Fructose metabolizing enzymes in the rat liver and metabolic parameters: Interactions between dietary copper, type of carbohydrates, and gender

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This study was conducted to determine the effects of nutrient interactions between dietary carbohydrates and copper levels on fructose-metabolizing hepatic enzymes in male and female rats. Male and female rats were fed diets for 5 weeks that were either adequate or deficient in copper that contained either starch or fructose. Rats of both sexes fed fructose as compared with those fed starch showed higher activity of hepatic fructose metabolizing enzymes. There were also significant differences in fructose metabolism of liver between the male and female rats. Female rats had lower hepatic ketohexokinase and triose kinase but higher triosephosphate isomerase activities compared with male rats. Male rats fed copper-deficient diets had lower aldolase B activity compared with those fed copper-adequate diets. Female rats fed copper-deficient diets had higher triosephosphate isomerase activity compared with rats fed copper-adequate diets. Our data suggest that gender differences in hepatic fructose metabolism may not be the primary reason for the severity of copper deficiency syndrome in male rats fed copper-deficient diet with fructose. (J. Nutr. Biochem. 6:373–379, 1995.)

Introduction

Fructose is widely used as a commercial sweetener in foods, sweets, confectionery, and soft drinks. The introduction of high-fructose corn syrup in 1967 has led to an exponential increase in the consumption of free fructose in the food supply of the United States.[1] Dietary fructose has adverse effects on certain segments of the population. Fructose is more lipogenic than glucose or starch. It elevates uric acid and lactic acid in blood. It also causes greater elevation in plasma triglycerides and cholesterol than other dietary carbohydrates.[2]

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In the last decade attention has been focused on the effects of interactions between dietary fructose and copper on metabolic, biochemical, and endocrine parameters. The effects of these interactions are sex-dependent.[3,4] Male rats fed low-copper diets with fructose show more severe signs of copper deficiency than females even though indicators of copper deficiency such as levels of hepatic copper and serum ceruloplasmin activity are similar.[3] In addition, only male rats fed the copper-deficient diets with fructose die of cardiac rupture.[3–5] Gonadal sex hormones do not appear to be involved in the sexual difference in response to copper deficiency and fructose feeding.[4,5]

Fields et al.[6] have shown the differential effect of copper status and type of dietary carbohydrates on some of the enzymes in rats. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities are elevated in liver and heart diseases. It is possible that the activities of these enzymes may therefore increase in copper deficiency. Uric acid, blood urea nitrogen (BUN), and creatinine are elevated by fructose[2] and hence may be altered by copper–carbohydrate interaction.
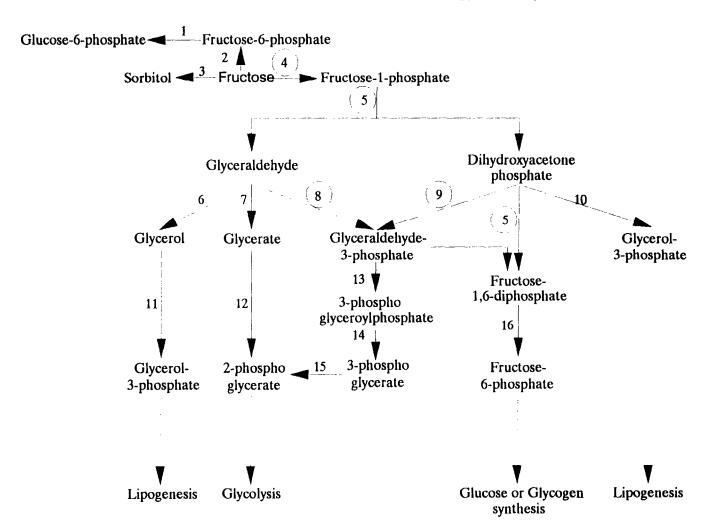
The severity of copper deficiency in the male rat is also dependent on the type of dietary carbohydrate. Substituting starch for fructose or supplementing the diet with copper, partly reverses the severity of the syndrome.[7] Since the diets of Western societies contain relatively high amounts of refined sugars,[1,2,8] especially fructose,[1] and marginal levels of copper,[9,10] these observations suggest a potential health risk for the human population.

