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Effect of high fiber intake on pancreatic lysosomal stability in ethanol-fed rats

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Chronic ethanol consumption increases the fragility of pancreatic lysosomes, but the effect of a high fiber intake, alone or combined with alcohol abuse, on the lysosomal stability has not been studied. Furthermore, it is not yet known whether these treatments could predispose the exocrine pancreas to a greater damage after cerulein-induced acute pancreatitis. Cytosolic specific activity of three lysosomal enzymes, N-acetyl-β-D-glucosaminidase (NAG), cathepsin B, and β-glucuronidase were measured, as an index of lysosomal stability in pancreas from control rats and rats under chronic alcohol and/or high fiber intake. Cathepsin B is the only enzyme with significantly increased specific activity after chronic ethanol consumption and, moreover, its specific activity undergoes the highest increase after cerulein-induced acute pancreatitis, in all the groups of rats, when compared with the remainder enzymes. When pancreatitis was induced by cerulein, the combination of chronic alcohol and high fiber intake produces a significant decrease in the cytosolic specific activity of N-acetyl-β-D-glucosaminidase and β-glucuronidase when compared with chronic alcohol alone. Our results suggest that fiber partially avoids the damage of ethanol on pancreatic lysosomes, reducing the effects of pancreatitis. (J. Nutr. Biochem. 9:164–169, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Though many authors have established a cause-effect relationship between alcohol and acute and chronic pancreatitis,[1] there is a great deal of controversy about the precise role of ethanol in the etiology of this disease. Furthermore, one of the most known hypothesis on the origin of pancreatitis points to the role of the lysosomal enzymes, particularly proteases, as activators of the digestive proenzymes, this being one of the primary events that could subsequently develop the disease.[2–5] In fact, many authors[5–11] have reported that one of the early events in a particular case of acute experimental pancreatitis, that is induced by cerulein, is a redistribution of the lysosomal enzymes to the zymogen granules, with a colocalization of both types of enzymes, which could finally produce the activation of the digestive proenzymes. Moreover, Wilson et al.[12] have shown that ethanol consumption increases the fragility of pancreatic lysosomes and Haber et al.[13] have recently found that ethanol administration also increases the fragility of pancreatic zymogen granules, proposing that zymogen granule fragility may play an early part in the pathogenesis of alcoholic pancreatitis, by permitting a direct contact between digestive and lysosomal enzymes. However, the pathogenic role of lysosomal enzymes in the induction of the pancreatic damage has been questioned recently[10,14] and some authors[2,3] suggest that lysosomal hydrolases may not play a role in initiating enzyme activation, being rather more important in the intracellular degradation of active enzymes and hence suggesting this is a defensive mechanism against pancreatitis, and therefore not a cause but a consequence of the disease. Anyway, many authors point to the involvement of pancreatic lysosomal enzymes in the pathophysiology of alcoholic pancreatitis, though their real role remains obscure.

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On the other hand, confusing results have been reported concerning the effect of a high fiber diet on the exocrine pancreatic function. Some authors[15,16] consider that fiber does not affect the exocrine pancreatic secretion, whereas others state that fiber stimulates this secretion[17–20] and some establish a possible correlation between the fiber ingestion and pancreatic injury.[19,21] However, the action of the ingestion of a high fiber diet, alone or combined with alcohol abuse, on the stability of pancreatic lysosomes has not yet been studied.

This prompted us to study the stability of pancreatic lysosomes under chronic alcohol and high fiber intake, also trying to know whether these treatments could in some way predispose the exocrine pancreas to a greater damage when acute pancreatitis is induced by cerulein.

Methods and materials

Animals and treatments

Male Wistar rats, initially weighing 125 to 150 g received the following treatments for 6 months: NW group: rats fed a standard laboratory chow diet and water for drinking ad libitum; (2) FW group: rats fed a high fiber diet (15% cellulose) and water for drinking ad libitum; (3) NE group: rats fed a standard laboratory chow diet and 20% (vol/vol) ethanol for drinking ad libitum; and (4) FE group: rats fed a high fiber diet (15% cellulose) and 20% (vol/vol) ethanol for drinking ad libitum.

