Effects of essential fatty acid deficiency on gamma-glutamyltranspeptidase activity of rat pancreas

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The effect of feeding rats with an essential fatty acid-deficient or -sufficient diet for a total of 29½ weeks (1½ weeks of pregnancy, 4 weeks of lactation and 24 weeks postweaning) on gamma-glutamyltranspeptidase activity and fatty acid composition of pancreas were determined. Enzyme activity of essential fatty acid-deficient rats was significantly higher than in controls. In a group of 24-week-old, essential fatty acid-deficient rats, the sufficient diet was fed for 8 weeks. Pancreatic gamma-glutamyltranspeptidase activity and fatty acid composition were restored to normal, suggesting a modulating effect of the diet.

The results indicated that elevated gamma-glutamyltranspeptidase activity of essential fatty acid-deficient rat pancreas was reverted to normal by feeding an essential fatty acid-sufficient diet.

Keywords: gamma-glutamyltranspeptidase, pancreas, essential fatty acid deficient rats

Introduction

Gamma-glutamyltranspeptidase (5 glutamyl)-peptide: aminoacid 5 glutamyltranspeptidase (E.C.2.3.2.2.), is a plasma membrane ectoenzyme. Its activity is notably high in the apical region of the plasmalemma of cell populations involved in absortive or secretory processes such as brush border of kidney proximal tubules,[1] exocrine portion of the pancreas,[2] enterocyte striated border[3] and biliary tract epithelia,[4] as well as in lymphocytes[5] and macrophages.[6]

Gamma-glutamyltranspeptidase is a broad specificity transferase that catalyses the transfer of gamma-glutamyl groups from a large variety of peptide donors to a wide range of amino acid and peptide acceptors.[7-9] The enzyme plays a central role in the metabolism of glutathione and its conjugates and mediates the intra- and interorgan circulation of these compounds upon their leaving the cell.[10-12] Gamma-glutamyltranspeptidase is a phase II (drug detoxifying) enzyme.[13]

Essential fatty acid (EFA)-deficiency can be achieved in rats by manipulating the lipid content of the diet.[14,15] This results in ultrastructural,[16] immunologic,[17,18] enzymatic,[19-22] metabolic, and functional[23-25] alterations that are probably dependent on changes in membrane fatty acid composition induced by the deficient diet.[26,27]

Materials and methods

Gamma-glutalmyltranspeptidase assay

Gamma-glutamyltranspeptidase activity was measured with 0.0024 mol/L L-gamma-glutamyl-p-nitroanilide as substrate and 0.038 mol/L glycylglycine as acceptor in 0.05 mol/L Tris buffer, adjusted to pH 8.5 with HCl according to Gardell and Tate.[28] All determinations were made at 37° C, in duplicate. The amount of protein and the time of incubation were within the linear range of the enzyme reaction. Specific activity was expressed as mkat (kg protein)$^{-1}$; 1 mkat = 1 mmol of measured p-nitroaniline liberated/s. Proteins were determined by the method of Lowry et al. using crystalline bovine serum albumin as standard.[29]

Gamma-glytamyltranspeptidase kinetics

Kinetic parameters (K_m and V) were calculated from a Lineweaver-Burk plot, on a postnuclear membrane fraction of pancreas of EFA-deficient and -sufficient rats according to Cameron et al.[30]

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Fatty acid composition

Lipids, extracted from homogenates using Folch's procedure,[31] were transesterified with boron trifluoride-methanol following the procedure of Morrison and Smith.[32] The fatty acid composition of methyl-esters was determined by gas liquid chromatography on 180 × 0.2 cm glass column packed with 10% DEGS on 80/100 Supelcoport. The column temperature was maintained at 180° C. Standard fatty acid methyl-esters were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 20:3 (n9) to 20:4 (n6) ratio was calculated.[33]

Statistical analysis

Results on enzyme activities were processed with analysis of covariance.[34,35]

Animal experiments

Adult albino rats of a Wistar strain were mated, fed a commercial diet, and maintained on alternated 12-hour periods of light and dark. At day 10 of pregnancy and through lactation, dams were fed one of the two diets of the following composition:

Essential fatty acid deficient diet. The composition of the EFA-deficient diet was similar to the diet utilized by Hill et al.[36] but no hydrogenated coconut oil or fiber was added, as in the original (casein, 27.77%; sucrose, 66.94%; salt mixture, 4.22%; vitamin mixture, 0.52%; and choline chloride, 0.3%). Salt mixture (g per 100 Kg of diet) contained $CaCO_3$, 1,555; $CuCO_4$, 5.0; $FeCl_3$, 29; $MgCO_3$, 230; $MnCl_2$, 17.20; KCl, 730; KI, 0.13; NaH_2PO_4, 827.93; Na_2HPO_4, 600; $ZnCl_2$, 5.74. Vitamin mixture (g per 100 kg of diet) consisted of calcipherol, 0.01; B_{12}, 0.005; biotin, 0.02; folic acid, 0.10; thiamine hydrochloride, 2; riboflavin, 3; pyridoxine, 10; paraminobenzoic acid, 10; inositol, 20; tocopheryl acetate, 40; calcium pantothenate, 10; niacin, 10 and vitamin A, 1,817,000 IU.

Essential fatty acid sufficient diet. The mixture consisted of the EFA-deficient diet supplemented with 5% corn oil.

Food and water were administered ad libitum for the total length of the experiments.

Seven pups, chosen randomly from three dams from each group, were weaned at 4 weeks of age and fed the same diet previously administered to their mothers for 24 weeks. Animals were sacrificed under chloroform anesthesia at 28 weeks of age. Pancreas and kidneys were resected and promptly sliced and homogenized at high speed in ice-cold 0.05 mol/L Tris buffer adjusted to pH 7.5 with HCl, in a Potter-Elvejhem Teflon-glass homogenizer.

Seven rats of a group of rats fed the EFA-deficient diet for 20 weeks postweaning were placed on the EFA-sufficient diet for 8 weeks. Seven rats fed the EFA-sufficient diet and seven fed the EFA-deficient diet served as controls. At 32 weeks of age, all rats were sacrificed; each pancreas was resected and homogenized as indicated above.

Homogenates were used for determining gamma-glutamyltranspeptidase activity and kinetic parameters, as well as total proteins and fatty acid composition.

Materials

All organic solvents were of analytical reagent grade. The dietary ingredients were obtained from a commercial source, except casein, which was a gift of Sancor, Córdoba and corn oil, which was purchased from a local supermarket. All biochemical reagents and enzyme substrates were obtained from Sigma Chemical Co.

Results

Pancreatic gamma-glutamyltranspeptidase activity was significantly greater in 28-week-old, EFA-deficient rats than the EFA-sufficient group of the same age. Kidney gamma-glutamyltranspeptidase did not differ in the two nutritional conditions (*Table 1*). Pancreatic gamma-glutamyltranspeptidase activity of 24-week-old, EFA-deficient rats fed the sufficient diet for 8 weeks exhibited similar values to the EFA-sufficient group. The pancreatic gamma-glutamyltranspeptidase activity of 24-week-old, EFA-deficient rats fed the sufficient diet (6.53 ± 0.12; n = 7) for 8 weeks was similar to the EFA-sufficient group (6.79 ± 0.004; n = 6). Enzymatic activity in both groups was significantly diminished ($P < 0.001$) in respect to the EFA-deficient group (8.56 ± 0.14; n = 7).

Fatty acid composition of pancreas in the three conditions is shown in *Table 2*. Pancreas of EFA-depleted-repleted rats showed normal fatty acid pattern.

Kinetic parameters. The V [mkat. (kg protein)$^{-1}$] was significantly greater ($P < 0.01$) in the EFA-deficient group (32,207 ± 2,228; n = 4) than in EFA-sufficient rats (21,428 ± 1,813; n = 4). K_m (L-gamma-glutamyl-p-nitroaniline) did not differ in pancreas of both nutritional conditions (EFA-deficient; 0.015 ± 0.001, n = 4; EFA-sufficient: 0.016 ± 0.001, n = 4).

Discussion

The results demonstrated that administering an EFA-deficient diet for a total of 29½ weeks (1 and ½ week of pregnancy, 4 weeks of lactation, and 24 weeks post-weaning) increased gamma-glutamyltranspeptidase activity of pancreas. In this research it was also shown that the increase in pancreatic gamma-glutamyltranspeptidase observed in EFA-deficiency is entirely reversed by feeding EFA-deficient rats the deficient diet supplemented with corn oil, a source of linoleic acid, suggesting a direct relationship with EFA-levels. Kinetic studies indicated that EFA-deficiency had an effect on V but not on K_m.

