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Effects of Different Dietary Carbohydrates on Hepatic Enzymes of Copper-Deficient Rats (42018)

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Abstract. The present study was undertaken to measure the activities of several hepatic enzymes of regulatory importance in the pathways of lipogenesis and gluconeogenesis in rats fed diets marginally deficient in copper (1.2 μg Cu/g of diet) and containing either fructose, glucose, or starch as the carbohydrate sources. Although all copper-deficient rats exhibited the characteristic signs of copper deficiency, they were more pronounced in rats fed the diet containing fructose. Except for the activity of phosphoenolpyruvate carboxykinase which was unaffected either by copper deficiency or by the type of dietary carbohydrate, the hepatic activities of glucose-6-phosphate dehydrogenase, malic enzyme, L-α-glycerophosphate dehydrogenase and fructose 1,6-diphosphatase were unaffected by copper deficiency but were affected by the type of carbohydrate in the diet. Fructose produced the greatest increase in enzymatic activities, whereas starch produced the least activity and glucose induced an intermediate effect. These results indicate that the deleterious effects of a fructose diet deficient in copper on biochemical and physiological indices could not be due to an immediate metabolite of fructose. However, the involvement of a subsequent metabolite of fructose in the mechanism of copper utilization and/or requirement cannot be excluded.

Dietary fructose has been shown to exert a greater degree of severity on clinical and enzymatic indices in copper-deficient rats than does starch or glucose (1–6). The mechanisms by which fructose feeding as compared with starch or glucose aggravates the symptoms associated with copper deficiency are as yet unidentified. Dietary fructose (7) and copper deficiency (1, 8) have been shown to provoke a greater glycemic and insulin response when compared with starch in the rat. In addition, increased serum triglycerides and cholesterol have been reported after ingestion of carbohydrates containing fructose (9) and in copper deficiency (2, 4, 10). As the metabolism of fructose and copper occur mainly in the liver, it would be expected that indices of liver function would be impaired by copper deficiency in rats fed a fructose diet deficient in copper. Increased liver weight (1–6), decreased hepatic adenosine triphosphate (ATP) (1, 4, 6), decreased plasma albumin (2, 4), increased blood ammonia (4), decreased activities of the copper metalloenzyme superoxide dismutase (SOD), (4) and the selenoenzyme glutathione peroxidase (GSH-Px) (4) could be attributed to impaired liver function. The reduced activities of SOD and GSH-Px have been shown to accentuate the increased lipid peroxidation of tissue membranes (6). Thus, it is expected that in copper deficiency the activities of several hepatic enzymes of regulatory importance in the metabolism of fructose could also be affected by the severity of the deficiency. The purpose of the present study was to measure the effects of copper-deficient diets containing starch, fructose, or glucose on the activities of hepatic enzymes involved in lipogenesis and gluconeogenesis in the rat.

Materials and Methods. Weanling male Sprague–Dawley rats weighing approximately 40–45 g each were housed individually in stainless-steel cages with wire-mesh bottoms in a temperature–humidity controlled room with 12-hr periods of alternating light and dark. The rats were randomly divided into six groups fed different sources of dietary

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carbohydrates and levels of copper: group 1, cornstarch, copper deficient (15 rats); group 2, cornstarch, copper supplemented (10 rats); group 3, fructose, copper deficient (40 rats); group 4, fructose, copper supplemented (10 rats); group 5, glucose, copper deficient (15 rats); and group 6, glucose, copper supplemented (10 rats).

All diets contained (g/kg diet) 622 carbohydrate, 200 egg white, 95 corn oil, 30 nonnutritive fiber (cellulose), 35 AIN-76 salt mix, (copper omitted), 10 vitamin mix AIN-76A (11) supplemented with 2 mg biotin and 2.7 choline bitartrate. The copper-supplemented groups (controls) were given 5 μg Cu/ml in the form of anhydrous $CuSO_4$ added to the deionized drinking water. All copper-deficient diets contained 1.2 ± 0.02 μg Cu/g diet as analyzed by flame atomic absorption spectrophotometry. Multiple mineral analysis of the diets showed similar levels of sodium, potassium, calcium, magnesium, iron, zinc, and manganese. To assure accuracy, certified standard reference materials were analyzed along with the diets. During the fifth week, the fructose in the copper-deficient diet of 20 randomly selected rats was replaced by either starch (10 rats) or glucose (10 rats). The experiment was terminated during the 11th week. Five rats from each group were randomly selected, fasted overnight, and killed by decapitation. The livers were removed, weighed, and homogenized and aliquots were used to determine the activities of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), malic enzyme (ME, EC 1.1.1.40), and L-α-glycerophosphate dehydrogenase (LαGPD, EC. 1.1.99.5) as described by Freedland (12), fructose-1-6 diphosphatase (FDPase, EC. 3.1.3.11) and phosphoenopyruvate carboxykinase (PEPCK, EC 4.1.1.32) (13). The protein content was determined by the method of Lowry *et al.* (14) using bovine serum albumin as a standard. In order to assess copper status, blood ceruloplasmin activity was measured as described by Schosinsky *et al.* (15), liver copper was assayed using flame absorption spectrophotometry, and liver SOD activity was assayed as described by Misra and Fridovich (16). Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (17). Differences of $P < 0.05$ are reported as statistically significant.

