise specified, the values presented in the text, tables, and figures are expressed as mean ± standard error. Differences between the dietary groups were tested for significance by the independent samples Student's t-test (IBM SPSS, version 22.0, 2013), and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Body and pancreatic weight

Initial values of body weight were similar in rabbits fed the high cholesterol and lard diet (group HCHOL: 3521 ± 35.9 g, n = 6) and those given the standard chow (group C: 3375 ± 132.1 g, n = 6). However, after the experimental time, body weight was significantly lower in group HCHOL (3494 ± 80.2 g, n = 6) versus group C (3760 ± 151.9 g, n = 6). In fact, body weight in group HCHOL showed a slight decrease below the initial value (–27.7 ± 35.9 g, n = 6), whereas a marked increase (+385.6 ± 92.1 g, n = 6) was found in group C. Neither the absolute nor relative pancreatic weights were influenced by the experimental diets (Table 2). The macroscopic examination of the glands did not show evidence of pancreatic alteration in either group.

3.2. Protein and cholesterol content in pancreatic homogenates

As shown in Table 2, no differences existed between the groups for pancreatic protein concentration. In contrast, feeding with the diet rich in cholesterol and saturated fat for 50 days was associated with greater values of total cholesterol in the pancreas compared with the standard chow (Table 2).

3.3. Activity of pancreatic enzymes

For all enzymes determined, we calculated activity (U/g pancreas), specific activity (U/mg protein), and total gland activity (U/pancreas). Since the results revealed the same differences regardless of how they were expressed, we only show specific activity data.

Pancreatic amylase activity was similar in groups C and HCHOL (Figure 1A). In contrast, feeding the rabbits diets high in cholesterol and lard resulted in values of lipase and

Table 2. Pancreatic weight and content of protein and total cholesterol in rabbits after 50 days of feeding with different diets.

	C	HCHOL
Pancreatic weight (g)	2.802 ± 0.460	3.001 ± 0.410
Relative pancreas weight (pancreas weight/body weight)	$7.703\ 10^{4} \pm 1.42\ 10^{-4}$	$10.310\ 10^{-4} \pm 2.11\ 10^{-4}$
Protein (mg/g pancreas)	7.754 ± 0.855	7.292 ± 1.229
Total cholesterol (mg/g pancreas)	0.021 ± 0.001*	0.183 ± 0.048

Group C was fed a standard rabbit chow; group HCHOL was fed a diet rich in cholesterol and lard. Data are expressed as mean ± standard error, n = 6 animals per group. *Statistical significance ($P < 0.05$) between groups.

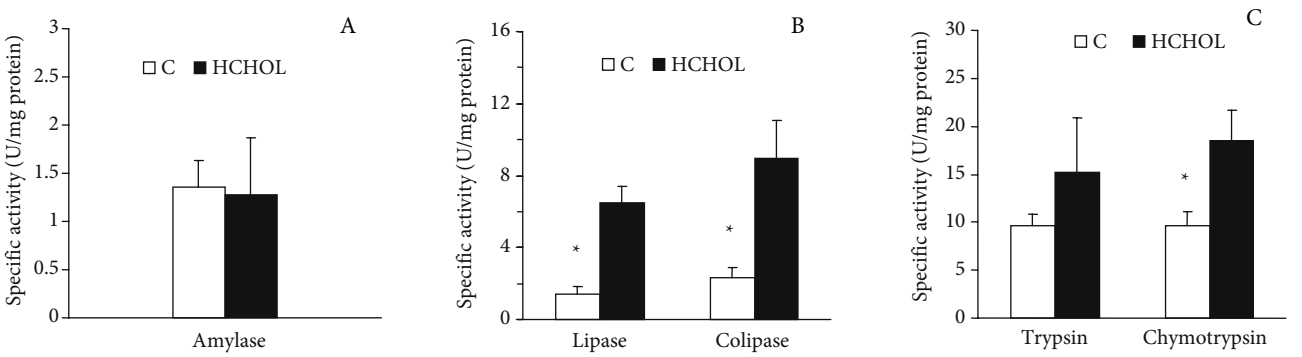

Figure 1. Pancreatic enzyme activity in New Zealand rabbits fed a standard chow (C diet) or a diet rich in cholesterol and lard (HCHOL diet) for 50 days. Values are expressed as mean ± standard error (n = 6 for both groups). *Statistical significance ($P < 0.05$) between dietary groups; (A) amylase specific activity; (B) lipase and colipase-specific activity; (C) trypsin and chymotrypsin-specific activity.

colipase activity significantly greater than those in chow-fed animals (Figure 1B). Specific activity of the proteases trypsin and chymotrypsin was also higher in rabbits from group HCHOL, although statistical significance was only reached for chymotrypsin (Figure 1C).

