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The Severity of Copper Deficiency in Rats is Determined by the Type of Dietary Carbohydrate (41832)

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Abstract. The purpose of this investigation was to study the interaction between copper and dietary carbohydrates on clinical and enzymatic indices associated with copper deficiency. Copper deficiency was produced in rats by feeding diets adequate in all nutrients including selenium and chromium, but marginal in copper (1.2 μg/g diet) containing 62% of either starch, fructose,or glucose. During the fifth week, the fructose of the copper-deficient diet (20 rats) was replaced by either starch (10 rats) or by glucose (10 rats). The experiment was terminated after 11 weeks. Copper deficiency in rats fed fructose significantly lowered body weight and hematocrit, but increased liver weight, blood urea nitrogen, ammonia, cholesterol, and triglycerides when compared to rats fed starch or glucose. The copper metalloenzyme, superoxide dismutase, the selenoenzyme, glutathione peroxidase, and hepatic ATP were decreased in the copper-deficient rats fed fructose as compared to copper-deficient rats fed starch or glucose. These results indicate that fructose may be the dietary component which has a deleterious effect on copper and selenium status. Changing the type of dietary carbohydrate in copper-deficient rats from fructose to either starch or glucose ameliorated the severity of the deficiency. The protective effects were more pronounced with starch than with glucose.

An interaction between dietary carbohydrate and copper status of copper-deficient rats has been recently reported (1–3). The degree of the severity is dependent on the type of dietary carbohydrate; fructose produces the most severe, whereas starch produces the least severe deficiency, and glucose produces an intermediate deficiency (2, 3). The diets used in those previous studies did not include supplemental chromium or selenium in the mineral mix. Chromium has been implicated in glucose homeostasis (4, 5) and its deficiency impairs glucose metabolism (6). Copper deficiency has also been shown to adversely affect glucose tolerance (1, 2, 7–9). Selenium has been shown to interact with copper status (10–15) and copper-deficient rats have reduced activity of the selenoenzyme glutathione peroxidase (12–14). The copper metalloenzyme superoxide dismutase (SOD), and the selenoenzyme glutathione peroxidase (GSH-Px) are scavengers of intermediates of oxygen metabolism and are protectors against cellular damage (16). Thus the deficiency of other essential trace minerals in addition to copper, may contribute to the severity of the copper deficiency.

The average daily dietary intake of copper for humans living in the United States is believed to be only marginal (17–19) and well below the recommended level considered to be adequate and safe (17–20). In order to more closely simulate a marginal copper deficiency in rats, the level of dietary copper used in the present study was increased from 0.9 μg/g diet used in previous studies (1, 2) to 1.2 μg/g diet.

The major purpose of the present study was to determine whether the differential effects of dietary carbohydrate on indices of copper deficiency exists in rats fed diets containing supplemental selenium and chromium and only marginally deficient in copper. In addition, it was important to determine whether the severity of copper deficiency in rats fed fructose could be reversed by changing the dietary carbohydrate to either glucose or starch.

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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- and 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 carbohydrates and levels of copper: (A) cornstarch, copper deficient (15 rats); (B) cornstarch, copper supplemented (10 rats); (C) fructose, copper deficient (40 rats); (D) fructose, copper supplemented (10 rats); (E) glucose, copper deficient (15 rats); (F) glucose, copper supplemented (10 rats). The cornstarch was obtained from Teklad, Madison, Wisconsin, the fructose from Hoffman-La Roche, Nutley, New Jersey, and the glucose from Clinton Corn Processing Company, Clinton, Iowa.

All diets contained (g/kg diet) 622 carbohydrate, 200 egg white, 95 corn oil, 30 nonnutritive fiber (cellulose), 35 AIN-76 salt mix, (21) (copper omitted), 10 vitamin mix AIN-76A, and 2.7 choline bitartrate. The copper-supplemented groups (controls) were given 5 μg Cu/ml in the form of $CuSO_4$ added to the deionized drinking water. All copper-deficient diets contained 1.20 ± 0.02, 1.25 ± 0.02, and 1.26 ± 0.01 μg Cu/g diet for starch, fructose, and glucose, respectively, as analyzed by flame atomic absorption spectrophotometry, using acid digestion prior to analysis. Multiple mineral analysis of the diets showed similar levels of sodium, potassium, calcium, magnesium, iron, zinc, and manganese. To assure accuracy, National Bureau of Standards standard reference materials were analyzed along with the diets.

