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Benfluorex, a Hypotriglyceridemic Drug, Reduces Lipid Peroxidation and Alleviates Adverse Metabolic Complications of Copper Deficiency

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ABSTRACT

The pathologies associated with copper deficiency in rats fed fructose may be induced, in part, by hypertriglyceridemia and lipid peroxidation. Reducing triacylglycerol levels in plasma may result in lowering lipid peroxidation, which in turn could ameliorate metabolic effects resulting from the combination of fructose feeding and copper deficiency. Benfluorex, a hypolipidemic factor able to reduce hypertriglyceridemia, was administered to weanling male rats fed either copper-deficient (0.6 μg Cu/g) or adequate (6.0 μg Cu/g) diets containing fructose as the sole dietary carbohydrate. In copper-deficient rats, benfluorex (50 $\mu g \cdot kg^{-1} \cdot d^{-1}$) reduced plasma triacylglycerols from 45 to 31 mg/dL, reduced lipid peroxidation by approximately 50%, and prevented the enlargements of heart and liver size and the atrophy of the pancreas, and ameliorated anemia. It is suggested that lipid peroxidation associated with hypertriglyceridemia may be responsible for the pathologies induced by the combination of fructose consumption and copper deficiency. *Nutrition* 1997;13:895–899. ©Elsevier Science Inc. 1997

Key words: benfluorex, hypertriglyceridemia, lipid peroxidation, copper, iron, pancreas

INTRODUCTION

Copper deficiency is associated with anemia, hypercholesterolemia, hypertriglyceridemia, abnormal glucose tolerance, pancreatic atrophy, heart hypertrophy, and premature mortality.[1–5] These abnormalities are due to the presence of simple sugars, such as sucrose or fructose in the diet.[1–5] When rats consume a copper-deficient diet that contains complex carbohydrates, such as starch, the pathologies associated with copper deficiency are either ameliorated or prevented, and the animals survive.[1–6] Since copper deficiency by itself is not sufficient to induce these pathologies, we hypothesized that metabolic pathways that are specific for fructose but not for starch are responsible for the dramatic differences between fructose-fed and starch-fed rats.

Fructose feeding has been shown to produce elevations in plasma triacylglycerols.[7–10] Increased superoxide production has been demonstrated in patients with hypertriglyceridemia.[11] Similarly, increased levels of reactive oxygen species have been reported to be induced in copper deficiency due to the reduced activity of the copper-containing enzyme superoxide dismutase (CuSOD) and the selenoenzyme glutathione peroxidase (GSH-Px).[12–14] The combination of fructose with copper deficiency produced reactive oxygen species detected by electron spin resonance (ESR) and increased lipid peroxidation.[12,15,16] The consumption of a copper-deficient, starch-based diet did not have this effect.[12,15,16]

Because reactive oxygen species are suspected to play an important role in the initiation of cellular damage, we investigated whether lowering hypertriglyceridemia will reduce levels of lipid peroxidation, which in turn should alleviate some metabolic and physiologic abnormalities associated with copper deficiency. Benfluorex, (2-[[1-methyl-2 [3-(trifluoromethyl)-phenyl]ethyl]amino] ethanol benzoate ester), a pharmaceutical hypolipidemic agent, was able to reduce hepatic triacylglycerol in alcoholic rats and in rats fed fructose and fat.[17,18] It also inhibited de novo synthesis of

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TABLE I.

FINAL BODY MASS, RELATIVE ORGAN SIZE, HEMATOCRIT AND HEPATIC COPPER AND IRON CONCENTRATIONS

	Copper-deficient		Copper-adequate	
	−Benfluorex	+Benfluorex	−Benfluorex	+Benfluorex
Body mass (g)	159 ± 3	140 ± 4	194 ± 3	172 ± 2
Heart mass (g)	0.99 ± 0.05	0.75 ± 0.02	0.75 ± 0.02	0.66 ± 0.02
Relative heart size (g/100 g)	0.62 ± 0.03	0.53 ± 0.02	0.39 ± 0.09	0.39 ± 0.01
Liver mass (g)	7.34 ± 0.12	5.97 ± 0.18	7.07 ± 0.18	6.27 ± 0.13
Relative liver size (g/100)	4.6 ± 0.10	4.2 ± 0.12	3.6 ± 0.07	3.6 ± 0.06
Hematocrit (%)	25.8 ± 0.6	32.5 ± 1.4	44.3 ± 0.5	44.3 ± 0.5
Liver Cu (μg/g wet wt)	0.93 ± 0.07	1.15 ± 0.10	5.59 ± 0.21	5.71 ± 0.18
Liver Fe (μg/g wet wt)	174.8 ± 11.3	184.3 ± 19.5	102.4 ± 9.5	101.2 ± 2.9
Hepatic lipid peroxidation (nmol MDA/g)	33.8 ± 3.1	17.7 ± 3.5	21.7 ± 3.2	12.7 ± 2.7