3.4. Fatty acid composition of pancreatic plasma membranes

Fatty acids in pancreatic plasma membranes were markedly influenced by the diets (Table 3). Membranes of group C were characterised by significantly higher levels of total SFA as compared with group HCHOL. This was mainly due to the contribution of 16:0, since the percentages of 14:0 and 18:0 were similar. Values for total monounsaturated fatty acids (MUFA) were higher in HCHOL membranes, which related to the more elevated proportion of 18:1ω-9 in this group. In contrast, other MUFA, such as 14:1ω-5 and 16:1ω-7, were significantly greater in the membranes of group C. The percentage of total polyunsaturated fatty acids (PUFA) and ω-6 PUFA in pancreatic plasma membranes was not influenced by the diets. However, intake of cholesterol and lard resulted in a significantly lower level of ω-3 PUFA in group HCHOL membranes due to a sharp decrease in 18:3ω-3. The composition of experimental diets affected the SFA/MUFA and SFA/PUFA ratios, both of which were significantly higher in group C than in group HCHOL.

3.5. Fatty acid composition of pancreatic microsomal membranes

As shown in Table 4, there were no differences in all SFA and MUFA regardless of the diet. Compared with intake of standard chow (group C), consumption of the diet rich in cholesterol and lard (group HCHOL) evoked a significant decrease in 18:3ω-3 content, but this did not translate into significantly different levels of ω-3 PUFA. Nevertheless, it should be noted here that total PUFA, ω-6 PUFA, and ω-3 PUFA were numerically lower (and SFA were numerically greater) in group HCHOL, which may explain the finding of a significantly lower unsaturation index in this group of rabbits.

4. Discussion

Recent changes in lifestyles are associated with consumption of high-lipid diets especially rich in saturated fat and cholesterol. In this report we show, for the first time, the effects of these diets on the exocrine pancreas by using rabbits fed a cholesterol- and lard-rich diet as a model.

Table 3. Fatty acid profile of pancreatic plasma membranes in rabbits after 50 days of feeding with different diets.

	C	HCHOL
14:0	3.02 ± 0.172	2.98 ± 0.105
16:0	34.85 ± 1.410*	30.71 ± 0.906
18:0	6.87 ± 0.342	7.32 ± 0.244
14:1ω-5	0.42 ± 0.016*	0.30 ± 0.046
16:1ω-7	5.79 ± 0.491*	4.43 ± 0.529
18:1ω-9	26.99 ± 0.597*	31.32 ± 0.479
18:2ω-6	14.45 ± 1.746	16.15 ± 0.800
18:3ω-3	1.23 ± 0.247*	0.74 ± 0.083
20:4ω-6	0.56 ± 0.047	0.70 ± 0.098
20:5ω-3	0.05 ± 0.012	0.05 ± 0.006
22:6ω-3	0.08 ± 0.011	0.07 ± 0.006
SFA	47.54 ± 1.774*	44.04 ± 0.808
MUFA	34.47 ± 0.679*	37.51 ± 0.269
PUFA	17.99 ± 1.963	18.46 ± 0.855
ω-6 PUFA	16.24 ± 1.774	17.12 ± 0.857
ω-3 PUFA	1.46 ± 0.226*	0.96 ± 0.067
SFA/MUFA	1.38 ± 0.056*	1.17 ± 0.023
SFA/PUFA	2.98 ± 0.497*	0.88 ± 0.016
Unsaturation index	1.56 ± 0.134	1.72 ± 0.066