During the 5th week, the fructose in the copper-deficient diet of 20 randomly selected rats was replaced by either starch (10 rats) or glucose (10 rats). During the 11th week, hemoglobin (1) and hematocrit (22) were measured on blood obtained from the tail vein. Two days later five rats from each group were randomly selected, and were killed by decapitation. Blood was collected into citrated test tubes and the plasma was stored at $-20°C$ prior to the analyses of ceruloplasmin (23), albumin (24), blood urea nitrogen (BUN) (25), ammonia (26), creatinine (27), cholesterol (28), and triglyceride (29). Five other rats from each dietary regimen were anesthetized with ether and liver homogenates (10% w/v) were immediately prepared from one portion of the liver in 0.2% v/v Triton X-100 as described by Paynter (30). The homogenates were treated with chloroform–ethanol to inactivate the manganese-dependent SOD. The homogenates were mixed well and centrifuged at 6000*g* for 20 min. The supernatant obtained was stored at $-20°C$ overnight and then assayed for SOD activity. Activity of SOD was determined by the method of Misra and Fridovich (31) using a photochemical *o*-dianisidine riboflavin assay. The other portion of the liver was homogenized in 0.15 *M* KCl, fractionated by centrifugation, and the supernatant obtained was assayed for Se-dependent GSH-Px activity using a final concentration of 0.3 m*M* *t*-butyl hydroperoxide as the substrate according to the method of Paglia and Valentine (32) as modified by Levander *et al.* (33). The protein content was determined by the method of Lowry *et al.* (34) using bovine serum albumin as a standard. The remainder of the liver was used to measure ATP levels. Hepatic ATP was extracted (35) and determined enzymatically (36).

Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (37). Differences of $P < 0.05$ are reported as statistically significant.

Results. The effects of dietary carbohydrate on body, liver, and heart weights are presented in Table I. Copper deficiency resulted in impairment of growth only in rats fed fructose. Liver weight was increased in rats fed the fructose or glucose diet deficient in copper as compared to their copper-supplemented controls and to rats fed starch. However, liver weight was further increased in rats fed fructose as compared to those fed glucose. Heart weight was increased due to copper deficiency only in rats fed fructose. Body weight was increased and liver weight decreased when starch or glucose replaced fructose. Heart weight was decreased by starch and increased by glucose when compared to rats continuously fed fructose.

The results of copper deficiency on blood parameters are reported in Table II. Ceruloplasmin activity, an indicator of copper status, was reduced by copper deficiency (Mean $\pm$ SEM = 7.8 ± 0.6 U/liter) in all copper-de-

TABLE I. EFFECT OF DIETARY CARBOHYDRATES (CHO) AND COPPER (Cu) NUTRITURE ON BODY, LIVER, AND HEART WEIGHTS OF RATS

	Starch		Fructose		Glucose		ANOVA[d]		
	−Cu	+Cu	−Cu	+Cu	−Cu	+Cu	Cu	CHO	Interaction
(A)[f] Body wt (g)	327 ± 5[a]	332 ± 8[a]	290 ± 6[b]	322 ± 4[a]	297 ± 6[b]	313 ± 9[a,b]	S	NS	NS
Liver wt[e]	2.4 ± 0.08[a]	2.5 ± 0.02[a]	3.5 ± 0.1[b]	3.1 ± 0.04[c]	2.9 ± 0.08[c]	2.6 ± 0.07[a]	S	S	S
Heart wt[e]	0.39 ± 0.01[a,b]	0.34 ± 0.01[a,b]	0.40 ± 0.01[b]	0.30 ± 0.01[c]	0.45 ± 0.03[b]	0.40 ± 0.04[b]	S	S	NS
(B)[g] Body wt (g)	331 ± 7[a]		290 ± 6[b]		305 ± 2[c]				
Liver wt[e]	2.7 ± 0.03[a]		3.5 ± 0.1[b]		3.1 ± 0.1[c]				
Heart wt[e]	0.38 ± 0.01[a]		0.40 ± 0.1[a]		0.48 ± 0.01[b]				

Note. Values within parameter rows are expressed as means ± SEM.
[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.
[e] g/100 g body wt.
[f] Rats fed starch, fructose, or glucose throughout the experiment (11 weeks).
[g] All rats were fed the fructose diet deficient in copper for 4 weeks. The rats were then divided into three groups which were fed the copper-deficient diet containing either starch, fructose, or glucose for an additional 7 weeks.

