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HISTOLOGICAL CHANGES IN THE RAT EXOCRINE PANCREAS DUE TO DIFFERENT LEVELS OF SUNFLOWER OIL IN THE DIET

VESNA KOKO*, RADMILA GLIŠIĆ**, MAJA ČAKIĆ***, LJILJANA PETRONIJEVIĆ****, JELENA RADOVANOVIĆ***, VANJA RISTIĆ*, ANA LABAN***** and ZORICA STOJŠIĆ*****

**Institute for Medical Research, Belgrade, **Institute for Biological Research, Faculty of Science, Kragujevac, ***Institute of Zoology, Faculty of Science, Belgrade, ****Institute of Digestive Disease, Medical Faculty, Belgrade *****Institute for Pathology, Medical Faculty, Belgrade.*

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Male Wistar rats were fed cereal-based diets which were enriched with 5 (F_5) and 20% (F_{20}) of sunflower oil. A foodmatched control group (CR) was evaluated in addition. After 6 months of experimental treatment all animals were sacrificed during Nembutal anaesthesia. The pancreas was rapidly removed, weighed in air and processed for light and electron microscopic examination. Weibel's test lattice was used for stereological investigation of the exocrine pancreas. Histological results obtained showed that there were many acini with small and large vacuoles in the rats fed with F_5 and F_{20} diets. In the controls (C and CR rats) there were no such alterations in the acinar cells. Ultrastructural investigations confirmed that those vacuoles represented typical autophagic vacuoles which were located near to the acinar lumen and lateral plasma membrane. Large interstitial spaces between acini were present. Signs of very intensive secretion were also observed. All results obtained indicated that a mild form of acute pancreatitis existed.

Key words: Oil diets; Pancreas; Rat; Histology

INTRODUCTION

Factors proposed to play a role in the pathogenesis of acute pancreatitis include a secretagogue (such as cholecystokinin), lysosomal enzymes, bile, alcohol, phospholipase A_2, enterokinase, trypsin, oxygen-derived free radicals, imbalance between protease and protease inhibitors (Niederau et al., 1985; Singh and Simsek, 1990; Powers et al., 1986; Rutledge et al., 1987; Sanfey et al., 1985; Terry et al., 1985; Toouli et al., 1985; Wedgwood et al., 1986; Largman et al., 1986).

Experimental pancreatitis can be induced by sodium taurocholate, duct ligation, supramaximal stimulation, with excessive doses of synthetic pancreatic secretagogue (caerulein) and by different diets (Aho et al., 1980; Aho and Nevalainen, 1980; Nevalainen and Seppa, 1975; Lampel and Kern, 1977; Kioke et al., 1982).

Extensive experimental evidence has implicated oxygen-derived free radicals as important mediators in the pathogenesis of numerous forms of tissue

injury (Slater, 1984; Mc Cord and Roy, 1982), as well as in the early pathogenesis of acute pancreatitis (Sanffey et al., 1986).

Oxigen-derived free radicals can produce peroxidation of biological membranes due to alteration of their fluidity (Poli et al., 1987). Cell membrane fluidity can be altered by the fat content of the diet (Periago et al., 1988; Chi-Ru Lee et al., 1989). Diets rich in unsaturated fatty acid have been reported to increase cellular membrane fluidity (Berlin et al., 1980) and can promote the growth of mammary tumors through the generation of lipid peroxy radicals and/or oxygen radicals (Hillyard et al., 1980).

Rao and Reddy cited Boorman's data that rats given corn oil (rich in polyunsaturated fatty acid) as a control vehicle, developed acinar cell hyperplasia, acinar cell adenoma and acinar carcinoma in the pancreas (Rao and Reddy, 1985).

There are no histological and stereological data on the rat pancreas after a long- lasting experiment with a high-fat diet. For this reason, the aim of this study was to examine the histological picture of the rat pancreas after long-term treatment of rats with different levels (5 and 20%) of sunflower oil which is also rich in polyunsaturated fatty acids.