Group C was fed a standard rabbit chow; group HCHOL was fed a diet rich in cholesterol and lard. Results are expressed as percentage of total fatty acids (mean ± standard error, n = 6 animals per group). *Statistical significance ($P < 0.05$) between groups. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Substantial body weight gain occurred after 50 days in animals given the chow diet (group C), whereas body weight remained unchanged in rabbits fed on the high cholesterol and lard diet (group HCHOL). Initially, both control and HCHOL rabbits completely consumed the offered food and gained weight. However, for the last part of the study, mean body weight of HCHOL rabbits decreased in such way that at the end of the experimental period values returned to the initial ones. This can be due in part to the fact that during the last 1–2 weeks of the dietary period some HCHOL rabbits occasionally refused to eat the whole amount offered. However, other factors may have accounted for the observed weight loss. One of them is a moderate impairment of hepatic function evoked by cholesterol feeding, as indicated by previous results of our group (Aguilera et al., 2005) using the very same experimental model. We confirmed that livers from rabbits fed with the HCHOL diet developed steatohepatitis, characterised by mild-to-moderate neutrophilic and lymphocytic infiltration in association with intracytoplasmic lipid deposits, as well as moderate central vein sclerosis and pericentral spidery fibrosis (Aguilera et al., 2005). Our previous research (Aguilera et al., 2005) also showed that the molar percentage of bile acids in gallbladder bile was significantly lower in rabbits fed the HCHOL diet than in those fed the standard rabbit chow. This may have caused mild lipid malabsorption and contributed to weight loss in this group of animals.

Chronic intake of excessive amounts of dietary fat has been said to exert deleterious effects on pancreatic

Table 4. Fatty acid profile of pancreatic microsomal membranes in rabbits after 50 days of feeding with different diets.

	C	HCHOL
14:0	2.47 ± 0.193	2.75 ± 0.112
16:0	29.58 ± 0.942	32.20 ± 0.902
18:0	9.05 ± 1.037	8.56 ± 0.496
14:1ω-5	0.30 ± 0.063	0.31 ± 0.010
16:1ω-7	4.99 ± 0.421	3.52 ± 0.418
18:1ω-9	27.17 ± 1.091	29.29 ± 1.117
18:2ω-6	16.64 ± 1.368	15.64 ± 0.378
18:3ω-3	1.01 ± 0.120*	0.55 ± 0.127
20:4ω-6	1.30 ± 0.257	1.31 ± 0.222
20:5ω-3	0.27 ± 0.041	0.41 ± 0.090
22:6ω-3	0.31 ± 0.028	0.21 ± 0.045
SFA	44.63 ± 1.418	45.83 ± 1.440
MUFA	34.12 ± 1.315	34.35 ± 1.527
PUFA	21.25 ± 1.417	19.99 ± 0.618
ω-6 PUFA	18.91 ± 1.428	17.56 ± 0.647
ω-3 PUFA	1.93 ± 0.186	1.47 ± 0.186
SFA/MUFA	1.35 ± 0.099	1.31 ± 0.086
SFA/PUFA	2.16 ± 0.194	2.30 ± 0.107
Unsaturation index	2.19 ± 0.089*	1.76 ± 0.098

Group C was fed a standard rabbit chow; group HCHOL was fed a diet rich in cholesterol and lard. Results are expressed as percentage of total fatty acids (mean ± standard error, n = 6 animals per group). *Statistical significance ($P < 0.05$) between groups. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

morphology and physiology in rats (Chowdhury et al., 2000). Moreover, the effects of dietary fat on pancreatic cancer are controversial; increased risk with saturated fat and cholesterol has been communicated in some epidemiological studies (Lin et al., 2005) but not in others (Nothlings et al., 2005). In our study, all pancreata looked macroscopically normal, and protein content was comparable in both groups. Gland weight and protein data confirm the absence of hypertrophy/hyperplasia in rabbits fed the HCHOL diet, which is consistent with previous experimental work showing that saturated-fat–rich diets do not initiate nor promote pancreatic tumours (Roebuck et al., 1981; Jacobs, 1983). In addition, the current results suggest that the pancreas of rabbits given lard and cholesterol displays a greater resistance towards the development of lesions than do other organs of the same animals (Aguilera et al., 2005).

The lipid membrane composition influences its organisation and properties, so it is not surprising that disorders in transport and lipid metabolism have a decisive role in human disease (Maxfield and Tabas, 2005).