TABLE II. EFFECT OF FEEDING COPPER-DEFICIENT DIETS CONTAINING STARCH, FRUCTOSE, OR GLUCOSE ON HEMOGLOBIN, HEMATOCRIT, PLASMA ALBUMIN, BUN, AMMONIA, CREATININE, CHOLESTEROL (Chol), AND TRIGLYCERIDES (TG)

Diet	Hemoglobin (g/dl)	Hematocrit (%)	Albumin (g/dl)	BUN (mg/dl)	Ammonia (μg/ml)	Creatinine (mg/dl)	Chol (mg/dl)	TG (mg/dl)
Starch								
−Cu	13.7 ± 0.5[a]	44.0 ± 0.9[a]	2.8 ± 0.2[a]	12.5 ± 0.7[a]	0.4 ± 0.1[a]	0.72 ± 0.11	93 ± 5[a]	40 ± 4[a]
+Cu	14.1 ± 0.4[a]	45.2 ± 0.8[a]	2.7 ± 0.2[a]	12.7 ± 0.4[a]	0.6 ± 0.3[a]	0.71 ± 0.09	73 ± 4[b]	33 ± 5[a]
Fructose								
−Cu	12.5 ± 0.3[b]	39.2 ± 0.6[b]	2.1 ± 0.1[b]	27.2 ± 4.6[b]	4.3 ± 0.4[b]	0.73 ± 0.01	147 ± 9[c]	65 ± 11[b]
+Cu	13.9 ± 0.3[a]	44.2 ± 0.8[a]	3.5 ± 0.2[c]	20.1 ± 0.8[c]	1.7 ± 0.2[c]	0.74 ± 0.03	85 ± 5[b]	32 ± 2[a]
Glucose								
−Cu	13.2 ± 0.4[a,b]	43.2 ± 0.8[a]	2.4 ± 0.2[b]	15.3 ± 0.6[d]	3.0 ± 0.4[d]	0.73 ± 0.02	113 ± 12[a]	39 ± 3[a]
+Cu	13.4 ± 0.3[a,b]	43.6 ± 0.6[a]	3.0 ± 0.2[c]	11.6 ± 1.0[a]	1.5 ± 0.4[c]	0.69 ± 0.09	89 ± 4[b]	37 ± 3[a]
ANOVA[e]								
Copper	S	S	S	S	S	NS	S	S
Carbohydrate	NS	NS	NS	S	S	NS	NS	NS
Interaction	NS	NS	NS	NS	S	NS	S	S

Note. Values within parameter column are expressed as means ± SEM.
[a–d] Means with different superscript letters are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.
[e] A 2 × 3 analysis of variance. Effects and interactions significant (S) ($P < 0.05$). Nonsignificant (NS).

TABLE III. EFFECT OF REPLACING FRUCTOSE WITH STARCH OR GLUCOSE IN COPPER-DEFICIENT DIETS ON HEMOGLOBIN, HEMATOCRIT, PLASMA ALBUMIN, BUN, AMMONIA, CREATININE, CHOLESTEROL (Chol), AND TRIGLYCERIDES (TG) OF RATS

Fructose replacement	Hemoglobin (g/dl)	Hematocrit (%)	Albumin (g/dl)	BUN (mg/dl)	Ammonia (μg/ml)	Creatinine (mg/dl)	Chol (mg/dl)	TG (mg/dl)
None	12.9 ± 0.2[a]	39.2 ± 0.6[a]	2.1 ± 0.1[a]	27.2 ± 4.6[a]	4.3 ± 0.4[a]	0.73 ± 0.01	147 ± 9[a]	65 ± 11[a]
Starch	13.7 ± 0.1[b]	45.0 ± 0.6[b]	2.5 ± 0.1[b]	15.4 ± 0.9[b]	1.7 ± 0.2[b]	0.72 ± 0.04	87 ± 8[b]	37 ± 5[b]
Glucose	13.6 ± 0.1[b]	45.0 ± 1.3[b]	2.7 ± 0.1[b]	18.7 ± 0.6[c]	2.5 ± 0.5[b]	0.70 ± 0.09	127 ± 9[c]	52 ± 3[a]

Note. Values within parameter column are expressed as means ± SEM.
[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.

ficient rats regardless of the nature of dietary carbohydrates as compared to copper-supplemented controls (mean = 118 ± 16 U/l). Hemoglobin and hematocrit were reduced due to copper deficiency only in rats fed fructose. Plasma albumin was reduced by copper deficiency in rats fed fructose and glucose. Copper deficiency increased BUN and ammonia in rats fed fructose and glucose, but the increase was more pronounced in rats fed fructose. Creatinine was neither affected by copper deficiency nor by the type of dietary carbohydrates. Plasma cholesterol was increased by copper deficiency in all rats regardless of the type of dietary carbohydrates, but the increase was greater in rats fed fructose. Triglyceride was increased only in rats fed the fructose diet deficient in copper.