MATERIAL AND METHODS

The experiment was performed on male Wistar rats (Institute Colony, Vinča) weighing approximately 200 g at the beginning of the experiment. Each group consisted of ten animals. They were housed individually in wire -mesh cages under controlled conditions of temperature (23 ± 1^0C), humidity and lighting (light on 0600-1800 hours). The animals were fed on of three cereal based diets: a nutritionally complete experimental diet for rats and mice, including the vitamin and mineral mixture described in J. Nutr. 107: 1340-1348, 1977, (diet C), a diet with 5% (wt) of sunflower oil added (F_5), and a diet with 20% (wt) of sunflower oil added (F_{20}) The quantity of all ingredients in diets F_5 and F_{20} was identical as in diet C, except for starch and fat. The composition of the diets is shown in Table 1.

The control group of rats (C) was fed diet C ad libitum and consumed 21.4 ± 0.40 g of food per day. The mean gross energy intake per day was 348 ± 6.5 kJ. The F_5 and F_{20} groups of rats were fed 15 g per day of diet F_5 and F_{20}, respectively. The mean gross energy intake per day was 253 kJ for the rats of group F_5 and 302 kJ for F_{20} rats. A food- matched control group (CR. control restricted 15 g of diet C per day and mean gross daily energy intake - 244 kJ) was evaluated in addition. All animals were offered drinking water ad libitum.

The experiment lasted 6 months. After an overnight fast all animals were sacrificed during i. p. Nembutal anaesthesia. The pancreas was rapidly removed and weighed in air. The tissue was fixed in Bouin's solution, dehydrated and embedded in paraffin Sections (6 μm) were stained by hematoxylin- eosin for the light microscopic examination. Samples of pancreatic tissue were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer at pH= 7.4 for 24 h and post

fixed in 2% osmium tetroxide for 1 h. The samples were washed in the buffer, dehydrated in alcohol, and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and studied in a Philips CM 12 microscope. For orienting purposes, semi -thin (1μm) Araldite sections were stained with alkaline toluidine blue.

Table 1. Composition of diets

Ingredients	Control	Sunflowrer oil diets	
	C	F_5	F_{20}
	g%	5%	20%
Dried skimmed milk	8.0	8.0	8.0
Fish meal	8.0	8.0	8.0
Meat meal	4.0	4.0	4.0
Wheat standard middlings	10.0	10.0	10.0
Cornstarch	20.0	20.0	20.0
Alfalfa meal dehydrated	4.0	4.0	4.0
Soybean meal	14.0	14.0	14.0
Torula yeast-dried	3.0	3.0	3.0
Starch	24.2	21.5	6.5
Sunflower oil	0.80	5.0	20.0
Lard	1.50	—	—
Ground limestone	0.50	0.50	0.50
Dicalcium phosphate	1.25	1.25	1.25
Sodium chloride	0.50	0.50	0.50
Premixes[1]	0.25	0.25	0.25
kJ/100 g	1627.38	1666.16	20.12.71
Crude protein	21.23	21.23	21.23
Crude fat	5.04	7.74	22.74
Crude carbohyfrate	62.12	59.42	44.42
Crude fiber	3.71	3.71	3.71
Ash	5.40	5.40	5.40

1. Vitamin and mineral premixes provided per kg diet: vitamin A (stabilized), 6.050 IU; vitamin D_3 5.060 IU; vitamin K, 3.1 mg; a-tocopheryl acetate, 22 IU; choline, 0.6 g; folic acid, 2.4 mg; niacin, 33 mg; d-pantothenic acid, 20 mg; riboflavin, 3.7 mg; thiamin, 11 mg; vitamin B-12, 4.4 mg; pyridoxine, 1.9 mg; biotin, 0.15 mg; cobalt, 0.44 mg; copper, 4.4 mg; iron, 132 mg; manganese, 66 mg; zinc, 1.8 mg; iodine, 1.5 mg, BHT (antioxidant), 88.5 mg.

Histological examinations of the pancreas were then performed and pancreatic lesions were classified according to Takahashi and Pour (1978). We observed that the main lesion in the pancreas of the F_5 and F_{20} groups of rats was acinar cell vacuolisation.