	ANOVA (*P* values)		
	Copper	Benfluorex	Copper × Benfluorex
Body mass	0.0001	0.0001	NS
Heart mass	0.0001	0.0001	0.0156
Relative heart size	0.0001	0.0348	0.0240
Liver mass	NS	0.0001	NS
Relative liver size	0.0001	NS	NS
Hematocrit	0.0001	0.0004	0.0004
Liver Cu	0.0001	NS	NS
Liver Fe	0.0001	NS	NS
Lipid peroxidation	0.0150	0.0010	NS

Means ± SEM of 10 observations/group. MDA, malondialdehyde; ANOVA, analysis of variance; NS, not significant.

fatty acids, triacylglycerol synthesis and improved insulin sensitivity.[17–19] We predicted that these suppressive effects of benfluorex on lipid metabolism might alleviate the pathologies associated with copper deficiency in rats fed fructose. The work presented here was designed to test this hypothesis.

MATERIALS AND METHODS

Weanling male rats weighing approximately 40–50 g each were randomly assigned to four dietary groups according to copper levels and benfluorex: group 1, copper-deficient; group 2, copper-deficient + benfluorex; group 3, copper-adequate; group 4, copper-adequate + benfluorex. The copper-deficient diets contained 0.6 μg Cu/g diet, and the copper-adequate diets contained 6.0 μg Cu/g diet as determined by atomic absorption spectrophotometry. The description of the diets has been previously reported.[20]

Benfluorex hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was introduced to the diet on day 2 at a concentration designed to provide 12.5 $mg \cdot kg^{-1} \cdot d^{-1}$ and gradually increased to 50 $mg \cdot kg^{-1} \cdot d^{-1}$ over a 3-d period.

The rats were fed their respective diets for 4 wk. Following an overnight fast, they were decapitated. Blood was collected into capillary tubes for hematocrit. Blood was also collected into heparinized test tubes and centrifuged. Plasma was used to measure the concentration of triacylglycerols by the automated procedure of the centrifichem. Plasma insulin was measured by using a radio immunoassay (RIA) using rat antibody (Linco Research Inc., St. Charles, MO, USA). Livers, pancreata, and hearts were removed and weighed, and portions of livers were used for the determination of copper and iron concentrations.[21] Other liver portions were used to assess lipid peroxidation by measuring malondialdehyde formation using the thiobarbituric acid (TBA) reactive technique as described elsewhere.[22,23]

The pancreas was trimmed of fat and weighed again. It was homogenized in phosphate-buffered saline (PBS) using a polytron homogenizer equipped with stainless steel blades. Aliquots of homogenate were used to measure the concentration of insulin using the same RIA kit that utilizes an antibody made specifically against rat insulin (Linco Research Inc.). Other aliquots were used to measure the activities of pancreatic amylase using Sigma enzymatic procedure no. 577, respectively, and pancreatic copper and iron concentrations by atomic absorption spectrophotometry.[21]

All data were subjected to a 2 × 2 analysis of variance (ANOVA), two levels of copper, and two levels of benfluorex. Main effects and interactions of significance level of $P < 0.05$ were considered statistically significant.

RESULTS

Final body mass, relative organ sizes, hematocrit, and hepatic copper and iron concentrations are summarized in Table I. Body mass was reduced by copper deficiency. Rats fed benfluorex exhibited lower body mass than rats that did not consume the drug. Food intake was not affected by benfluorex (data not shown). The largest heart and liver sizes were found in copper-deficient rats that did not receive benfluorex. Heart weight and relative heart sizes were reduced by benfluorex in copper-deficient rats. Liver weight was reduced by benfluorex. When expressed per 100 g body weight, the largest relative liver size was noted in copper-deficient rats that were not

TABLE II.