In humans and in the rat, fructose is absorbed in the small intestine and metabolized primarily in the liver. Although both the small intestinal mucosa and the kidney contain the enzymes necessary for the metabolism of the ketohexose,[11] the utilization of fructose in extrahepatic tissues is minimal.[12] Fructose utilization is unique in that it bypasses the known metabolic pathways of glucose and therefore does not require insulin. The initial step in fructose metabolism is the phosphorylation of fructose by ketohexokinase (KHK) to produce fructose 1-phosphate (F-1-P). The second step is the splitting of F-1-P by aldolase B to dihydroxyacetone phosphate (DHAP) and glyceraldehyde (GAH). GAH is at the center of metabolic crossroads

(*Figure 1*) and has three possible metabolic routes. It can be phosphorylated by triose kinase (TK) to glyceraldehyde 3-phosphate (GAH3P) which continues through either glycolysis or gluconeogenesis for glycogen storage; converted to glycerate and then enter glycolysis after phosphorylation to 2-phosphoglycerate; and reduced to glycerol which can be phosphorylated and continue through lipogenesis or be converted to DHAP. DHAP can also follow three metabolic pathways. It can be isomerized to GAH3P by triosephosphate isomerase (TPI); reduced to glycerol 3-phosphate; or condensed with GAH3P by aldolase B to form fructose 1,6-diphosphate (F-1,6-P$_2$), and ultimately produce glucose or glycogen.

Gender differences in the metabolism of fructose may be partly responsible for the differences in the severity of copper deficiency between male and female rats. Other studies have implicated pathways involving sorbitol formation to explain the gender differences.[13,14] The purpose of the present study was to test the hypothesis that differences in hepatic fructose metabolism between male and female rats might account for the severity of copper deficiency in fructose-fed rats.

Figure 1 Possible pathways of fructose metabolism. (1) Phosphohexose isomerase; (2) Fructokinase or hexokinase (brain and muscle); (3) sorbitol dehydrogenase; (4) Ketohexokinase (liver); (5) aldolase B; (6) alcohol dehydrogenase or aldose reductase; (7) aldehyde dehydrogenase; (8) triose kinase; (9) triosephosphate isomerase; (10) glycerol-3-phosphate dehydrogenase; (11) glycerol kinase; (12) glycerate kinase; (13) glyceraldehyde-3-phosphate dehydrogenase; (14) phosphoglycerate kinase; (15) phosphoglyceromutase; (16) fructose diphosphatase. The enzymes measured in the present study are indicated by circled numbers.

Methods and materials

Thirty-two female and 32 male weanling Sprague–Dawley rats (Hilltop Lab Animals, Scottdale, PA USA) weighing 40 to 45 g were randomly assigned to four groups. The rats were housed individually in stainless-steel cages with wire-mesh bottoms in a temperature- and humidity-controlled room with 12 hr periods of light and dark. The rats were fed ad libitum for 5 weeks the diets containing either starch or fructose as the sole carbohydrate source. The diets were either deficient (0.6 mg of Cu/kg of diet) or adequate (6 mg of Cu/kg of diet) in copper as analyzed by atomic absorption spectrophotometry. The composition of the diets was as follows (g/kg of diet): fructose (A.E. Staley Corp., Decatur, IL USA) or cornstarch (CPC International, Inc., Englewood Cliffs, NJ USA), 627; egg white (ICN Biochemicals, Cleveland, OH USA), 200; corn oil (CPC International, Inc.), 95; cellulose (ICN Biochemicals), 30; AIN-76[15] mineral mixture prepared without copper (Teklad, Madison, WI USA), 35; AIN-76A[16] vitamin mixture (Teklad), 10; choline bitartrate (Teklad), 2.7; and biotin (Aldrich Chemicals, Milwaukee, WI USA), 0.002. Copper was added to the appropriate diets as copper carbonate (Fisher Scientific, Pittsburgh, PA USA). All animals were allowed free access to distilled deionized drinking water.

Body weights were recorded weekly. At the end of the feeding period the rats were fasted for 14 hr and decapitated. Trunk blood was collected and kept on ice before centrifugation at 1,500g for 25 min at 4°C. Serum was separated and stored at -70°C until used. Livers were quickly removed, blotted, weighed, and placed on dry ice and stored at -70°C until analyzed. Diet and liver samples, 1 g each, were digested by dry heat and acid[17] and copper was measured by using flame atomic absorption spectrophotometry (model 5000, Perkin Elmer, Norwalk, CT USA). Bovine liver 1577a, obtained from the National Institute of Standards and Technology (Gaithersburg, MD USA), was digested and analyzed along with samples to verify accuracy.