Differences in the fatty acid profiles of rabbit pancreatic plasma membranes confirmed that all rabbits adapted to changes induced by experimental dietary lipids. Compared with the standard chow, intake of the high-cholesterol and lard diet resulted in a significant decrease in SFA (due to 16:0) and a significant increase in MUFA (due to 18:1ω-9). Similar results have been described in hepatic mitochondria using the same animal model (Aguilera et al., 2003). In our study, a slightly higher level of 18:1ω-9 in

the HCHOL diet could explain, in part, the higher 18:1ω-9 and MUFA proportions in pancreatic plasma membranes of HCHOL animals, although we should also consider other factors. The HCHOL diet had more (18:0), but this did not translate into increased incorporation of this fatty acid in plasma membranes of HCHOL group. Rather, our results suggest stimulation of 18:1ω-9 synthesis in the HCHOL group by induction of Δ9-desaturase. The HCHOL diet may induce this enzyme through increased cholesterol feeding (Muriana et al., 1992) and decreased 18:2ω-6 intake (Ntambi, 1999). It is worth noting that the level of 18:2ω-6 in HCHOL rabbits was well preserved (and even tended to increase) in spite of the lower amount in the fat source, which may be explained by depressed Δ6-desaturase activity evoked by high cholesterol consumption (Muriana et al., 1992). Overall, the observed changes in the fatty acid composition of pancreatic plasma membranes of HCHOL animals may have occurred as part of a homeostatic mechanism to control membrane fluidity following overload of SFA and cholesterol. In previous experiments in rats, we found that pancreatic plasma membranes show a marked enrichment with those fatty acids most abundant in the diet (Yago et al., 2004, 2006). This did not happen in the present study in rabbits, probably because of the above-mentioned compensatory mechanism.

With only the exception of 18:3ω-3, the fatty acid composition of rabbit pancreatic microsomes was the same regardless of diet, which contrasts with a clear influence of diet upon plasma membrane. This different behaviour could be in relation to the amount of cholesterol incorporated by both types of membrane.

Cholesterol is one of the most important regulators in said lipid organisation of cell membranes (Sullan et al., 2010; Kraft, 2013), having developed complex mechanisms to maintain the levels of the molecule within a normal range. However, when these homeostatic mechanisms are outside of this control, as in the case of atherosclerosis, the consequences can be severe. Thus, different studies have demonstrated the molecular and cellular basis of membrane lipids disorders such as Alzheimer disease (Allinguant et al., 2014; Marshall et al., 2014) and atherosclerosis (Uyy et al., 2013; Cortes et al., 2014). Increases in cholesterol content promote cell signalling cascades and growth, inhibiting apoptosis in prostate cancer cells (Zhuang et al., 2005).

There is little in the literature addressing how changes in cholesterol content in acinar cell membranes affect the secretory activity of the exocrine pancreas after a high intake of cholesterol. In fact, cholesterol is necessary for the correct formation of zymogen granules in the acinar cells of the pancreas (Wang et al., 2000; Gondré-Lewis et al., 2006). Cholesterol-deficient mouse models have exhibited a 30% decrease in the number of secretory granules and a deregulation of secretion, which is suggestive of an impaired secretory function (Gondré-Lewis et al., 2006). Lippincott-Schwartz and Phair (2012) showed the influence of lipids and cholesterol on endomembrane trafficking.

Moreover, Hao et al. (2007) have shown results that support a possible link between high cholesterol and type 2 diabetes. These authors showed that insulin secretion by pancreatic beta cells is directly affected by the levels of cholesterol in their membranes, crops C57BL/6J mouse. Too much cholesterol in the same plays a crucial role in pancreatic islet dysfunction. Bogan et al. (2012) show how cholesterol accumulation in crop secretory granules of pancreatic beta cells (MIN6) alters the size thereof, affecting their properties; this, in turn, can affect the pathophysiology of type 2 diabetes.

In our study, total cholesterol in pancreatic homogenates was significantly higher in rabbits fed the HCHOL diet than in those fed chow (C). Due to the low yield, we could not measure cholesterol in membrane fractions. However, most cellular free cholesterol (65%–80%) resides in the plasma membrane, whereas the endoplasmic reticulum (ER) is a cholesterol-poor organelle, commonly containing 0.1%–2% cellular free cholesterol (Prinz, 2002; Soccio and Breslow, 2004). This distribution persists after high cholesterol feeding, and only when the plasma membrane capacity to hold free cholesterol is exceeded does cholesterol activate the ER enzyme acyl-coenzyme A:cholesterol acyltransferase (ACAT) to form cholesterol esters which are then stored in the cores of cytosolic lipid droplets (Prinz, 2002; Soccio and Breslow, 2004). Our cholesterol and fatty acid results are consistent with the above "unequal" cholesterol distribution; if only the plasma membrane (but not the microsomes) has significantly increased its cholesterol content, it is then reasonable that only the plasma membrane (and not the microsomes) displays a fatty acid compensatory mechanism to keep membrane fluidity within physiological limits.