The effects of replacing dietary fructose with either starch or glucose on blood parameters in copper deficient rats are presented in Table III. Ceruloplasmin activity (mean = 7.8 ± 1.1 U/liter) was not affected by changing the type of dietary carbohydrates. Hemoglobin, hematocrit, and albumin were increased by replacing the dietary fructose by either starch or glucose as compared to rats continuously fed the fructose diet. BUN was decreased by replacement of fructose with either starch or glucose; the decrease being greater with starch than with glucose. Ammonia was decreased by replacing fructose with either glucose or starch; in contrast, creatinine was unaffected. Plasma cholesterol was significantly decreased in rats previously fed fructose, but switched to either starch or glucose; however, the decrease was significantly greater for feeding starch compared to glucose. Plasma triglycerides were decreased only in rats changed to the starch diet.

As ceruloplasmin activity was reduced to the same extent by all copper deficient diets, it was impossible to differentiate between the degrees of the severity on this parameter alone. Therefore, the tissue activity of the copper metalloenzyme SOD was measured. In addition, the activity of the selenoenzyme GSH-Px was measured to provide an index of selenium status. Table IV presents liver activities of SOD, GSH-Px, and ATP concentrations. All copper deficient rats exhibited reduced SOD compared to the copper-supplemented controls; however, SOD was further reduced

in rats fed fructose. GSH-Px activity was decreased only in the rats fed fructose-25 and 50%, for those fed the copper-supplemented or -deficient diet, respectively. Hepatic ATP was reduced in copper deficiency by feeding the rats fructose or glucose when compared to copper-supplemented controls and to rats fed starch. However, the decrease in ATP was of much greater magnitude in the copper-deficient rats fed fructose than those fed glucose.

The effect of replacing fructose with starch or glucose in the copper-deficient diets on hepatic SOD and GSH-Px activities and ATP concentration is also presented in Table IV. SOD and GSH-Px activities and ATP concentrations were significantly increased due to changing the dietary fructose to either starch or glucose as compared to rats continuously fed fructose.

Discussion. The results of this study demonstrate that although the copper-deficient diets were only marginally deficient in copper, and the diet included all known essential nutrients including recommended levels of selenium and chromium, dietary fructose aggrevated the copper deficiency. In addition, fructose impaired selenium status. The results obtained after dietary fructose was replaced by either starch or glucose further supports this contention. Thus, it is possible to markedly increase the severity of copper deficiency by feeding fructose and to decrease the severity by feeding glucose or even more so by starch. A comparison of the severity of copper deficiency in rats fed diets containing fructose, starch, or glucose as the sole source of dietary carbohydrate has been recently reported (3). Although it has been suggested that dietary fructose increases the requirement and/or utilization of copper, these effects could not be demonstrated in rats fed a diet adequate in copper. Thus, the increased requirement and/or utilization appears to be manifested only when the level of dietary copper falls below a certain critical level.

The mechanisms by which the feeding of fructose as compared with starch or glucose aggrevate the symptoms associated with copper deficiency are as yet unidentified. Dietary fructose has been shown to provoke a greater glycemic and insulin response when compared to starch in the rat (38). Copper deficiency has a similar effect on glucose tolerance (1, 2,

TABLE IV. EFFECT OF DIETARY CARBOHYDRATE (CHO) AND COPPER (Cu) NUTRITURE ON THE ENZYMATIC ACTIVITIES OF SOD, GSH-Px, AND ATP CONCENTRATION IN THE LIVER

	Starch		Fructose		Glucose		ANOVA[d]		
	−Cu	+Cu	−Cu	+Cu	−Cu	+Cu	Cu	CHO	Interaction
(A)[h] SOD[e]	1038 ± 60[a]	1384 ± 100[b]	660 ± 40[c]	1330 ± 85[b]	992 ± 86[a]	1212 ± 67[a]	S	S	S
GSH-Px[f]	1.00 ± 0.15[a]	1.10 ± 0.15[a]	0.50 ± 0.10[b]	0.75 ± 0.02[c]	0.95 ± 0.11[c]	0.90 ± 0.04[a]	S	S	S
ATP[g]	0.96 ± 0.12[a]	1.04 ± 0.06[a]	0.55 ± 0.10[b]	1.22 ± 0.10[a]	1.04 ± 0.12[a]	1.49 ± 0.08[c]	S	NS	S
(B)[i] SOD[e]	972 ± 62[a]		660 ± 40[b]		871 ± 68[a]				
GSH-Px[f]	0.98 ± 0.08[a]		0.50 ± 0.10[b]		0.93 ± 0.07[a]				
ATP[g]	0.87 ± 0.10[a]		0.55 ± 0.12[b]		1.02 ± 0.06[a]				