Serum uric acid, blood urea nitrogen, and creatinine levels, and serum alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) activities were measured by the Centrifichem automated procedure (Trace-America, Miami, FL USA).

Fructose metabolizing enzymes were extracted from livers by the following procedure: one part of liver was homogenized with four parts (wt/vol) of ice-cold 10 mM Tris-HCl buffer, pH 7.4, containing 0.15 M KCl, 5 mM EDTA, and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 30,000g for 20 min at 4°C. The resulting supernatant served as the source of the enzymes. The protein content of the supernatant was measured according to Lowry et al.,[18] using bovine serum albumin as standard.

All auxiliary enzymes and chemicals used in this work were obtained from Sigma Chemical Co. (St. Louis, MO USA). All enzymes, except fructokinase, were studied spectrophotometrically at 340 nm at 25°C.

KHK, EC 2.7.1.3, was measured by the method described by Hers.[19] The reaction mixture contained, in a volume of 1.20 mL, reagents in final concentration as follows: 50 mM imidazole buffer, pH 7.0; 1.85 mM fructose, as substrate; 4 mM NaATP; 4 mM MgCl$_2$; 1 M KAc; 20 mM N-acetylglucoseamine (to inhibit competitively the phosphorylation of fructose by hexokinase); and 40 mM NaF. A control without NaATP and MgCl$_2$ was carried out in parallel. The reaction was started by the addition of 0.1 mL of enzyme extract containing 1 mg of protein. The mixture was incubated for 16 min at 37°C. The reaction was stopped by the addition of 3 mL of 0.15 M ZnSO$_4$ followed by 3 mL of 0.1 M Ba(OH)$_2$. After thorough mixing and centrifugation at 5,500g for 15 min, residual fructose was determined in the supernatant according to Roe.[20]

Triose kinase (TK), EC 2.7.1.28, was assayed by a modification of the method of Frandsen and Grunnet.[21] The reaction mixture contained, in a volume of 2.56 mL, reagents in final concentration as follows: 50 mM imidazole buffer, pH 7.0; 7 mM NaATP; 7 mM MgCl$_2$; 5 mM arsenate; 100 mM KCl; 4 U of glycerophosphate dehydrogenase (GDH); 10 U of triosephosphate isomerase (TPI); 5 U of lactic dehydrogenase (LDH); 0.3 mM NADH and enzyme extract containing 1 mg of protein. The reaction was started by the addition of 5 mM D-glyceraldehyde as substrate and was monitored for 20 min.

Aldolase B, EC 4.1.2.13—In order to have an appropriate evaluation of the aldolase, both F-1-P and F-1,6-P$_2$ were used as substrates.[22,23] The reaction mixture contained, in a volume of 2.76 mL, reagents in final concentration as follows: 50 mM collidine buffer, pH 7.4; 0.3 mM iodoacetate; 4 U of GDH; 10 U of TPI; 5 U of LDH; 0.3 mM NADH; and an enzyme extract containing 1 mg of protein. The reaction was started by the addition of 10 mM F-1-P or 2 mM F-1,6-P$_2$ as substrates, and was monitored for 20 min.

TPI, EC 5.3.1.1, was measured as described by Bergmeyer et al.[24] The reaction mixture contained, in a volume of 2.56 mL, reagents in final concentration as follows: 300 mM triethanolamine buffer, pH 7.6; 4 mM GAH3P, as substrate; 0.3 mM NADH; and 3 U GDH. The reaction was started by the addition of 0.01 mL liver extract containing 0.1 mg of protein, and was monitored for 2 min. Enzyme activities were expressed as nkat/mg protein (nkat = nM substrate/sec).

All data were analyzed by a $2 \times 2 \times 2$ ANOVA design to study the interactions between copper, carbohydrate, and sex.[25]

Results

Table 1 summarizes the influence of sex, type of carbohydrate, and copper status on hepatic copper, serum metabolites, and enzyme activities. In both genders, hepatic copper concentration was lower in rats fed the copper-deficient diet regardless of dietary carbohydrate. Regardless of the type of carbohydrate, serum uric acid levels were higher in rats fed the copper-deficient diets compared with those fed the copper-adequate diets. The levels were significantly higher in male than in female rats. Blood urea nitrogen levels in both sexes were higher in copper-deficient rats fed fructose than in copper-adequate rats. The levels were higher in male rats compared with female rats and in rats fed fructose compared with those fed starch. Creatinine levels were higher in copper-deficient rats than in copper-adequate rats. Male rats had higher ALT activities than female rats. Serum AST activity was not affected by copper level, type of carbohydrate fed, or gender.

