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Hepatic fructose-metabolizing enzymes and related metabolites: Role of dietary copper and gender

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The purpose of this study was to further examine the hypothesis that variations in hepatic fructose-metabolizing enzymes between males and females might account for the differences in the severity of copper (Cu) deficiency observed in fructose-fed male rats. Weanling rats of both sexes were fed high-fructose diets either adequate or deficient in copper for 45 days. Cu deficiency decreased sorbitol dehydrogenase activity and dihydroxyacetone phosphate levels and increased glyceraldehyde levels in both sexes. Gender effects were expressed by higher activities of glycerol 3-phosphate dehydrogenase and aldehyde dehydrogenase in male than in female rats and higher levels of dihydroxyacetone phosphate and fructose 1,6-diphosphate (F1,6DP) in female than in male rats. The interactions between dietary Cu and gender were as follows: alcohol dehydrogenase activities were higher in female rats and were further increased by Cu deficiency in both sexes; aldehyde dehydrogenase activities were decreased by Cu deficiency only in male rats; sorbitol levels were higher in male rats and were further increased by Cu deficiency in male rats; fructose 1-phosphate (F1P) levels were increased by Cu deficiency in both sexes, but to a greater extent in male rats; glyceraldehyde 3-phosphate levels were higher in female rats, but were decreased by Cu deficiency in female and increased in male rats. Though most of the examined hepatic fructose-metabolizing enzymes and metabolites showed great differences between rats fed diets either adequate or deficient in Cu, it is the activity of fructokinase and aldolase-B, and the concentrations of their common metabolites, F1P and notably F1,6DP, that could be in part responsible for differences in the severity of pathologies associated with Cu deficiency observed between female and male rats. (J. Nutr. Biochem. 11: 374–381, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

Keywords: copper deficiency; gender; fructose; metabolism; liver; rats

Introduction

Copper (Cu), an important trace element, plays a vital role in various biological, physiologic, and structural integrity functions in animals.[1] Expression of the signs of dietary Cu deprivation in rats is dependent on the age of the animal at the time of inducing Cu deficiency,[2] gender,[3] type of dietary carbohydrate,[4] and growth rate.[5–8] During fetal, weaning, and early postweaning periods, rats are extremely susceptible to Cu deprivation.[9] Furthermore, only weaned male rats, but not female, consuming a low Cu diet containing fructose exhibit severe signs of Cu deficiency. These include reduced body weight, hepatic and cardiac enlargement, atrophy of the pancreas, anemia, hypercholesterolemia, hypertriglyceridemia, and cardiac and kidney abnormalities that eventually lead to early death.[4,7,10]

The lethal combination of low-Cu, high-fructose diets should raise concern in industrialized societies for several reasons. First, diets in Western societies are low in Cu.[11] Second, fructose is widely used as a commercial sweetener in foods, sweets, confectioneries, and soft drinks. Third, the introduction of high-fructose corn syrup in 1967 has lead to an exponential increase in the presence of free fructose in the food supply.[12] Fructose is more lipogenic than are glucose or starch. It elevates uric acid and lactic acid in blood and causes greater elevation in plasma triglycerides and cholesterol than other dietary carbohydrates.[13] In humans and rats, fructose is primarily metabolized in the liver,

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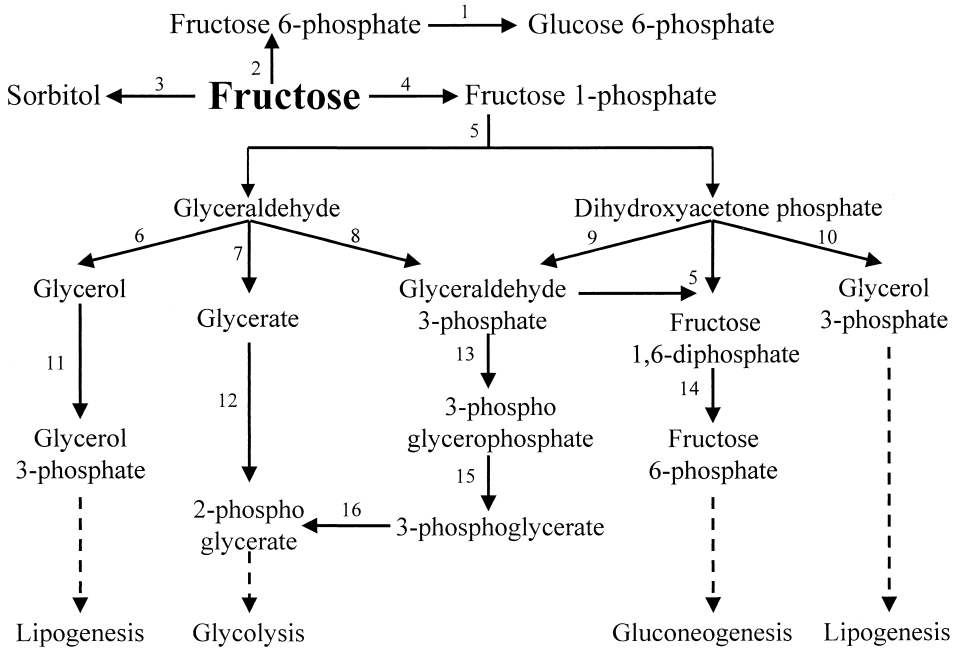