Adaptation of pancreatic enzymes to dietary fat has been mostly examined in rats (Snook, 1971; Sabb et al., 1986; Wicker and Puigserver, 1990; Okada et al., 1993; Diaz et al., 2003; Martinez et al., 2004), with less work conducted in other animal species (Flores et al., 1988; Ballesta et al., 1990; López-Palomo et al., 1997), and very little in humans (Emde et al., 1985; Yago et al., 1997); studies tackling this issue in rabbits are almost nonexistent (Borel et al., 1991). We show in the present study that intake of the experimental diets influences the enzyme content of rabbit pancreas. Feeding the animals with the diet rich in cholesterol and lard (HCHOL) evoked the typical adaptation of the exocrine pancreas to enhanced levels of dietary fat. Rat pancreatic lipase has shown an adaptive

response to increasing amounts of dietary fat consisting of stimulation of the synthetic rate and elevation of mRNA and enzyme levels (Snook, 1971; Sabb et al., 1986; Wicker and Puigserver, 1990). In rabbits, approximately doubling the amount of fat (6% versus 2.7%) did not modify pancreatic lipase activity after a 2-week feeding, but a significant enhancement was observed when the level of dietary fat was further increased to 12% (Borel et al., 1991). In the present study, feeding rabbits for 50 days with diets containing 5.9% triglycerides (HCHOL) instead of 3.0% (C) increased lipase activity in the pancreas significantly, supporting the view that adaptation of this enzyme depends on both the amount of fat and the length of the feeding period.

This robust response of pancreatic lipase to the level of increased fat in the rabbit is greater than that reported in other species. In weanling rats, increasing the amount of fat in the diet from 5% to 23% (by weight) significantly elevated specific lipase activity. However, lipase was not stimulated at or below a threshold of 20% fat (Sabb et al., 1986). Another important point is that, compared with rabbits, rats can be fed much higher levels of dietary fat (up to 32% by weight) without compromising growth (Sabb et al., 1986).

Our results also show enhanced colipase levels in animals fed the HCHOL diet compared with those fed the standard chow diet. The response of colipase to high-fat diets is controversial. Some authors reported an increase in colipase activity and mRNA levels resulting from high-fat diets (Wicker and Puigserver, 1990; Okada et al., 1993) and others reported no change (Saraux et al., 1982). It is interesting that the HCHOL diet increased both lipase and colipase in our study. Since optimal activity of lipase during triacylglycerol hydrolysis is obtained with a lipase:colipase (1:1) molar activity, our results can be viewed as a real mechanism to increase the lipolytic capacity of the pancreatic secretion of HCHOL rabbits in order to optimise fat digestion.

Even though our two experimental diets had practically the same amount of protein (13.5% in chow versus 12.9% in HCHOL diet), chymotrypsin content in gland homogenates was higher in rabbits fed the HCHOL diet compared to group C animals. Similarly, earlier reports in rats and pigs showed that moderate elevations in dietary fat content result in a clear increase in the pancreatic activity of chymotrypsin (Snook, 1971; Flores et al., 1988). The physiological relevance of this change is unknown, but it illustrates the phenomenon that enzyme elevations evoked by changes in a dietary constituent are not necessarily limited to the enzyme responsible for digesting that constituent.

Pancreatic amylase synthesis and content is typically enhanced when animals are fed a high-carbohydrate diet and reduced when carbohydrate is replaced with fat (Sabb et al., 1986; Snook, 1971). We were able to show only a slight, not significant attenuation in pancreatic amylase activity in HCHOL rabbits compared to control animals. Although lower than in the standard chow (50%), the still high carbohydrate content of the HCHOL diet (47.9%) may have been enough to maintain amylase activity in this group.

The diet rich in cholesterol and lard used in our study is known to produce in rabbits a combined hyperlipidemic state manifested as increased plasma cholesterol and triacylglycerols (Aguilera et al., 2005; Gonzalez-Santiago et al., 2006). Hypertriglyceridemia is an established cause of acute pancreatitis (Yadav and Pitchumoni, 2003) and so it could be argued that enzyme changes observed by us do reflect some pancreatitis-associated disturbances. However, while pancreatitis is characterised by altered pancreatic protein content with all major enzymes changing in the same direction, i.e. either increasing or decreasing (Rydzewska et al., 2001; Rakonczay et al., 2003), the consensus of pancreatic adaptation studies is that the enzyme content changes according to a pattern, and that depends on the amount of the corresponding dietary substrate. For this reason we interpret our results as nonpathological, purposive adaptive changes to diet composition.