Results. Body and liver weights, hepatic copper, and SOD are presented in Table I. Growth rate was significantly decreased by copper deficiency only in rats fed fructose compared to their copper-supplemented controls. Relative liver weight was increased by

TABLE I. GROWTH RATE, LIVER WEIGHT, HEPATIC COPPER AND SOD ACTIVITY IN RATS FED STARCH, FRUCTOSE, OR GLUCOSE FOR 11 WEEKS

Diet	Body wt (g)	Liver wt (g/100)	Hepatic copper (μg/g wet wt)	Hepatic SOD (U/g wet wt)
Starch				
−Cu	327 ± 4[a]	2.4 ± 0.08[a]	2.7 ± 0.2[a]	1038 ± 60[a]
+Cu	332 ± 8[a]	2.5 ± 0.02[a]	5.3 ± 0.2[b]	1384 ± 100[b]
Fructose				
−Cu	290 ± 6[b]	3.5 ± 0.1[b]	1.3 ± 0.2[c]	660 ± 40[c]
+Cu	322 ± 4[a]	3.1 ± 0.04[c]	5.9 ± 0.05[d]	1330 ± 85[b]
Glucose				
−Cu	297 ± 6[b]	2.9 ± 0.08[c]	2.1 ± 0.2[a]	992 ± 86[a]
+Cu	313 ± 9[a,b]	2.6 ± 0.07[a]	5.0 ± 0.08[b]	1212 ± 67[b]
ANOVA[d]				
Copper	S	S	S	S
Carbohydrate	NS	S	S	S
Interaction	NS	S	S	S

Note. Values within parameter column are expressed as means ± SEM from five rats.

[a–c] Means with different superscript letters are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

[d] A 2 × 3 analysis of variance. Effects and interactions significant (S) ($P < 0.05$), nonsignificant (NS).

TABLE II. LIVER ENZYMES IN RATS FED STARCH, FRUCTOSE, OR GLUCOSE FOR 11 WEEKS

Diet	G6PD	ME	FDPase	LαGPD	PEPCK
			U/g protein		
Starch					
−Cu	20 ± 1[a]	11 ± 1[a]	17 ± 0.8[a]	615 ± 22[a]	10 ± 2[a]
+Cu	19 ± 1[a]	11 ± 0.5[a]	17 ± 0.3[a]	620 ± 11[a]	8 ± 1[a]
Fructose					
−Cu	69 ± 7[b]	54 ± 7[b]	69 ± 7[b]	1035 ± 120[b]	9 ± 1[a]
+Cu	72 ± 3[b]	51 ± 3[b]	69 ± 2[b]	1049 ± 88[b]	11 ± 1[a]
Glucose					
−Cu	34 ± 2[c]	18 ± 1[c]	28 ± 2[c]	736 ± 45[c]	7 ± 2[a]
+Cu	35 ± 1[c]	17 ± 0.5[c]	29 ± 1[c]	769 ± 36[c]	8 ± 1[a]
ANOVA[d]					
Copper	NS	NS	NS	NS	NS
Carbohydrate	S	S	S	S	NS
Interaction	NS	NS	NS	NS	NS

Note. Values within parameter column are expressed as means ± SEM from five rats.

[a-c] Means with different superscript letters are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

[d] A 2 × 3 analysis of variance. Effects and interactions significant (S) ($P < 0.05$) nonsignificant (NS).

copper deficiency only in rats fed fructose and glucose but not starch. However, liver weight was increased by fructose feeding in rats fed the copper-deficient or supplemented diets compared to rats fed either starch or glucose. Hepatic copper and SOD activity were reduced by copper deficiency in all rats fed the copper-deficient diets, but the decrease was much greater in rats fed fructose. Ceruloplasmin activity was reduced by copper deficiency (mean ± SEM 8 ± 1 U/liter) in all copper-deficient rats regardless of the nature of dietary carbohydrates as compared to copper-supplemented controls (118 ± 16 U/liter).

Hepatic enzyme activities are presented in Table II. Except for PEPCK, all other hepatic enzymes showed a significant dependence on the nature of dietary carbohydrates. The lowest hepatic enzyme activity was noted in rats fed starch and the highest activity in rats fed fructose. Values of enzymatic activities in rats fed glucose were between those of starch and fructose. None of the enzymes studied were affected by copper status.

The effects of changing dietary carbohydrates in copper-deficient diets from fructose to either starch or glucose on body and liver weights, hepatic copper, SOD, and enzymatic activities are presented in Table III. Body weight was increased and liver weight decreased by changing the diet from fructose to either starch or glucose, when compared to rats continuously fed fructose. Starch feeding as compared to glucose was more effective. Ceruloplasmin activity was not changed due to changes of dietary carbohydrates, but hepatic copper was increased by starch, and SOD was increased by both starch and glucose when compared to rats continuously fed fructose. The activities of G6PD, ME, and FDPase were decreased due to changing dietary carbohydrates from fructose to starch. There was a trend toward a reduction in the activities of G6PD, ME, FDPase, and LαGPD when dietary fructose was replaced by glucose, however, only changes in ME were significant.