Morphometric examinations were performed in the teleinsular regions of the exocrine pancreas. Weibel's test lattice (M_{42}) was used to determine the volume fraction of the acini, small blood vessels, intralobular ducts, connective tissue fibres and interstitial spaces around the acini (Weibel et al, 1966). The

number of acini per unit area (mm^2) was calculated from the formula NA=N/AT, where N is the number of sectioned profiles of acini and AT the area of section. The numerical density of acini was determined using the method of DeHoff and Rhines as sescribed by Aherne and Dunnill (1982). Ten different fields, randomly selected, were measured for each animal, using an Olympus light microscope at an objective magnification of 100 x.

The mean radius of the acini and nuclei was determined using an eyepiece micrometer.

All results were analyzed by using one- way analysis of variance (ANOVA) and Tuckey's t-test for multiple comparisons.

RESULTS

The final body weights of the rats were significantly different (Table 2).

Table 2. Effect of different levels (5 and 20%) of sunflower oil in the diet on growth in rats during 6 months of treatment

Measurement	C (n=10)	CR (n=10)	F_5 (n=10)	F_{20} (n=10)
Final body weight (g)[a, b]	462±12.99*	350±9.33	339±11.09	372±7.41
Pancreatic weight (g)[a, b]	1.38±0.06	1.14±0.08	1.15±0.04	1.11±0.07
Relative pancr. wt. (%)	0.303±0.015	0.327±0.018	0.342±0.017	0.295±0.019

*Values are means ± SEM for ten animals in each group.
Significantly different ($p<0.05$ or less): a=food effect
b=fat effect

Thus, the growth curves for groups CR, F_5 and F_{20} rats were decreased, as compared with the control group of rats (Figure 1).

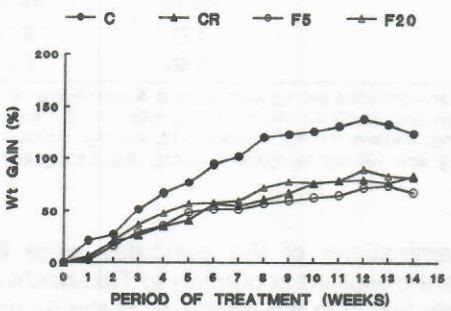

Figure 1. Body weight gain of C, CR, F_5 and F_{20} groups of rats.

Pancreatic weight was also decreased in animals of groups CR, F_5 and F_{20} and those differences reached statistical significance. Relative pancreatic weights were similar in all groups of rats.

Stereological investigations of the rat exocrine pancreas are summarized in Table 3.

Table 3. Stereological parameters of the exocrine pancreas in rats fed with diets containing 5 and 20% of sunflower oil for 6 months

	C (n=10)	CR (n=10)	F_5 (n=10)	F_{20} (n=10)
	Volume density (mm^0)			
Acini	0.80±0.006*	0.82±0.008	0.78±0.007	0.80±0.005
Intralobular ducts[b]	0.04±0.003	0.03±0.003	0.02±0.002	0.02±0.002
Small blood vessels[b]	0.02±0.002	0.02±0.001	0.01±0.001	0.01±0.001
Connective tissue fibres	0.12±0.004	0.11±0.004	0.12±0.005	0.11±0.006
Interstitial spaces[b]	0.02±0.002	0.02±0.003	0.06±0.005	0.06±0.003
	Numerical density (mm^{-3})			
Acini (x 10^2)	361.2±12.84	359.6±8.02	347.8±12.45	342.2±11.5
	Number of acini per unit area (mm^2)			
Acini[a, b]	1317±15.29	1031±29.33	1165±26.15	1148±18.98
	Mean diameter of acini and nuclei (μm)			
Acini[a, b]	18.4±0.52	14.4±0.33	16.8±0.30	16.9±0.41
Nuclei [b]	3.6±0.11	3.0±0.17	4.2±0.28	4.0±0.17

* Values are MEAN ± SEM for ten animals in each group
Significantly different ($p<0.05$ or less) a= food effect;
b= fat effect

The results obtained for volume density of acini in all groups of rats were similar. Volume density of connective tissue fibres did not differ much between the controls (C, CR) and those given more fat. A decrease in the volume density of small blood vessels, as well as of intralobular ducts was observed in both groups of rats fed with sunflower oil. Statistically significant differences were found both for the volume density of intralobular ducts and small blood vessels.