In alcohol-treated groups, the ethanol was given to the rats as 5% concentration (vol/vol) on the first week, 10% (vol/vol) on the second week, 15% (vol/vol) on the third week, and 20% (vol/vol) from the fourth week to the end of the experimental period.

The composition of diets, expressed as percentage of total energy, is the following: 16% total protein, 4% total fat, 8% minerals, 1% calcium, 0.64% phosporus, 0.3% sodium, 12,000 Ul/Kg vitamin A, 1200 Ul/Kg vitamin D3, 10m/Kg vitamin E (alphatocopherol), 4 mg/Kg copper (pentahydrated sulfate), traces of antioxidants. Both diets differ only in their content of fiber. The high fiber diet contains 15% cellulose, whereas the standard laboratory diet contains 8% cellulose. The higher percentage of cellulose was obtained by decreasing the amount of other carbohydrates from 23.6% starch in standard diet to 15.7% starch in high fiber diet.

After the experimental treatment, rats were killed by decapitation. The pancreas was quickly removed, trimmed of adipose tissue, and samples were taken for light microscopy and for isolation of enriched lysosomal fraction.

In some animals of each group, acute pancreatitis was induced according to the method of Lampel and Kern.[22] Briefly, four subcutaneous injections of cerulein (20 μg/Kg body weight) were applied at 1-hr intervals. Five hours after the last dose, the animals were killed by cervical dislocation and the pancreas was removed. These groups of animals were named PNW, PFW, PNE, and PFE.

Isolation of an enriched lysosomal fraction

The isolation of an enriched lysosomal fraction was done according to the method of Wilson et al.[12]

Briefly, 100 mg of pancreatic tissue from each animal was homogenized in a medium containing 0.25 M sucrose and 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.025% soybean trypsin inhibitor in a Omni 1,000 homogenizer during 20 sec at 20,000 rpm. The homogenate was then centrifuged at 540 g for 6 min at 4°C to remove unbroken cells and nuclear debris. The resulting pellet was resuspended, rehomogenated, and centrifuged as described above. Supernatants were combined and centrifuged at 18,000 g for 20 min at 4°C to obtain the lysosome-enriched pellet. Subsequently lysosomal marker enzymes were measured in both the supernantant (cytosolic fraction) and the pellet.

Determination of lysosomal enzyme activities

The lysosomal enzyme activities of N-acetyl-β-D-glucosaminidase (NAG), cathepsin B, and β-glucuronidase were measured.

NAG and β-glucuronidase activities were measured according to the original technique of Robinson et al.[23] by means of an spectrofluorimeter, using methylumbelliferone substrates.

Cathepsin B activity was measured according to the method of Barret[24] by means of an spectrofluorimeter, using 7-amino-4-metilcumarine substrate.

To know the specific activities of the three enzymes, protein concentrations were determined by the method of Bradford.[25]

Assessment of lysosomal stability

The lysosomal stability was assessed by calculating the percentage of lysosomal enzyme activities remaining in the supernatant after the last centrifugation at 18,000 g for 20 min. Increased lysosomal fragility is indicated by an increase in the percentage of enzymatic activity in the supernantant, according to Wilson et al.[12]

Histological studies

Pieces of pancreas were removed and fixed in 10% formaldehyde buffered to pH 7.4 for 24 hr. Pieces were processed and embedded in paraffin, sliced at a thickness of 7 μm, and stained with haematoxilin-eosin. The stained slices were coded and histological alterations were graded in a blind manner. Lesions were graded from 0 to 3 according to the following: 0: No lesions; 1: Up to 20% of the acini showed some kind of lesion; 2: 20 to 50% of the acini were affected; 3: More than 50% of the acini showed some lesions.

Statistical analysis

Data are expressed as means ± SEM. Results were compared by a multifactor analysis of variance (ANOVA) with three main effects, pancreatitis, fiber, and ethanol, followed by the Scheffé test. Differences were considered statistically significant at $P < 0.05$.

Results

Animals and treatments

The body weight average in the four groups during the treatment is showed in the *Figure 1*. As seen in the figure, rats fed a high fiber diet (FW and FE) presented a significant lower increase in their body weight compared to the rats fed a standard laboratory chow diet (NW and NE). Regarding the effect of ethanol ingestion, as seen in *Figure 1*, rats receiving ethanol (NE and FE) showed a significant reduced increase in their body weight than rats receiving water (FW and NW).