PANCREAS SIZE, COPPER, IRON AND INSULIN CONCENTRATIONS AND AMYLASE ACTIVITY

	Copper-deficient		Copper-adequate	
	−Benfluorex	+Benfluorex	−Benfluorex	+Benfluorex
Pancreas weight (g)	0.73 ± 0.05	0.96 ± 0.04	1.40 ± 0.03	1.32 ± 0.03
Pancreas size (g/100 g)	0.46 ± 0.03	0.69 ± 0.02	0.72 ± 0.02	0.76 ± 0.02
Copper (μg/g wet wt)	1.78 ± 0.12	1.66 ± 0.11	2.31 ± 0.12	1.29 ± 0.09
Iron (μg/g wet wt)	51.6 ± 3.8	49.4 ± 2.3	17.0 ± 0.9	18.9 ± 0.8
Insulin (ng/pancreas)	6348 ± 1650	1718 ± 458	332 ± 87	351 ± 57
Amylase (U/pancreas)	2988 ± 587	5783 ± 889	7695 ± 431	9912 ± 860

	ANOVA (*P* values)		
	Copper	Benfluorex	Copper × Benfluorex
Pancreas weight	0.0001	0.0001	0.0002
Pancreas size	0.0001	NS	0.0006
Copper	NS	0.0001	0.0006
Iron	0.0001	NS	NS
Insulin	0.0001	0.0046	0.0047
Amylase	0.0001	0.0005	NS

Means ± SEM of 10 observations/group. NS, not significant.

treated with benfluorex. Copper-deficient rats exhibited the lowest hematocrits. Benfluorex raised hematocrits in copper-deficient rats. Liver copper was the lowest in rats that consumed the low copper diet. Liver iron was elevated by copper deficiency. Benfluorex had no effect on either liver copper or liver iron concentrations. The highest degree of lipid peroxidation was evident in copper-deficient rats that did not receive benfluorex. Benfluorex reduced hepatic lipid peroxidation in copper-deficient and copper-adequate rats.

Table II summarizes data pertaining to pancreas size and contents. The smallest pancreas weight and size was found in copper-deficient rats. Benfluorex raised the weight and relative size of the pancreas in copper-deficient rats. The concentration of copper in the pancreas was lowered but that of iron was elevated by copper deficiency. The highest insulin concentration of the pancreas was found in copper-deficient rats not receiving benfluorex. The administration of benfluorex greatly reduced the concentration of pancreatic insulin. The lowest insulin content was recorded in copper-adequate controls. The administration of benfluorex doubled the activity of amylase in the pancreas of copper-deficient rats.

Fasting levels of triacylglycerols and insulin in plasma are summarized in Table III. The highest levels of triacylglycerols were found in plasma of copper-deficient rats. Concentration of triacylglycerols was reduced by benfluorex in copper-deficient and adequate rats. Copper-deficient rats had the lowest level of plasma insulin. The administration of benfluorex raised fasting levels of insulin in all rats.

DISCUSSION

The present study was designed to determine whether a reduction of plasma triacylglycerols will lower lipid peroxidation,

TABLE III.

FASTING PLASMA TRIACYLGLYCEROLS AND INSULIN

	Copper-deficient		Copper-adequate	
	−Benfluorex	+Benfluorex	−Benfluorex	+Benfluorex
Triacylglycerols (mg/dL)	45.1 ± 3.5	31.3 ± 3.3	30.7 ± 1.3	22.3 ± 1.2
Insulin (ng/mL)	0.165 ± 0.02	0.313 ± 0.06	0.454 ± 0.09	0.520 ± 0.06

	ANOVA (*P* values)		
	Copper	Benfluorex	Copper × Benfluorex
Triacylglycerol	0.0001	0.0001	NS
Insulin	0.0603	0.0015	NS

Means ± SEM of 10 observations/group. NS, not significant.

which in turn will prevent some of the pathologies associated with copper deficiency such as liver enlargement, heart hypertrophy, pancreatic atrophy, and anemia. The administration of benfluorex, a hypolipogenic and hypotriacylglycerolmic drug reduced plasma triacylglycerols. It also lowered hepatic lipid peroxidation and prevented abnormalities induced by copper deficiency. It is suggested that suppressing effects of benfluorex on lipid metabolism were responsible for the amelioration of pathologies associated with copper deficiency.