A significant increase of pancreatic gamma-glutamyltranspeptidase activity in 80–100-week-old rats fed for life an EFA–deficient or EFA-sufficient

Table 1 Effect of feeding an EFA-deficient or -sufficient diet on gamma-glutamyltranspeptidase activity of pancreas and kidneys of 28-week-old rats

	EFA-sufficient group	EFA-deficient group
Pancreas[a]	6.32 + 0.33 (7)	8.20 + 0.26 (6)
Kidneys[b]	53.08 + 2.12 (7)	56.21 + 3.29 (5)

Enzyme activity was expressed as mkat. (kg prot)$^{-1}$. The values are mean + SEM of (n) rats.

[a] EFA-sufficient group versus EFA-deficient group; significance, $P < 0.001$.

[b] EFA-sufficient group versus EFA-deficient group; not significant.

Table 2 Fatty acid composition of pancreas of 32-week-old rats fed on EFA-sufficient, -deficient or -deficient/sufficient diets

Organ	Experimental condition	Fatty acid									
		14:0	16:0	16:1	18:0	18:1	18:2	20:3 (n9)	20:3 (n6)	20:4 (n6)	20:3 (n9) / 20:4 (n6)
Pancreas	EFA-sufficient group	1.397	26.993	7.977	13.267	27.961	11.581	0.577	0.988	8.760	0.066
	EFA-deficient group	1.547	24.966	13.285	5.493	42.819	2.650	5.812	0.545	2.487	2.336
	EFA-deficient/sufficient group	1.354	24.472	8.462	11.489	29.760	12.921	0.714	1.014	9.414	0.076

Values are area percent (mean of two determinations on pooled samples for each group).

diet[27] suggested the possibility that the increase of pancreatic enzyme activity of EFA-deficient rats might be due to a particular response of the enzyme during aging. We have shown that a distinct rise of enzyme activity is present concomitantly with the characteristic fatty acid pattern of EFA-deficiency. Furthermore, adult enzyme levels are established at about 30 days of age.[37] Here we show that similar values are seen in adult animals, with no significant differences in respect to old animals, indicating no age-dependent effect on gamma-glutamyltranspeptidase activity of pancreas in EFA-deficient rats.

Present data on pancreatic gamma-glutamyltranspeptidase are in contrast with observations showing no changes in gamma-glutamyltranspeptidase activity of kidney, liver, and submandibular salivary gland in EFA-deficiency.[27,38,39]

Fatty acid composition of pancreas in EFA-deficient rats revealed that 16:1 and 18:1 (n9), were higher than in the controls; 18:2 (n6) and 20:4 (n6) were lower, and there was an accumulation of 20:3 (n9). Fatty acid pattern of EFA-deficiency was restored to normal profile by feeding the EFA-sufficient diet to deficient rats for 8 weeks, in agreement with studies on submandibular salivary glands.[22,39]

Concomitant variations of certain membrane-bound enzyme activities and fatty acid composition have been reported. Ouabain sensitive ($Na^+ + K^+$)-ATPase and adenylate cyclase activities were higher in salivary glands of EFA-deficient rats.[39] Adenylate cyclase and 5′ nucleotidase activity were decreased and (Na^+, $K^+ + Mg^{2+}$)-ATPase activity was increased after feeding a diet enriched in unsaturated acyl groups.[40]

Present results, as well as those of other authors, indicate that there are organ-related variations of activity of certain membrane-bound enzymes in EFA-deficiency, whereas this condition causes similar changes in fatty acid composition of tissue lipids.[15,19,21,26,27,37-40] Therefore, the association between levels of gamma-glutamyltranspeptidase activity and changes in the fatty acid composition could be suggested for certain cell populations but not for others. Possible explanations for variation of activity of membrane-bound enzymes in EFA-deficiency have been discussed; it was proposed that they are due to the following: 1) an increase in the number of enzyme molecules per mg of protein; 2) an increase in the activity of the individual gamma-glutamyltranspeptidase molecules; and 3) change in the physical state of the membrane containing the gamma-glutamyltranspeptidase molecules that affect the measurement of the activity.[39] Nevertheless, the data presented would be most consistent with an increased quantity of enzyme.

We conclude that rat pancreatic gamma-glutamyltranspeptidase activity was significantly increased in EFA-deficiency. The change was completely reverted by feeding an EFA-sufficient diet.

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