The food intake average in the four groups during the 6-month treatment is shown in the *Figure 2A*. Generally, in all the groups the food intake, expressed as g/100 g body weight, decreased during the first 4 to 8 weeks of treatment and then reached a plateau, as a result of the rat physiological growth curve. There is an effect of the ethanol intake on the food intake average. Rats receiving ethanol (FE and NE) always presented lower food intake than rats drinking water

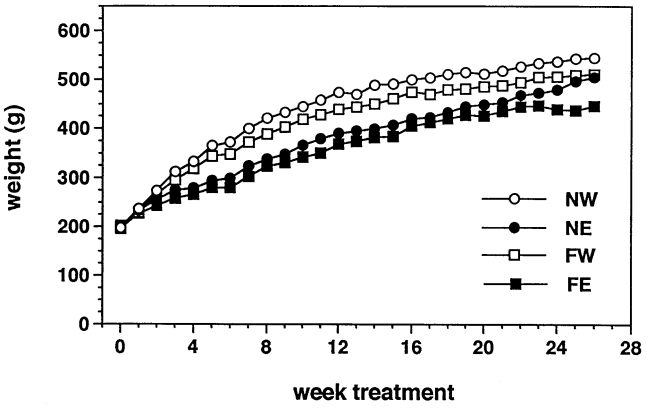

Figure 1 Body weight average (g) in the four groups of rats during the 6-month treatment. Data are mean (n = 40–60). NW: normal-water group; FW: fiber-water group; NE: normal-ethanol group, and FE: fiber-ethanol group.

(NW and FW) during the experimental period. There are not differences in the food intake regarding the type of diet.

The liquid intake average in the four groups of rats during the 6-month treatment is shown in the *Figure 2B*. As well as the food intake, the liquid (ethanol or water) intake average, expressed as ml/100 g body weight, decreased during the first 4 to 8 weeks of treatment attributable to the normal rat development. There is an effect of the ethanol intake on this parameter; thus, rats receiving ethanol (FE and NE) presented lower liquid intake average compared to rats receiving water (NW and FW). There is not an effect of high fiber diet on the liquid intake average and, therefore, on the ethanol intake.

Groups NW, FW, NE, and FE

Concerning the percentage of enzyme activity found in the supernatant after the last centrifugation, which is an index of the lysosomal fragility, the only enzyme which showed statistically significant differences among the four groups of rats, was cathepsin B; according to the Scheffé test, these differences were found between NW and, respectively, NE and FE groups. These two groups showed an increase in the percentage of activity. These percentages are shown in *Figure 3*.

Groups PNW, PFW, PNE, and PFE

Figure 4 shows the cytosolic percentage of each enzyme after inducing pancreatitis by cerulein in all the four groups of rats. All the enzymes analyzed showed an increase in the percentage of its cytosolic activity in the PNE group, though these differences were statistically significant only for the enzymes NAG and β-glucuronidase. In these two latter enzymes, Scheffé test showed statistically significant differences between PNE and, respectively, PNW and PFE groups.

Comparison between animals without and after cerulein-induced acute pancreatitis

Pancreatitis induced an increase in the percentage of cytosolic activity in all the enzymes analyzed. *Table 1* shows the