Discussion. The results of the present study clearly demonstrate that although the three diets deficient in copper contained the same concentration of copper, dietary fructose aggravated the deficiency as measured by body and liver weight, and hepatic copper and SOD. This is in agreement with our previous studies where fructose increased the severity of the deficiency when compared to starch or glucose (1–6). Although it is well established that hepatic ATP is reduced (1, 4, 18, 19) and protein synthesis is inhibited (18) upon feeding diets containing fructose defi-

TABLE III. EFFECT OF CHANGING OF DIETARY FRUCTOSE IN THE COPPER-DEFICIENT DIET FROM FRUCTOSE TO EITHER STARCH OR GLUCOSE ON BODY AND LIVER WEIGHTS, HEPATIC COPPER, SOD, G6PD, ME, FDPase, LαGPD, AND PEPCK

	Fructose replacement		
	None	Starch	Glucose
Body weight, g	290 ± 6[a]	331 ± 7[b]	305 ± 2[c]
Liver, g/100	3.5 ± 0.1[a]	2.7 ± 0.03[b]	3.1 ± 0.1[c]
Hepatic Cu, μg/g wet wt	1.3 ± 0.2[a]	3.2 ± 0.3[b]	1.8 ± 0.05[a]
Hepatic SOD, U/g wet wt	660 ± 40[a]	972 ± 62[b]	871 ± 68[b]
G6PD[d]	69 ± 7[a]	33 ± 4[b]	56 ± 17[a]
ME[d]	54 ± 7[a]	15 ± 1[b]	26 ± 5[b]
FDPase[d]	69 ± 7[a]	29 ± 3[b]	52 ± 13[a]
LαGPD[d]	1035 ± 120[a]	810 ± 100[a]	1102 ± 88[a]
PEPCK[d]	9 ± 1[a]	11 ± 1[a]	10 ± 0.5[a]

Note. Values with parameter row are expressed as mean ± SEM from five rats.

[a-c] Means with different superscript letters are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

[d] U/g protein.

cient in copper, the regulatory enzymes in the liver, participating in metabolizing fructose, were not affected by the copper status. This is in agreement with our previous studies in which G6PD and ME activities were not different in rats fed diets containing fructose either deficient or supplemented with copper (20). The dietary level of copper in that study (20) was 0.9 μg Cu/g diet, compared with 1.2 μg Cu/g diet in the present study. When a more severe deficiency was produced by feeding rats diets containing milk deficient in copper, the deficiency did not affect the activity of enzymes participating in glucose oxidation, fatty acid oxidation, and tricarboxylic acid cycle (21). This is also in agreement with Kopp *et al.* (19) who showed that all hepatic metabolites of glucose except for glycerol-3-phosphate were not affected by copper deficiency. Copper deficiency in rats fed diets containing either starch or glucose similarly showed no decrease in the activities of hepatic enzymes participating in the metabolism of glucose.

A two- to fourfold increase in the activities of most of the liver lipogenic and gluconeogenic enzymes was obtained in rats fed the fructose diet as compared to rats fed starch or glucose. This is in agreement with other studies that follow long-term feeding of fructose containing sugars (22–25). In agreement with Cohen *et al.* (22) the activity of PEPCK was unaffected by the type of dietary carbohydrate. The changes in the pattern of liver enzymes in rats fed fructose compared to starch or glucose are due to the large share of the liver in the metabolism of fructose as compared with the role of the liver in glucose metabolism (26, 27).

As the hepatic enzymatic pattern is dependent on the nature of dietary carbohydrates and not on copper status, it was possible to alter the activities of some of the enzymes by merely changing the type of dietary carbohydrates from fructose to either starch or glucose. Starch has been shown to be more effective than glucose in decreasing the activities of some of the hepatic enzymes involved in glucose metabolism in rats previously fed a fructose copper-deficient diet. This dependency on the type of dietary carbohydrate has been recently shown to affect glucose homeostasis and metabolism and to restore blood glucose to its normal levels following the oral glucose load (5). When comparing starch with glucose, starch has been shown to be more protective (2, 4–6). The reason why differences exist in metabolic and enzymatic indices between starch and glucose could be due to differential effects on gastric emptying, induction of enteric hormones, and intestinal hydrolytic enzyme activity. In addition, a delay in hydrolysis of starch in the small intestine could in turn influence the rate of intestinal absorption of glucose (28).

Thus, the many undesirable metabolic effects observed following feeding the fructose

diet deficient in copper cannot be due to the immediate metabolism of fructose via gluconeogenesis and lipogenesis. However, the higher levels of triglycerides and cholesterol in copper-deficient rats fed fructose as compared to starch or glucose (1, 4) might increase the requirement and/or utilization of copper. Although these effects could not be demonstrated in rats fed a diet adequate in copper, such a possibility cannot be excluded.

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