The effects of dietary copper level and carbohydrates on the specific activities of fructose-metabolizing enzymes in the liver of female and male rats are presented in *Table 2*. Regardless of dietary copper intake, the activities of hepatic KHK and TK were higher in males than in female rats. Dietary fructose significantly increased KHK and TK activities in both genders. Hepatic aldolase-B activity with F-1-P as substrate was higher in rats fed fructose as compared with those fed starch. Copper deficiency decreased the activity in male rats fed either starch or fructose but not in female rats. Hepatic aldolase-B activity with F-1,6-P$_2$ as substrate was higher in male as compared with female rats and was increased by fructose feeding as compared with starch. Rats fed a copper-deficient diet showed significantly

Table 1 Liver copper and serum parameters in female and male rats fed diets with fructose or starch and either adequate or deficient in copper, for 5 weeks*

Sex	CHO	Copper	Hepatic copper (mmol/g wet wt)	Uric acid (mmol/L)	BUN (mmol/L)	Creatinine (mmol/L)	ALT (U/L)	AST (U/L)
F	Fru	CuA	100.9 ± 7.7	79.8 ± 18.5	3.66 ± 0.93	22.3 ± 7.7	30.1 ± 6.3	120.5 ± 21.9
F	Fru	CuD	24.7 ± 4.4	92.2 ± 20.6	5.74 ± 0.99	28.9 ± 7.2	34.4 ± 4.4	130.1 ± 41.8
F	Sta	CuA	110.3 ± 11.8	80.7 ± 16.9	2.97 ± 0.83	21.5 ± 9.3	28.2 ± 6.7	138.0 ± 16.2
F	Sta	CuD	40.4 ± 3.0	91.8 ± 19.9	3.83 ± 0.82	28.2 ± 4.1	30.4 ± 3.8	149.0 ± 30.0
M	Fru	CuA	97.1 ± 6.3	120.8 ± 20.6	4.22 ± 0.64	24.5 ± 3.6	42.9 ± 10.3	123.8 ± 25.5
M	Fru	CuD	24.6 ± 2.8	136.8 ± 27.7	8.45 ± 1.70	29.0 ± 3.3	39.0 ± 4.3	132.0 ± 24.7
M	Sta	CuA	97.3 ± 8.2	107.8 ± 19.4	3.62 ± 0.76	21.9 ± 7.2	40.4 ± 7.5	135.5 ± 16.4
M	Sta	CuD	41.1 ± 4.9	118.6 ± 14.7	4.35 ± 0.02	24.1 ± 6.1	34.7 ± 2.8	144.9 ± 36.4

ANOVA†					P values			
Sex			S	S	S	NS	S	NS
CHO			S	NS	S	NS	NS	NS
Copper			S	S	S	S	NS	NS
Sex × CHO			NS	NS	S	NS	NS	NS
Sex × copper			S	NS	NS	NS	S	S
CHO × copper			S	NS	S	NS	NS	NS
Sex × CHO × copper			NS	NS	S	NS	NS	NS

CHO, carbohydrate; F, female; M, male; Fru, fructose; Sta, starch; CuA, copper-adequate; CuD, copper-deficient; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
†2 × 2 × 2 analysis of variance. S, significant ($P < 0.05$); NS, nonsignificant.
*Values are means + standard deviation (8 rats/group).

lower $F-1,6-P_2$ activity than those fed a copper-adequate diet. Triosephosphate isomerase activity was higher in female than in male rats. In female rats the TPI activity was significantly higher in rats fed a copper-deficient diet than in those fed a copper-adequate diet.