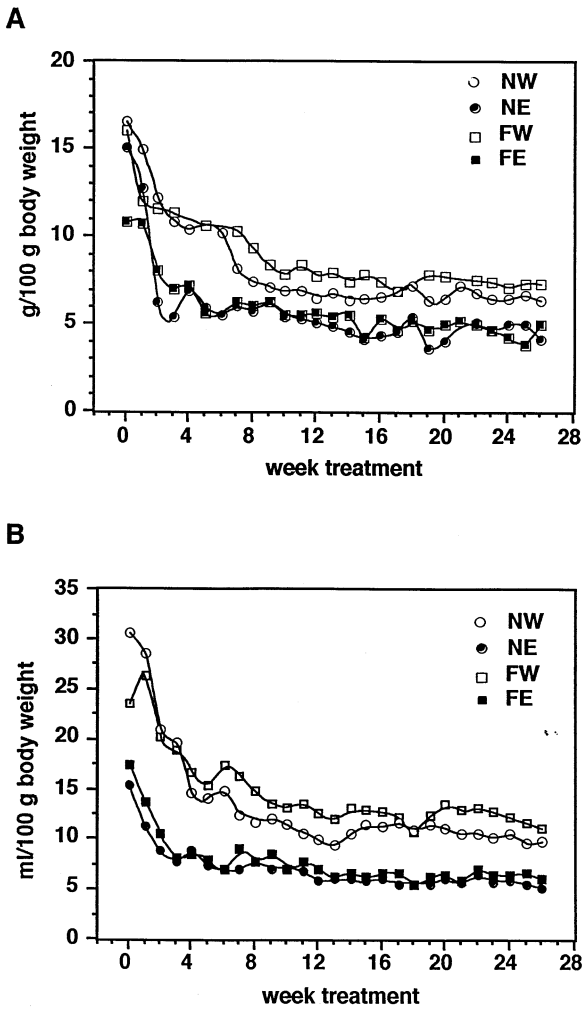

Figure 2 Total food (panel A) and liquid (panel B) intake average in the four groups of rats during the 6-month treatment. Data are expressed as g or mL/100 g body weight, respectively. Data are mean (n = 40–60). NW: normal-water group; FW: fiber-water group; NE: normal-ethanol group, and FE: fiber-ethanol group.

statistically significant differences according to the Scheffé test. Only the enzyme cathepsin B showed a significant increase in the four groups of rats. Moreover, NAG and β-glucuronidase showed a significant increase in the percentage of cytosolic activity between NE and PNE groups.

Histological results

The four groups of rats without pancreatitis (NW, FW, NE, and FE) showed a pancreas with a normal histological structure. The four groups of pancreatitic rats showed similar degree of damage in its histological structure. The mean (n = 6) scores of damage were as follows: PNW: 2.5 ± 0.22; PFW: 1.33 ± 0.21; PNE: 1.8 ± 0.37, and PFE: 2.16 ± 0.3.

No significant differences were found among the four groups of pancreatitic rats.

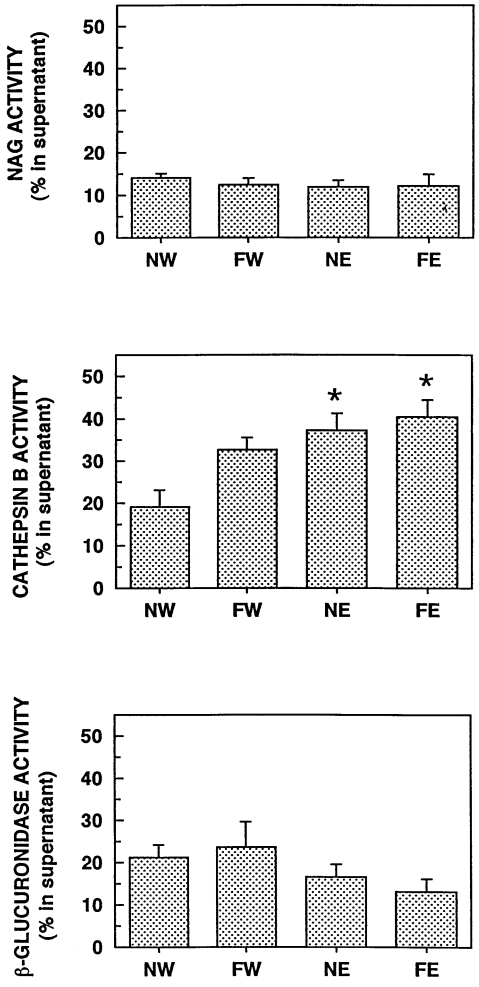

Figure 3 Percentage of different specific enzyme activities found in the supernatant after sedimentation of the lysosomal enriched fraction (see Methods and materials section). NW: normal-water group; FW: fiber-water group; NE: normal-ethanol group, and FE: fiber-ethanol group. Data are mean ± SEM of six animals in each group. Statistical significance is shown as $^*P < 0.05$ (3-factor ANOVA followed by the Scheffé test).