Volume density of the interstitial spaces around the acini was significantly increased in animals of groups F_5 and F_{20}, as compared with both control groups of animals. The volume density of interstitial spaces ranged between 0.014-0. 033 in animals of group C and 0. 010-0. 050 in animals of group CR. In the F_5 and F_{20} groups of rats those values were between 0.038-0. 083 and 0.050-0.076, respectively. The results obtained for the volume density of the interstitial spaces around the acini indicated that edema existed.

The numerical density of acini was similar in all groups of rats, but the number of acini per unit area (mm^2) was decreased in all groups of food restricted rats (CR, F_5 and F_{20}). Those values were significantly greater in groups F_5 and F_{20}, as compared with group CR which indicated that the level of fat in the diet had an effect.

The mean radius of the acini was significantly changed due to the restriction in food intake. Thus, daily food restriction during the whole experiment caused the acini in the CR, F_5 and F_{20} groups of rats to become smaller than

in the control (C). The mean radius of the acini and nuclei in the F_5 and F_{20} groups of rats was greater, as compared with the CR group of rats.

Histological examination of the exocrine pancreas after long-term feeding (6 months) with sunflower oil diets (F_5 and F_{20}) showed that marked vacuolisation of the acinar cells existed (Figure 2). Minimal cell necrosis also persisted, as well as minor cell infiltration. We observed that intralobular spaces were enlarged indicating that edema was also present.

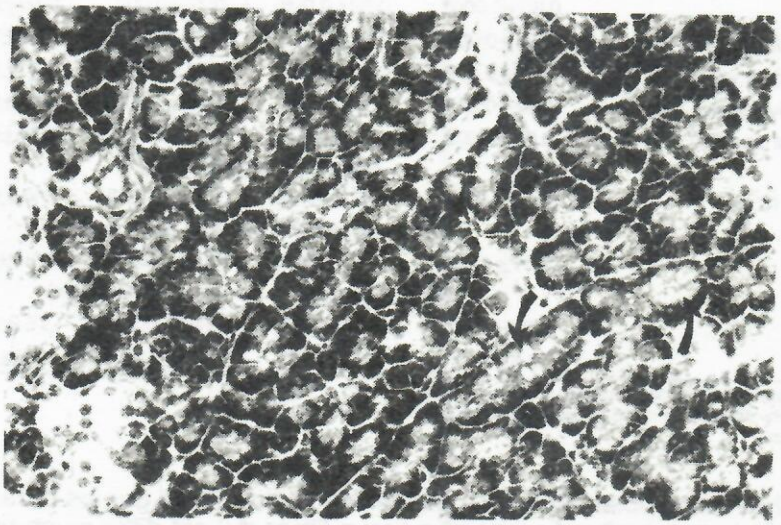

Figure 2. Marked vacuolization of acinar cells. Small and large vacuoles near to the acinar lumina (arrow). Magnification x 200.

We then analyzed the altered acinar cells and found that large and small vacuoles represented typical autophagic vacuoles. The vacuoles were observed predominantly in the Golgi region. Fusion of autophagic vacuoles was also observed. Regions of close apposition between the membrane of zymogen granules and secondary lysosomes were frequently seen. The large vacuoles in general contained a flocculent material. Zymogen granules were also present in those vacuoles (Figure 3). Fragments of endoplasmic reticulum, mitochondria and small vesicles were observed inside the limiting membrane, indicating the initiation of an autophagic process in the exocrine cells. Large vacuoles approached the basolateral plasma membrane.

Mature zymogen granules were crowded around the acinar lumen which may be separated from the large vacuoles only by a small rim of cytoplasm. Acinar lumina were enlarged and often filled by the blebs and spiralized membrane material.