In conclusion, we found that, as compared with a standard chow, feeding rabbits with diets enriched in cholesterol and saturated fat for 50 days influenced the enzyme content of the pancreas and the fatty acid profile of pancreatic plasma membranes, indicating an adaptation of the animal to the increased fat and cholesterol load. We also observed that the pancreas seems to display a greater resistance towards the development of lesions than do other organs such as the liver in this rabbit model.

Despite the well-known cardiovascular effects of an atherogenic diet, there are no data relating to its effect on exocrine pancreas. This study shows for the first time the consequence of this eating pattern on the exocrine pancreas. Further research on the long-term effects of this diet is required. In addition, it would be interesting to know whether the changes in membrane composition induced by an atherogenic diet affect transport mechanisms, intracellular calcium homeostasis, enzyme secretion processes, and cellular signalling pathways.

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References

Aguilera CM, Mesa MD, Ramírez-Tortosa MC, Quiles JL, Gil A (2003). Virgin olive and fish oils enhance the hepatic antioxidant defence system in atherosclerotic rabbits. Clin Nutr 22: 379–384.

Aguilera CM, Ramírez-Tortosa CL, Quiles JL, Yago MD, Martínez-Burgos MA, Martínez-Victoria E, Gil A, Ramírez-Tortosa MC (2005). Monounsaturated and omega-3 but not omega-6 polyunsaturated fatty acids improve hepatic fibrosis in hypercholesterolemic rabbits. Nutrition 21: 363–371.

Allinguant B, Clamagirand C, Potier MC (2014). Role of cholesterol metabolism in the pathogenesis of Alzheimer´s disease. Curr Opin Clin Nutr Metab Care 17: 319–323.

Ballesta MC, Mañas M, Matáix FJ, Martínez-Victoria E, Seiquer I (1990). Long-term adaptation of pancreatic response to dietary fats of different degrees of saturation: olive oil and sunflower oil. Br J Nutr 64: 487–496.

Bogan JS , Xu Y, Hao M (2012). Cholesterol accumulation increases insulin granule size and impairs membrane trafficking. Traffic 13: 1466–1480.

Borel P, Armand M, Senft M, Andre M, Lafont H, Lairon D (1991). Gastric lipase: evidence of an adaptive response to dietary fat in the rabbit. Gastroenterology 100: 1582–1589.

Chowdhury P, Nishikawa M, Blevins GW Jr, Rayford PL (2000). Response of rat exocrine pancreas to high-fat and high-carbohydrate diets. Proc Soc Exp Biol Med 223: 310–315.

Cortes VA, Busso D, Maiz A, Arteaga A, Nervi F, Rigotti A (2014). Physiological and pathological implications of cholesterol. Front Biosci 19: 416–428.

Díaz RJ, Yago MD, Martínez-Victoria E, Naranjo JA, Martínez MA, Mañas M (2003). Comparison of the effects of dietary sunflower oil and virgin olive oil on rat exocrine pancreatic secretion in vivo. Lipids 38: 1119–1126.

Emde C, Liehr RM, Gregor M, Pleul O, Riecken EO, Menge H (1985). Lack of adaptive changes in human pancreatic amylase and lipase secretion in response to high-carbohydrate, low-fat diet applied by a 10-day continuous intraduodenal infusion. Dig Dis Sci 30: 204–210.

Erlanson-Albertsson C, Larsson A, Duan R (1987). Secretion of pancreatic lipase and colipase from rat pancreas. Pancreas 2: 531–535.

Fernández ML, Webb D (2008). The LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk. J Am Coll Nutr 27: 1–5.

Flores CA, Brannon PM, Bustamante SA, Bezerra J, Butler KT, Goda T, Koldovský O (1988). Effect of diet on intestinal and pancreatic enzyme activities in the pig. J Pediatr Gastroenterol Nutr 7: 914–921.

Gondré-Lewis M, Petrache HI, Wassif CA, Harries D, Parsegian A, Porter FD, Loh YP (2006). Abnormal sterols in cholesterol-deficiency diseases cause secretory granule malformation and decreased membrane curvature. J Cell Sci 119: 1876–1885.