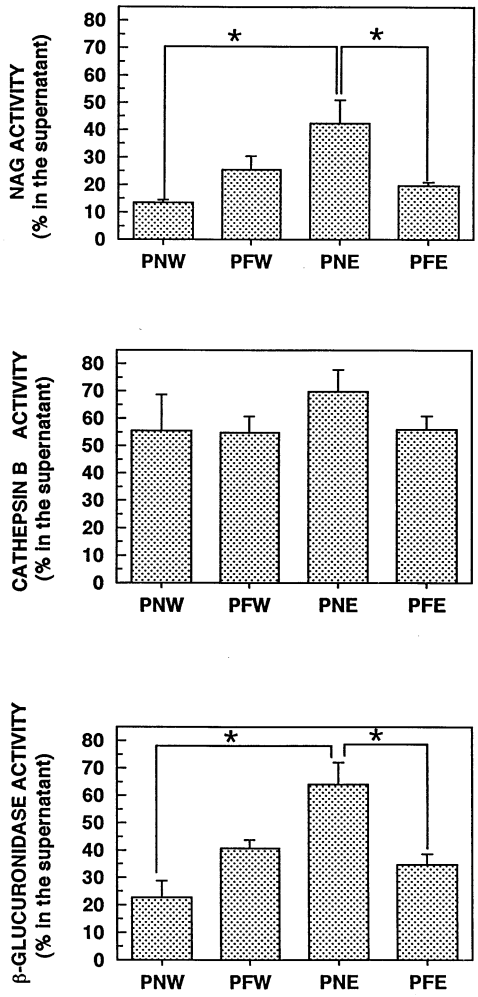

Figure 4 Percentage of different specific enzyme activities found in the supernatant after sedimentation of the lysosomal enriched fraction (see Methods and materials section), from pancreas of the four groups of rats after cerulein-induced acute pancreatitis. PNW: pancreatitis-normal-water group; PFW: pancreatitis-fiber-water group; PNE: pancreatitis-normal-ethanol group; and PFE: pancreatitis-fiber-ethanol group. Data are mean ± SEM of six animals in each group. Statistical significance is shown as $^*P < 0.05$ (3-factor ANOVA followed by the Scheffé test).

Discussion

In this work we observed that chronic ethanol consumption significantly increased the percentage of cytosolic cathepsin B activity; moreover, the chronic ethanol intake intensifies lysosomal fragility after cerulein-induced acute pancreatitis; on the other hand, when a high fiber diet is combined with alcohol intake, the lysosomal stability is higher and, therefore, it seems that a high fiber diet can protect in some way from the deleterious effect caused by pancreatitis on the lysosomal membranes of ethanol fed rats.

Wilson et al.[12,28] have reported that ethanol diminishes the lysosomal stability and Haber et al.[29] have suggested that chronic ethanol consumption produces increased amounts of fatty acids ethyl esters, which can account for the lysosomal membrane disorganization. Furthermore, Wilson et al.[28] have also reported that cholesteryl ester accumulation may mediate the effect of ethanol on the fragility of lysosomes. Recently, Apte et al.[30] have demonstrated that chronic ethanol consumption increases the capacity of the pancreatic acinar cell to synthesize digestive and lysosomal enzymes, particularly cathepsin B. In our study we found that the only enzyme affected by the ethanol treatment is cathepsin B (see *Figure 3*), this enzyme being significantly increased in the cytosolic fraction from both groups of rats (NE and FE) with chronic ethanol intake. Therefore, cathepsin B seems to be the lysosomal enzyme that indicates better the lysosomal damage after ethanol treatment.