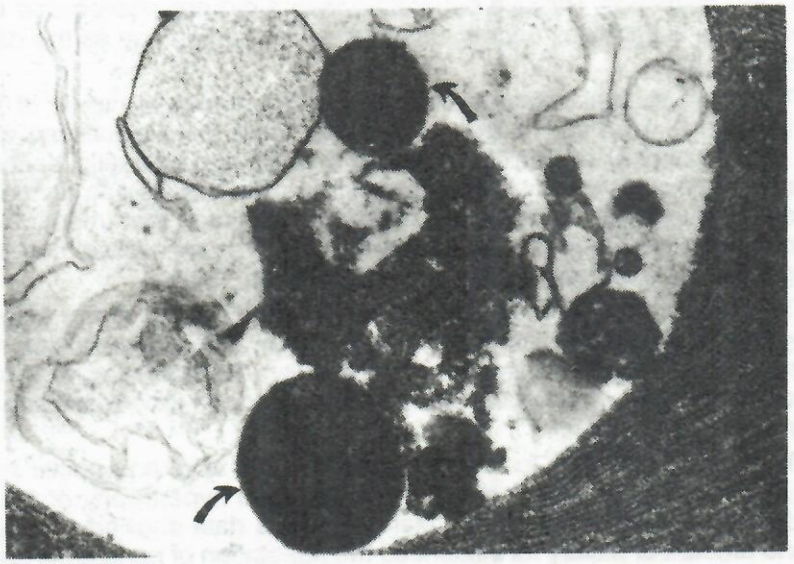

Figure 3. Flocculent material and mature zymogen granules (arrow) in the large vacuole. Magnification x 29900.

In the apical regions of the acinar cells many omega figures and mature zymogen granules were seen in the acinar lumen indicating intensive exocytotic activity along the luminal plasma membrane.

Animals that were fed with the F_{20} diet had abnormalities identical with those descibed above, except that the alterations were slightly more severe and affected a greater number of cells.

There was no accumulation of fat droplets in any acinar cells.

DISCUSSION

This work was primarily planned to be a histological and stereological examination of the rat exocrine pancreas after long-term high- fat diets (sunflower oil) because, to date, there have been no such morphological and morphometric studies in this field.

During the whole experimental period (6 months), the rats were fed diets enriched in fat (5 and 20% of sunflower oil) offered at 15 g of feed per day. Food intake was restricted to eliminate any influence of caloric excess that could be dangerous in combination with the high-fat diet, as reported earlier (Birt, 1987). For that reason a pair-fed control group of rats (CR, control restricted) was also evaluated in addition.

The results obtained for body and pancreatic weight indicated that a significant decrease had occurred in the CR, F_5 and F_{20} groups of rats due

to the food restriction. Relative pancreatic weight did not change and showed that pancreas weight was decreased in the same percentage as the decrease in body weight.

The stereological results demonstrated the strong influence of high-fat diets (F_5 and F_{20}) on the rat pancreas. Thus, there were significant changes in the volume density of small blood vessels, ducts and interstitial spaces. The significant increase in the volume density of interstitial speces with a simultanous decrease in the small blood vessels and ducts indicated that edema fluid was present and compressed the capillaries and ducts as mentioned earlier in the pathogenesis of pancreatitis (Tuzhilin and Dreiling, 1977).

The significant decrease in the number and diameter of the acini in the CR, F_5 and F_{20} groups of rats was not reflected in a decrease in their number per unit volume (as shown by numerical density). Probably this is a result of the reduction of the whole gland due to the continuous food restriction during the long-term experiment.

The results obtained for mean diameter of the acini and nuclei in the F_5 and F_{20} groups of rats showed that there was mild hypertrophy of the acinar cells due to the high- fat nutrition. Many literature data showed that both the type and amount of dietary fat influenced the adaptation of pancreatic enzymes, and that those two factors interacted (Houghton et al., 1983; Wicker and Puigserver, 1987; Deschodt-Lanckman et al., 1971; Sabb et al., 1986). Our high-fat diets (F_5 and F_{20}) had a high percentage of polyunstaturated fatty acid which could have acted on the endocrine cells in the rat intestinal mucosa. It was reported recently that polyunstaturated fatty acids (PUFA) significantly raised the tissue concentration of GIP and neurotensin (NT) in the rat duodenal mucosa (Ekeke et al, 1990) and that NT, together with CCK, had a trophic effect on the rat pancreas. (Nustede et al., 1990). On the other hand, it is also possible that the increased concentration of GIP, which has an insulinotrophic effect on the B cells of the islets of Langerhans, caused further enlargement of acinar cells.Information about the possible trophic effect of insulin on the exocrine pancreas is derived from a study of insulin deficiency (Gepts, 1965; Maclean and Ogilivie, 1955).