Note. Values within parameter row are expressed as means ± SEM.
[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) ($P < 0.05$) or not significant (NS).
[e] U/g wet wt.
[f] μmole NADPH oxidized/mg protein/min.
[g] μmole/g wet wt.
[h] Rats continuously fed starch, fructose, or glucose with or without copper supplementation for 11 weeks.
[i] After 4 weeks, the rats fed the fructose copper-deficient diet were divided into three groups and fed the copper-deficient diet containing either starch, fructose, or glucose for an additional 7 weeks.

7–9). In addition, increased serum triglycerides and cholesterol have been reported after ingestion of carbohydrates containing fructose (39) and by low copper intake (1, 2, 40–42). Decreased insulin binding (1, 43), decreased ATP content of the liver (2, 43–45) and increased activity of glucose-6-phosphatase in rat liver (46, 47) have been described both after sucrose or fructose intake. *In vitro,* the activity of glucose-6-phosphatase has been decreased due to low copper concentration (48). As the metabolism of copper and fructose occurs mainly in the liver, it would be expected that indices of liver functions would be impaired by copper deficiency in rats fed fructose. Increased liver weight, decreased plasma albumin, and increased plasma ammonia and BUN were noted in copper-deficient rats fed fructose. These impaired metabolic and clinical indices could not be attributed to impaired renal function, as all rats, regardless of the type of dietary carbohydrate and copper nutriture, had normal plasma creatinine concentration. Thus the increased levels of BUN in rats fed fructose could be due to the accelerated protein catabolism which is presumably a reflection of the more severe copper deficiency. On the other hand, decreased plasma albumin and increased levels of ammonia would indicate hepatocellular damage. In that regard, BUN and ammonia levels remained elevated after the carbohydrate source was changed from fructose to starch or glucose, and did not return to the lower levels of rats continuously fed glucose or starch.

The cellular activities of the copper metalloenzyme SOD and the selenoenzyme GSH-Px were reduced due to copper deficiency, but the reduction was more pronounced by dietary fructose. The interaction between copper deficiency and selenium status has been shown to occur in rats fed diets based on evaporated milk (12) powdered milk (13) and sucrose (14) but not glucose (15, 49). Also, recently a copper–selenium interaction was reported in fish (trout) (11). A 25% reduction in GSH-Px activity was also found in rats fed the fructose diet adequate in copper. The reduced SOD and GSH-Px activities have been shown to accentuate the increased lipid peroxidation of tissue membranes (15), which could result in uncoupling of oxidative phosphorylation and in the inhibition of the electron transport in the mitochondria of copper-deficient rat liver (50). However, the reduced tissue ATP observed in this study could be due to other metabolic processes such as the reduced activity of cytochrome oxidase (51), swelling of mitchondria (50), increased amount of unsaturated fatty acids (52) or reduced levels of mitochondria sulfhydryl (SH) groups (53).

As dietary fructose has been shown to aggrevate copper status when copper intake is inadequate, it was important to determine if replacing fructose with a more protective carbohydrate such as starch or glucose could reverse or lessen the degree of the deficiency, without increasing the dietary copper concentration. If the dietary copper deficiency is marginal, and the diet is deficient only in copper, fructose apparently has no adverse effect that is permanent and cannot be reversed. The interaction between dietary fructose and copper deficiency is specific for fructose; once fructose is removed from the diet, the deleterious effects cease to exist. The enhanced severity induced by feeding fructose was reversible and the impaired metabolic and cellular indices were ameliorated by altering only the type of dietary carbohydrate. Although both starch and glucose were protective against the severity of copper deficiency, starch was more effective.

It has been reported that the dietary copper intake in subjects living in industrialized societies may be marginal (17–20). Likewise, the copper-deficient diets used in the present study were only marginally deficient (1.2 μg Cu/g diet). Less than 1.0 μg Cu/g diet is considered to be copper deficient for rats. The introduction of high-fructose corn sweeteners in 1970 (54) and the consumption of high levels of fructose (54, 55) may result in similar interaction between dietary carbohydrate and copper status in humans as has been shown for experimental animals. Thus, the present data support the recommendation for modification of the United States diet calling for increased uptake of complex carbohydrates at the expense of simple sugars (56).

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