It has been described that pancreatitis induced by cerulein[26] or by a choline-deficient, ethionine-supplemented diet[27] increases the fragility of pancreatic lysosomes. In our study we have shown that the enzyme cathepsin B seems to be the most sensible to reflect lysosomal damage after cerulein-induced pancreatitis (see *Table 1*). The fact that

Table 1 Comparison of lysosomal enzyme activities before and after pancreatitis in the four groups of rats

	NW-PNW	FW-PFW	NE-PNE	FE-PFE
NAG			**	
Cathepsin B	**	**	**	**
β-Glucuronidase			**	

Results of a 3-factor ANOVA followed by the Scheffé test applied to compare the percentage of the different specific enzyme activities found in the supernatant after sedimentation of the lysosomal enriched fraction (see Methods and materials section) from the four groups of rats without and after cerulein-induced pancreatitis. NW: normal-water group; PNW: pancreatitis-normal-water group; FW: fiber-water group; PFW: pancreatitis-fiber-water group; NE: normal-ethanol group; PNE: pancreatitis-normal-ethanol group; FE: fiber-ethanol group; PFE: pancreatitis-fiber-ethanol group. Statistical significance is shown as $^{**}P < 0.05$.

cathepsin B seems to be the more suitable enzyme to reflect pancreatic damage after chronic ethanol consumption and cerulein-induced acute pancreatitis could be related to its particular molecular size: this enzyme has a smaller molecular weight (25 kDa) than NAG (150 to 160 kDa) and β-glucuronidase (220 kDa) and this points to the fact that cathepsin B could more easily leave the lysosomes when the lysosomal membranes are impaired in some way.

Concerning the predisposition of the pancreas to a greater damage after the induction of pancreatitis, it has been shown that chronic ethanol consumption intensifies the damage of the pancreas subsequent to pancreatitis induced in rats by infusion of bile into the pancreatic duct[31] or by cerulein.[32] However, Korsten et al.[33] have recently reported that the administration of alcohol did not increase the effects of supramaximal doses of cerulein on the pancreas. Our results show that a higher increase in the cytosolic activity of all enzymes analyzed was seen in the PNE group, though this increase was not significant for cathepsin B (*Figure 4*). This means that a previous chronic ethanol consumption intensifies the lysosomal fragility after cerulein-induced pancreatitis. Korsten et al.[33] have found that the effect of cerulein on pancreatic lysosomal fragility was not increased by previous alcohol administration, but the rats used in this study were treated with ethanol for only 4 weeks, which is a short time compared with the 6-month period of our study. On the other hand, a high fiber diet combined with ethanol, PFE group, reduced, in a significant way, the increase in the cytosolic activity of NAG and β-glucuronidase enzymes compared with those found in the PNE group (*Figure 4*). The fact that this decrease was not observed in the cathepsin B activity could be related again with its lower molecular size, because a partial restoration of the lysosomal membranes could be insufficient to avoid its leakage to the cytoplasm, though it could prevent the exit of other lysosomal enzymes. As it has been reported above, contradictory results have been described regarding the effect of the fiber on the exocrine pancreatic function.[15–21] Our results suggest that the fiber may attenuate the adverse effects of the ethanol only when acute pancreatitis is induced, but the mechanism involved in this protective effect is not clear. However, we have recently found[34] that, in normal rats, a high fiber diet, combined with chronic ethanol consumption, produces a remarkable improvement of the in vitro exocrine pancreatic secretion compared with that obtained in the exocrine pancreas from rats which receive only ethanol treatment. Therefore, a high fiber diet seems to improve the secretory function of the pancreas from ethanol-fed rats and seems to protect the pancreas against the deleterious effects of cerulein-induced acute pancreatitis on lysosomal enzymes from rats with chronic ethanol intake. The mechanism involved in this protective effect is not clear.

Histological study of the pancreas from the four groups of rats did not show significant alteration in any of them. Neither the chronic ethanol intake nor the high fiber diet or a combination of both produces any deleterious effect on the structure of the exocrine pancreas. Furthermore, we have not observed any significant difference when compared with the histological structure of the pancreas from all the four groups of pancreatitic rats, showing similar degrees of damage; therefore, studies on the fragility of lysosomes could reveal the existence of some alterations in the acinar cells that are not detected by histological studies.

In summary, our results suggest that the fiber partially avoids the damage of ethanol on the membrane of pancreatic lysosomes, thereby attenuating its adverse effects and reducing the effects of pancreatitis. Moreover, noteworthy is the fact that cathepsin B is the enzyme that reflects better the changes attributable to treatments that alter the lysosomal membranes.

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