Alpha lipoic acid attenuates high-fructose-induced pancreatic toxicity

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Objectives:

Dessert manufacturers prefer using high-fructose corn syrup (HFCS) rather than glucose and sucrose because of many advantages: it is sweet, it does not mask the original taste, it is cheaper and it retards satiety. HFCS commercially is named F30, F40 and F55, according to the ratio (30%, 40% and 55%) of fructose content. Prepared foods and soft drinks include these forms of HFCS in several countries.[1]Fructose is rapidly converted to triglycerides and stored in adipose tissue, and this causes obesity.[2] Some studies have shown that glucose tolerance and increased insulin resistance may occur by excessive consumption of sugary and fatty foods, which increases the risk of type 2 diabetes or metabolic syndrome.[3] Oxidative stress as a result of lipid peroxidation causes apoptosis, inducing of malondialdehyde (MDA), formation of Mallory bodies, neutrophil chemotaxis, collagen production and fibrosis.[4] In addition, HFCS increases AGE, which causes several complications such as reduction in the antioxidant mechanism.[5] Decrease of antioxidant enzymes activity and increase of oxidative stress parameters are responsible for decrease in hormone secretion because of pancreatic damage.[6]The goal of the study was to investigate the protective effects of ALA on pancreatic damage induced by chronic HFCS consumption.

Methods:

The protocol was carried out according to the Animal Care and Use Committee guidelines of Suleyman Demirel University (22/08/2013-03) and was performed in accordance with the National Institutes of Health Guidelines for the Care and Handling of Animals.In the study, 4-month-old, 24 Wistar Albino female rats (n = 24), each weighing 250–300 g, were included. F30 HFCS was obtained from Toposmanoglu (Isparta, Turkey), which contains approximately 24% fructose and 28% dextrose in the syrup of 73% total solids. Previous research has shown that subjects served meals with either 30% glucose beverages or 30% fructose beverages had differing hormonal and metabolic responses. Glycaemic excursions and insulin responses were reduced by 66% and 65%, respectively in the fructose-consuming subjects. For that reason in this study, the prepared 30% solution of F30 was added to drinking water for 10 weeks. Thioctacid 600 mg tablets (Meda Pharma, Turkey), a commercial form of ALA, were used for treatment. ALA was dissolved in distilled water. The single daily dose of 100 mg/kg was given orally for last 6 weeks of the experiment.Before the consumption, all the rats were randomly divided into three equal groups consisting of eight rats in each. Control group (given only standard commercial diet and tap water); HFCS group (given 30% F30 solution for 10 weeks); HFCS+ALA group (given 30% of F30 solution for 10 weeks and 100 mg/kg ALA by oral gavage for the last 6 weeks of the experiment). At the end of the experiment, 24 hours after the last ALA administration, blood samples were collected from the tail vein to determine the serum glucose, amylase, and lipase levels, and then rats were euthanized by cervical dislocation. After the abdominal incision, pancreatic tissue samples were taken. One half of partitioned pancreatic tissues were placed in formaldehyde solution for histopathological and immunohistochemical examinations. The other half of the tissues were homogenised and kept at −80 C for biochemical studies. Tissue samples were collected during the necropsy and fixed in 10% buffered formalin. After routine processing, tissues were embedded in paraffin, sectioned into 5-µm thickness, stained with haematoxylin–eosin (HE) and examined microscopically. Selected tissue sections were immunostained by active caspase-3 [Anti-Caspase 3 antibody (ab4051)], insulin [Anti-insulin+Proinsulin antibody (D6C4) ab8304] and glucagon [Anti-glucagon antibody, ab8055] expression in pancreatic tissue sections according to the manufacturer's instructions. The streptavidin–biotin peroxidase technique was used for immunohistochemistry.

Results:

The analysed results of biochemical parameters are shown in Table 1. Serum glucose levels were higher in the HFCS group (p<0.05) compared with the control and were lower in the ALA-treated group (p<0.05) compared with the HFCS group. Amylase (p<0.01) and lipase (p<0.01) levels were higher in the HFCS group compared with the control and lower after ALA treatment (p<0.001 and p<0.01, respectively) compared with the HFCS group.

MDA levels were higher in the pancreatic tissues of the HFCS group compared with control group and significantly lower in the ALA-treated group compared with the HFCS group (p<0.05) CAT activities were diminished in the pancreatic tissues of the HFCS group (p<0.05), and enhanced after ALA treatment (p<0.05) (Table 2).

Body weights were increased in the HFCS group and HFCS+ALA group during the first 4 weeks (before ALA treatment) comparing to the control group (p<0.05). ALA treatment decreased the body weight during the last 6 weeks of the study than HSCF group but results were statistically insignificant.

No gross lesions were found in the pancreatic tissue in any group throughout the experiment. Necrotic and degenerative histopathological changes were observed in islet cells in the HFCS group (Fig.1). Slight inflammatory cells were seen in the exocrine part of the pancreas in some rats in the HFCS group, indicating exocrine damage. In the control group, insulin immunostaining was centrally located, and glucagon immunostaining was located in the periphery of the islets. Insulin immunoreactive β cells and glucagon immunoreactive α cells were markedly reduced in the HFCS group (Fig. 2–3). In the ALA group, there were significantly more reversed histopathological lesions and enhanced immunostaining compared with the HFCS groups. Caspase-3 activity was markedly increased in both endocrine and exocrine cells in the HFCS group and diminished in the ALA-treated group.