Discussion

In laboratory animals, the severity of the copper deficiency syndrome depends on gender,[4] type of dietary carbohydrate[26,27] and the age of the animal at the time of induction

Table 2 Fructose metabolizing enzyme activities in the liver of female and male rats fed diets with fructose or starch and either adequate or deficient in copper for 5 weeks*

Sex	CHO	Copper	Ketohexokinase (KHK) (nkat/mg protein)	Triose Kinase (TK) (nkat/mg protein)	Aldolase B Isomerase		Triosephosphate (TPI) (nkat/mg protein)
					F-1-P (nkat/mg protein)	F-1,6-P$_2$ (nkat/mg protein)	
F	Fru	CuA	2.52 ± 0.33	0.040 ± 0.012	0.205 ± 0.021	0.216 ± 0.036	48.44 ± 11.45
F	Fru	CuD	2.44 ± 0.37	0.044 ± 0.004	0.186 ± 0.016	0.195 ± 0.031	62.13 ± 4.12
F	Sta	CuA	1.87 ± 0.22	0.035 ± 0.007	0.144 ± 0 020	0.153 ± 0.016	44.16 ± 7.44
F	Sta	CuD	1.77 ± 0.28	0.034 ± 0.006	0.139 ± 0.021	0.143 ± 0.029	54.01 ± 9.24
M	Fru	CuA	3.11 ± 0.36	0.056 ± 0.005	0.224 ± 0.022	0.246 ± 0.027	40.14 ± 12.96
M	Fru	CuD	2.90 ± 0.51	0.054 ± 0.003	0.169 ± 0.026	0.195 ± 0.048	40.36 ± 13.82
M	Sta	CuA	2.33 ± 0.33	0.049 ± 0.006	0.189 ± 0.009	0.203 ± 0.012	32.41 ± 8.49
M	Sta	CuD	2.22 ± 0.27	0.045 ± 0.004	0.154 ± 0.016	0.173 ± 0.016	32.20 ± 5.97

ANOVA†					P values		
Sex			S	S	S	S	S
CHO			S	S	S	S	S
Copper			NS	NS	S	S	S
Sex × CHO			NS	NS	S	NS	NS
Sex × copper			NS	NS	S	NS	S
CHO × copper			NS	NS	NS	NS	NS
Sex × CHO × copper			NS	NS	NS	NS	NS

CHO, carbohydrate; F, female; M, male; Fru, fructose; Sta, starch; CuA, copper-adequate; CuD, copper-deficient; F-1-P, fructose 1-phosphate; F-1,6-P$_2$, fructose 1,6-diphosphate; nKat, nM substrate/sec.
†2 × 2 × 2 analysis of variance. S, significant ($P < 0.05$); NS, nonsignificant.
*Values are means ± standard deviation (8 rats/group).

of the deficiency.[28] Although both male and female rats consumed the same copper-deficient diet containing fructose, only males exhibited numerous abnormalities and died prematurely of heart related pathologies.[3] We therefore hypothetized that differences in fructose metabolism between male and female rats may be in part responsible for the premature mortality of the copper-deficient male rats fed fructose. It is also possible that one or more of several risk factors recognized for heart pathology may be differently altered by gender, dietary copper level, and by the type of dietary carbohydrate.

In agreement with other studies,[4,26,29-31] we observed that only copper-deficient, fructose-fed male rats exhibited severe copper deficiency symptoms such as decreased weight gain, anemia, atrophy of the pancreas, hypertrophy of liver and heart, gross pathology of the heart, and premature mortality (data not shown). We and others have previously shown that substituting starch for fructose reduced the pathologies of copper deficiency in rats fed copper-deficient diets.[5,26] In the present study, fructose-fed, copper-deficient rats of both sexes had a lower hepatic copper concentration than those fed starch which may be due to reduced intestinal absorption of copper. Johnson and Gratzek[32] reported lower apparent copper absorption when copper-deficient rats were fed sucrose compared with starch. Fields et al.[33] did not observe significant difference in copper absorption between copper-deficient rats fed starch or fructose over a 24 hr period when given copper-67. However, the rats fed copper-deficient diet did show decreased absorption 48 to 96 hr following ingestion of radiolabeled copper.