González-Santiago M, Martín-Bautista E, Carrero JJ, Fonollá J, Baró L, Bartolomé MV, Gil-Loyzaga E, López-Huertas E (2006). One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. Atherosclerosis 188: 35–42.

Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW (2007). Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic β-cell dysfunction. Diabetes 56: 2328–2338.

Hickson JCD (1970). The secretion of pancreatic juice in response to stimulation of the vagus nerves in the pig. J Physiol 206: 275–297.

Jacobs LR (1983). Effect of short-term dietary fat on cell growth in rat gastrointestinal mucosa and pancreas. Am J Clin Nutr 37: 361–367.

Kraft ML (2013). Plasma membrane organization and function: moving past liquid rafts. Mol Biol Cell 24: 2765–2768.

Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA et al. (2001). Revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. J Nutr 131: 132–146.

Lepage G, Roy CC (1986). Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27: 114–120.

Lin Y, Tamakoshi A, Hayakawa T, Naruse S, Kitagawa M, Ohno Y (2005). Nutritional factors and risk of pancreatic cancer: a population-based case-control study based on direct interview in Japan. J Gastroenterol 40: 297–301.

Lippincott-Schwartz J, Phair RD (2010). Lipids and cholesterol as regulators of traffic in the endomembrane system. Annu Rev Biophys 39: 559–578.

López-Palomo V, Martínez-Victoria E, Yago MD, Lupiani MJ, Mañas M (1997). Regulation by diet of the pancreas enzyme content of suckling goats. Arch Physiol Biochem 105: 566–571.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275.

Marshall LL, Stimpson SE, Hyland R, Coorssen JR, Myers SJ (2014). Increased lipid droplet accumulation associated with a peripheral sensory neuropathy. J Chem Biol 7: 67–76.

Martínez MA, Lajas AI, Yago MD, Redondo PC, Granados MP, González A, Rosado A, Martínez-Victoria E, Mañas M, Pariente JA (2004). Dietary virgin olive oil enhances secretagogue-evoked calcium signaling in rat pancreatic acinar cells. Nutrition 20: 536–541.

Maxfield FR, Tabas I (2005). Role of cholesterol and lipid organization in disease. Nature 438: 612–621.

Meldolesi J, Jamieson JD, Palade GE (1971). Composition of cellular membranes in the pancreas of the guinea pig. I. Isolation of membrane fractions. J Cell Biol 49: 109–129.

Muriana FJ, Vázquez CM, Ruiz-Gutiérrez V (1992). Fatty acid composition and properties of the liver microsomal membrane of rats fed diets enriched with cholesterol. J Biochem 112: 562–567.

Noelting G, Bernfield P (1948). Sur les enzymes amylolytiques III. La β-amylase: dosage d'activité et contrôle de l'absence de α-amylase. Helv Chim Acta 31: 286–290.

Nothlings U, Wilkens LR, Murphy SP, Hankin JH, Henderson BE, Kolonel LN (2005). Meat and fat intake as risk factors for pancreatic cancer: the multiethnic cohort study. J Natl Cancer Inst 97: 1458–1465.

Ntambi JM (1999). Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res 40: 1549–1558.

Okada S, York DA, Bray GA (1993). Procolipase mRNA: tissue localization and effects of diet and adrenalectomy. Biochem J 292: 787–789.

Paeratakul S, Ferdinand DP, Champagne CM, Ryan DH, Bray GA (2003). Fast-food consumption among US adults and children: dietary and nutrient intake profile. J Am Diet Assoc 103: 1332–1338.

Prinz W (2002). Cholesterol trafficking in the secretory and endocytic systems. Semin Cell Dev Biol 13: 197–203.

Quiles JL, Mesa MD, Ramírez-Tortosa CL, Aguilera CM, Battino M, Gil A, Ramírez-Tortosa MC (2002). Curcuma longa extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. Arterioscler Thromb Vasc Biol 22: 1225–1231.

Rakonczay Z Jr, Jarmay K, Kaszaki J, Mandi Y, Duda E, Hegyi P, Boros I, Lonovics J, Takacs T (2003). NF-kappaB activation is detrimental in arginine-induced acute pancreatitis. Free Radic Biol Med 34: 696–709.

Reboud JP, Ben Abdeljlil A, Desnuelle P (1962). Variations in the enzyme content of the rat pancreas as a function of the composition of the diet. Biochim Biophys Acta 58: 326–337.