Table 1: Body weight changes and biochemical markers

		Control	HFCS	HFCS +ALA	P.value
Body weight change (g)	Pre-experiment	261 ± 11.2	259 ± 12.0	262.1 ± 12.8	CON-HFCS (NS) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (NS)
	Post-experiment	280.1 ± 10.9	293.5 ± 40.3	293 ± 43.5	CON-HFCS (<0.05) CON-HFSC+ALA (<0.05) HFCS-HFSC+ALA (NS)
	Change	19.1	34.5	30.9	CON-HFCS (<0.05) CON-HFSC+ALA (<0.05) HFCS-HFSC+ALA (NS)
Glucose (mg/dl)		150.92 ± 39.77	236.50 ± 18.28	164.15 ± 42.28	CON-HFCS (<0.05) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.05)
Amylase (U/L)		2165.00 ± 150.76	3027.66 ± 729.19	2216.78 ± 45.96	CON-HFCS (<0.01) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.001)
Lipase (U/L)		5.81 ± 2.22	11.51 ± 2.74	6.29 ± 2.60	CON-HFCS (<0.01) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.01)

Table 2: Body weight and AST changes between the groups.

	MDA (µmol/mg protein)	CAT (ku/mg protein)
Control	0.0167 ± 0.004[a]	0.0924 ± 0.029[a]
HFCS	0.0193 ± 0.006[b]	0.0359 ± 0.023[b]
HFCS +ALA	0.0179 ± 0.001[c]	0.0687 ± 0.006[c]

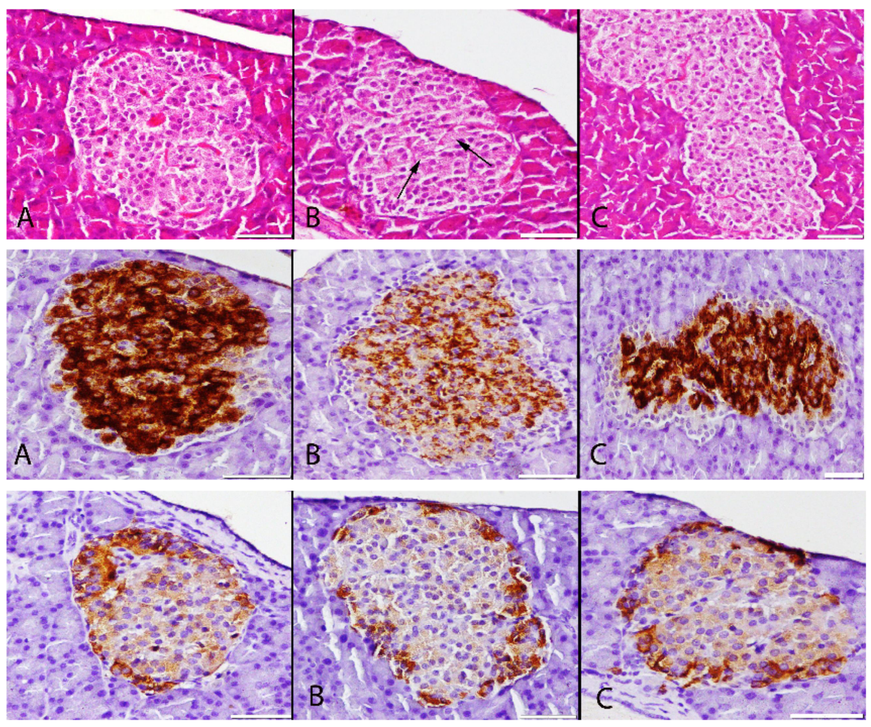

Figure 1: Histopathological appearance of the Langerhans islet; **(A)** Control group, completely normal islet, HE; **(B)** HFCS group, degenerative cells (arrows) generally localised in the central area of the islet, HE; **(C)** HFCS+ALA-treated group, sharing similar appearance to the control group, HE, Bars= 50µm.

Figure 2: Insulin immunoreactions between the groups; **(A)** normal insulin expression in the control group; **(B)** Marked decrease in insulin immunoreactions in the HFCS group; **(C)** At least normal insulin immunoreactions in Langerhans islet in the HFCS+ALA group, Streptavidin biotin method; Bars= 50µm.

Figure 3: Glucagon immunoreactions in groups; **(A)** Normal glucagon immunoreaction in the control group; **(B)** Decreased glucagon immunoreactions in the HFCS group; **(C)** Amelioration of glucagon synthesis cells in the HFCS+ALA group, Streptavidin biotin method, Bars= 50µm.

Conclusions:

Understanding of the regulation, secretion and molecular and cellular mechanisms of glucagon and insulin from pancreatic islets has been long awaited. Research on these cells and their role in nutrient metabolism has gained a renewed impetus in recent years. Based on our results, HFSC damages pancreatic cells, especially endocrine cells, and ALA has ameliorative effects on HFSC-induced pancreatic damage. ALA may be used to treat obesity and insulin resistance in humans, but further studies are needed on this subject.

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