Elevated plasma uric acid and BUN levels and elevated ALT and AST activities have been implicated as risk factors for heart disease. In the present study, the differences in the concentrations of some of these blood risk factor metabolites such as uric acid and BUN due to gender, type of dietary carbohydrate, or copper were significant and could explain in part the deleterious effects of fructose in male rats fed the low copper diet. It is important to note that urate in plasma and tissues has been shown to have an antioxidant activity[34-36] and proposed to have a physiologic role as a free radical scavanger in human tissues.[34] It is possible that the higher uric acid level observed in the present study in copper-deficient rats and in male rats may be in response to increased free radical damage. This, however, needs to be demonstrated. Dietary fructose and low copper diet both have been reported to increase uric acid in humans.[2,37]

In male rats, sucrose and fructose as compared with starch cause greater elevation in the activities of the enzymes of the pentose phosphate shunt.[38] In the present study, we focused on enzymes involved in fructose metabolism in liver. As expected, the specific activity of KHK which phosphorylates fructose to F-1-P, was significantly greater in rats fed fructose compared with those fed starch confirming the earlier reports in normal and diabetic animals.[39-41] The activity was higher in male than in female rats, but dietary copper level had no effect. Though KHK has a higher affinity for fructose with a K_m of approximately 0.5 mM,[42] it is not specific for fructose. Thus, it phosphorylates other ketoses such as galactoheptulose, sorbose, tagatose, and xylulose.[43] Since hexokinase activity is essen-

tially absent in liver,[44] phosphorylation of fructose to fructose 6-phosphate by hexokinase is unlikely to occur.

Though there are three homologs of fructoaldolase in vertebrates, namely A, B, and C,[45] only aldolase B is present in liver in large quantity. It acts on both F-1-P and F-1,6-P$_2$.[46] In liver, F-1-P is converted to two trioses by aldolase B, namely GAH and DHAP. In the present study we measured aldolase B activity using both substrates. Dietary fructose compared with starch produced higher activity of the enzyme using both substrates. Similarly, male rats had higher activity of aldolase B compared with female rats. However, in females the dietary copper level had no effect on the enzymes for either substrate, but in male rats the activity was lower for both substrates in copper-deficient rats compared with copper-adequate rats. Higher KHK activity and lower aldolase B activity with F-1-P as substrate in male rats fed copper-deficient diet will lead to greater accumulation of F-1-P in the liver of these rats.

Although glyceraldehyde can enter the glycolytic pathway via three different routes, its conversion to glyceraldehyde 3-phosphate by liver TK is the favored pathway.[41] We observed higher activity of TK in male rats compared with female rats, and dietary fructose elevated the activity of the enzyme compared with starch. There was, however, no effect of level of copper intake on the activity of this enzyme. Fields et al. showed that males had higher concentrations of glyceraldehyde than females and fructose feeding increased its levels compared with starch feeding.[14] Glyceraldehyde has been shown to generate free radicals which in turn can cause damage to tissues.[47]

Glyceraldehyde 3-phosphate can also be produced reversibly from dihydroxyacetone phosphate by TPI and the two of them can be converted to F-1,6-P$_2$ by aldolase. We measured TPI activity using glyceraldehyde 3-phosphate as substrate and observed lower activity in male rats compared with female rats. Dietary fructose appeared to increase the activity of the enzyme compared with starch. It is important to note that in female rats but not in male rats, feeding a copper-deficient diet with fructose caused a significant elevation in the activity of the enzyme compared with rats fed the copper-adequate diet. This may indicate a better utilization of fructose by females compared with males.

Though none of the fructose metabolizing enzymes showed large differences between rats fed diets either adequate or deficient in copper, all the enzymes involved in fructose metabolism measured in this study showed major differences due to gender and type of dietary carbohydrate. Fructose feeding induced higher activities of hepatic enzymes than starch feeding. Higher KHK activity and lower aldolase B activity with F-1-P as substrate will lead to greater accumulation of F-1-P in the liver of male rats compared with female rats. In addition, lower activity of aldolase B in rats fed a copper-deficient diet compared with those fed a copper-adequate diet will lead to further accumulation of F-1-P in male rats fed copper-deficient diets. Higher TK activity in male rats compared with female rats indicates better utilization of glyceraldehyde to form glyceraldehyde 3-phosphate. Similarly, decreased TPI activity and increased aldolase B activity with F-1,6-P$_2$ as substrate indicates higher convertion of dihydroxyacetone phosphate to F-1,6-P$_2$ in male rats compared with female rats. Thus,

interactions between gender and type of dietary carbohydrate in rats fed the low copper diet are responsible, in part, but are not the primary cause for the differences in the severity of pathologies associated with copper deficiency syndrome in the male rat. The exact metabolic pathway however is not clear and further studies are needed to understand the underlying mechanism.

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