Roebuck BD, Yager JD Jr, Longnecker DS, Wilpone SA (1981). Promotion by unsaturated fat of azaserine-induced pancreatic carcinogenesis in the rat. Cancer Res 41: 3961–3966.

Rydzewska G, Jurkowska G, Dzieciol J, Faszczewska A, Wroblewski E, Gabryelewicz A (2001). Does chronic ethanol administration have influence on pancreatic regeneration in the course of caerulein induced acute pancreatitis in rats. J Physiol Pharmacol 52: 835–849.

Sabb JE, Godfrey PM, Brannon PM (1986). Adaptive response of rat pancreatic lipase to dietary fat: effects of amount and type of fat. J Nutr 116: 892–899.

Saraux B, Girard-Globa A, Ouagued M, Vacher D (1982). Response of the exocrine pancreas to quantitative and qualitative variations in dietary lipids. Am J Physiol 243: G10–G15.

Serrano P, Yago MD, Mañas M, Calpena R, Matáix J, Martínez-Victoria E (1997). Influence of type of dietary fat (olive and sunflower oil) upon gastric acid secretion and release of gastrin, somatostatin, and peptide YY in man. Dig Dis Sci 42: 626–633.

Smith JG, Luk K, Schulz CA, Engert JC, Do R, Hindy G, Rukh G, Dufresne L, Almgren P, Owens DS et al. (2014). Association of low-density lipoprotein cholesterol-related genetic variants with aortic valve calcium and incident aortic stenosis. JAMA 312: 1764–1771.

Soccio RE, Breslow JL (2004). Intracellular cholesterol transport. Arterioscler Thromb Vasc Biol 24: 1150–1160.

Song Y, Joung H, Engelhardt K, Yoo SY, Paik HY (2005). Traditional v. modified dietary patterns and their influence on adolescents' nutritional profile. Br J Nutr 93: 943–949.

Snook JT (1971). Dietary regulation of pancreatic enzymes in the rat with emphasis on carbohydrate. Am J Physiol 221: 1383–1387.

Sullan RM, Li JK, Hao C, Walker GC, Zou S (2010). Cholesterol-dependent nanomechanical stability of phase-segregated multicomponent lipid bilayers. Biophys 99: 507–516.

Uyy E, Ivan L, Boteanu RM, Suica VI, Antohe F (2013). High-fat diet alters protein composition of detergent-resistant membrane microdomains. Cell Tissue Res 354: 771–778.

Wang Y, Thiele C, Huttner WB (2000). Cholesterol is required for the formation of regulated and constitutive secretory vesicles from the trans-Golgi network. Traffic 1: 952–962.

Wicker C, Puigserver A (1990) Expression of rat pancreatic lipase gene is modulated by a lipid-rich diet at a transcriptional level. Biochem Biophys Res Commun 166: 358–364.

World Health Organization (2003). Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. Geneva: World Health Organization.

Yago MD, Díaz RJ, Martínez MA, Audi N, Naranjo JA, Martínez-Victoria E, Mañas M (2006). Effects of the type of dietary fat on acetylcholine-evoked amylase secretion and calcium mobilization in isolated rat pancreatic acinar cells. J Nutr Biochem 17: 242–249.

Yago MD, Díaz RJ, Ramírez R, Martínez MA, Mañas M, Martínez-Victoria E (2004). Dietary-induced changes in the fatty acid profile of rat pancreatic membranes are associated with modifications in acinar cell function and signalling. Br J Nutr 91: 227–234.

Yago MD, González MV, Martínez-Victoria E, Matáix J, Medrano J, Calpena R, Pérez MT, Mañas M (1997). Pancreatic enzyme secretion in response to test meals differing in the quality of dietary fat (olive and sunflower seed oils) in human subjects. Br J Nutr 78: 27–39.

Yago MD, Martínez-Victoria E, Mañas M, Martínez MA, Matáix J (1997). Plasma peptide YY and pancreatic polypeptide in dogs after long-term adaptation to dietary fats of different degrees of saturation: olive and sunflower oil. J Nutr Biochem 8: 502–507.

Yadav D, Pitchumoni CS (2003). Issues in hyperlipidemic pancreatitis. J Clin Gastroenterol 36: 54–62.

Zhuang L, Kim J, Adam RM, Solomon KR, Freeman MR (2005). Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. J Clin Invest 115: 959–968.