The reduced hepatic lipid peroxidation and levels of plasma triacylglycerols were not due to changes in copper or iron concentrations, but rather to drug mechanism. Benfluorex via its metabolites inhibits de novo synthesis of fatty acids and triacylglycerol synthesis, and improves insulin sensitivity.[17–19] Because of these functions, benfluorex has been recommended as a potential tool for alleviating some of the adverse effects of diabetes, obesity, and atherosclerosis.[17–19]

It is well established that fatty liver leads to cirrhosis and fibrosis.[24,25] Similarly, in iron-storage diseases, the liver is the main target organ for injury, and hepatic fibrosis and cirrhosis are the major pathologic hallmarks of the disease.[24,25] Excess tissue iron provides a direct stimulus to collagen synthesis.[26] High stored iron by itself is not sufficient to cause tissue damage. It is well established that iron is highly reactive under a certain redox environment.[27–29] This reactivity can be associated with damage to cellular systems. Fructose consumption could provide this redox environment. Excess amounts of NADH and NADPH are known to occur during the metabolism of fructose.[30–32] In addition, fructose feeding should increase the production of superoxide since it stimulates the activity of xanthine oxidase.[30] In copper deficiency the combination of fructose feeding with hepatic iron retention is responsible for liver damage, as shown by fat vacuoles and foci of individual cell necrosis of hepatocytes.[33] Prevention of lipogenesis should reduce the availability of substrates necessary for lipid peroxidation by iron. Similarly, the reduction of hepatic iron in copper deficiency also lowers reactive oxygen species and lipid peroxidation.[15,16]

It is well established that liver injury, which is caused by hepatic iron overload, is capable of inducing pathologies to other organs such as the heart and the pancreas.[24] Liver iron overload has been associated with pancreatic damage and diabetes.[34–36] Hepatic iron retention occurs in copper deficiency.[37,38] Prevention of hepatic iron retention in copper deficiency either by administration of deferoxamine or lowering dietary intake of iron reduced the size of the liver and prevented hypertrophy of the heart and atrophy of the pancreas.[15,16] In the present study, however, levels of hepatic iron were not reduced by benfluorex, and yet liver enlargement and pancreatic atrophy were prevented. It is suggested that other mechanisms not related to iron but associated with prevention of hepatic lipogenesis were responsible for beneficial effects of benfluorex.

In addition to preventing pancreatic atrophy, functional ability of the pancreas to secrete insulin and to synthesize amylase was restored by benfluorex. In copper deficiency the exocrine pancreas atrophies, and the activity of digestive enzymes is reduced.[39–41] In addition, the ability of the endocrine pancreas to secrete insulin is impaired.[42] Copper-deficient rats of the present study accumulated insulin in the pancreas that was not secreted to the general circulation. The ability of the endocrine pancreas to secrete insulin was greatly restored by benfluorex. This improved capacity of the pancreas to release insulin was associated with higher levels of plasma insulin. Amylase activity was also raised by benfluorex. The improvement in insulin secretion may be responsible for amylase activity. It has been reported that amylase activity is regulated by insulin.[43,44]

Both copper-deficient and copper-adequate rats of the present study exhibited a reduction of body mass when treated with benfluorex. It is well established that growth rate affects essential metalloelement homeostasis. Due to a depressed growth rate, the requirements for copper are reduced and higher concentrations of stored copper but lower iron were reported.[45–47] The elevated levels of copper and the reduced levels of iron were responsible for the amelioration of the pathologies associated with copper deficiency.[45–47] In the present study, however, the reduced body mass of rats treated with benfluorex did not affect levels of copper or iron. Alleviated signs associated with copper deficiency in rats treated with benfluorex should be due to the direct effect of either benfluorex or its metabolites on metabolic pathways.

The results of this study show that rats fed a copper-deficient diet developed hypertriglyceridemia, lipid peroxidation, and numerous other pathologies. The ability of benfluorex to ameliorate or prevent these abnormalities may indicate that the mechanism involves alterations in lipid metabolism. Once circulating triacylglycerols were lowered and reactive oxygen species were reduced, a cluster of other abnormalities associated with copper deficiency were ameliorated.

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