Polyunsaturated fatty acids have a strong effect on secretin release in the gut (Brannon, 1990). Submaximal doses of secretin stimulated discharge of enzymes from acinar cells which was paralleled by a high frequency of exocytotic images at the luminal plasma membrane and accompanied by the occurrence of membrane fragments in the luminal space (Raush et al., 1985). Those results are in accordance with our histological observations and indicate that long-term high-fat diets can increase membrane shedding, as shown above and observed also in human pancreatic adenocarcinoma (Kern et al., 1986).

The altered acinar cells showed a mild form of acute interstitial pancreatitis. Jugding by the histological results obtained in our present work, this form of acute pancreatitis was identical with that found in rats treated with supramaximal doses of caerulein, an analogue of CCK (Lampel and Kern, 1977; Adler et al., 1979) which was produced by peroxidation of the biological membrane during liberation of oxygen- derived free radicals (Guice et al., 1986; Wisner et al., 1988; Schoenberg et al., 1990).

Adler at al., (1982) found that supramaximal doses of caerulein induced acute edematous pancreatitis in the rat. The earliest alterations were observed at the membrane of zymogen granules and the plasma membrane. Biochemical studies analyzing amino acid transport across the pancreatic plasma membrane demonstrated severe disturbances indicating changes in membrane integrity.

Previous data showed (Solcia et al., 1972) that supramaximal doses of caerulein during 6 months caused acute interstitial pancreatitis in rats which could recover after cessation of the treatment. These authors suggested that excessive release of pancreatic secretagogues might play a role in the onset of acute or chronic pancreatitis in man.

We conclude that a long-term high-fat diet (sunflower oir, rich in PUFA) causes hyperactivation of pancreatic acinar cells and, probably in combination with altered fluidity and peroxidation of biological membranes, in some regions can produce a mild form of acute pancreatitis.

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RAZLIČITI NIVOI SUNCOKRETOVOG ULJA U DIJETI I HISTOLOŠKE PROMENE U EGZOKRINOM PANKREASU

VESNA KOKO, RADMILA GLIŠIĆ, MAJA ČAKIĆ, LJILJANA PETRONIJEVIĆ, JELENA RADOVANOVIĆ, VANJA RISTIĆ, ANA LABAN i ZORICA STOJŠIĆ

SADRŽAJ

Mužjaci Wistar pacova hranjeni su standardnom peletiranom hranom koja je obogaćena s 5 (F_5) i 20% (F_{20}) suncokretovog ulja. Kontrolna grupa pacova (C) hranjena je standardnom peletiranom laboratorijskom hranom ad libitum. Druga kontrolna grupa životinja (CR) hranjena je hranom kao i grupa C pri čemu je unos hrane bio ograničen na 15 g dnevno kao što su hranjene životinje grupa F_5 i F_{20}. Posle 6 meseci eksperimentalnog tretmana sve životinje su žrtvovane u narkozi Nembutala. Pankreas je brzo izolovan, izmeren i sproveden za ispitivanja na svetlosnoj i elektronskoj mikroskopiji. Weibel-ova testna mrežica (M_{42}) je upotrebljena za stereološka ispitivanja na nivou svetlosne mikroskopije. Dobijeni histološki rezultati pokazali su prisustvo velikog broja malih i velikih vakuola u acinusnim ćelijama pacova grupa F_5 i F_{20}. U kontrolnih pacova (grupe C i CR) nisu nađene vakuole u acinusnim ćelijama. Ultrastrukturna ispitivanja su potvrdila da vakuole predstavljaju tipične autofagne vakuole koje su locirane u blizini acinusnih lumena i lateralne plazma membrane. Veliki intersticajalni prostori su postojali između acinusa, što je potvrđeno i stereološkom analizom. Dobijeni rezultati ukazivali su na postojanje blage forme akutnog pankreatitisa u pacova koji su jeli hranu obogaćenu suncokretovim uljem.