Figure 1 Pathways of fructose metabolism: (1) phosphohexose isomerase; (2) hexokinase; (3) sorbitol dehydrogenase; (4) fructokinase; (5) aldolase-B; (6) alcohol dehydrogenase; (7) aldehyde dehydrogenase; (8) triose kinase; (9) triosephosphate isomerase; (10) glycerol 3-phosphate dehydrogenase; (11) glycerol kinase; (12) glycerate kinase; (13) glyceraldehyde 3-phosphate dehydrogenase; (14) fructose diphosphatase; (15) phosphoglycerate kinase; (16) phosphoglyceromutase

although both the kidney and the small intestinal mucosa contain the enzymes necessary for the metabolism of the ketohexose.[14] Fructose metabolism is unique in that it bypasses the known metabolic pathway of glucose and therefore does not require insulin. In the liver, fructose can be metabolized in three different pathways (*Figure 1*): It can be (1) phosphorylated to fructose 6-phosphate by hexokinase; (2) reduced to sorbitol by sorbitol dehydrogenase (SDH); or (3) phosphorylated to fructose 1-phosphate (F1P) by fructokinase (FK). Because the affinity of hexokinase and SDH to fructose is lower than that of FK to fructose, fructose is mainly metabolized by FK.[15] Studies conducted to clarify the differences in the expression of the signs associated with Cu deficiency, when fructose is consumed, concluded that gonadal sex hormones do not appear to be involved in the sex-dependent differences.[16–18] Other studies failed to confirm the hypothesis that fructose, compared with starch, reduces Cu absorption and bioavailability.[19–22]

Several studies[5–8] showed that lowering growth rate by decreasing dietary energy density or food intake to 70 to 80% of normal levels ameliorates the severity of Cu deficiency and prevents mortality in male rats. Attempts also have been made to determine whether the exacerbation of Cu deficiency, in male versus female rats, during fructose feeding was due to some sex-related variations in hepatic fructose metabolism. Fields et al.[23] implicated pathways involving sorbitol formation to explain the gender differences. In our recent study[24] we examined the specific activities of several fructose-metabolizing enzymes, namely FK, triose kinase (TK), triosephosphate isomerase (TIM), and aldolase B, and observed significant differences in hepatic fructose metabolism between male and female rats.

The purpose of the present study is to further advance our earlier findings[24] that suggested that differences in hepatic fructose metabolism between male and female rats might account for the severity of Cu deficiency in fructose-fed male rats. We analyzed variations in the activities of an additional four enzymes and concentrations of seven metabolites involved in hepatic fructose metabolism.

Materials and methods

Weanling male and female Sprague-Dawley rats weighing 40 to 45 g each were obtained from the colony of the Department of Food Engineering and Biotechnology at the Technion – Israel Institute of Technology. Rats were randomly assigned to four groups of eight rats each, according to gender and dietary Cu level. Rats were housed individually in stainless-steel cages in a temperature-controlled room with 12-hr periods of light and dark. Rats were treated according to the Ethics Committee of the Technion for experimentation in animals. The rats were all fed the basal diet that contained fructose as the sole carbohydrate source. Diets were either deficient (CuD; 9.5 μmol Cu/kg of diet) or adequate (CuA; 96 μmol Cu/kg of diet) in Cu as analyzed by atomic absorption spectrophotometry.

The composition of the diets was as follows (g/kg diet): fructose, 627; egg white, 200; soybean oil, 95; cellulose, 30; AIN-93 mineral mixture, without Cu,[25] 35; AIN-93 vitamin mixture,[25] 10; choline bitartrate, 2.7; and biotin, 0.002. Cu was added to the appropriate diet as Cu carbonate. Rats were allowed free access to the diet and to distilled deionized drinking water and were maintained on their respective diets for 45 days. Body weights were recorded weekly.

At the end of the feeding period, rats were deprived of food for 14 hr and decapitated. Trunk blood was collected simultaneously in a capillary tube for hematocrit determination and in a centrifuge tube without anticoagulant for chemical analysis. Blood was kept on ice before centrifugation at 2,500 × g for 25 min at 4°C, and serum was separated and stored at −80°C until used. Livers were quickly removed, blotted, weighed, and stored immediately at −80°C until analyzed. Diets and liver samples, weighing 1 g each, were digested by dry heat and acid,[26] and Cu was measured by using a flame atomic absorption spectrophotometer (Instrumentation Laboratory Inc., AA/AE 157, Lexington, MA USA). Serum

Table 1 Body weight, relative liver size, liver copper (Cu), hematocrit, and ceruloplasmin activity in female (F) and male (M) rats fed high-fructose diets either adequate (CuA) or deficient (CuD) in Cu for 45 days

Study variables			Parameters				
Cu	Gender	*n*	Body weight *(g)*	Liver weight *(mg/g BW)*	Liver Cu *(nmol/g wet wt)*	Hematocrit *(volume fraction)*	Ceruloplasmin activity (U/L)
CuA	F	8	183 ± 6	38.3 ± 2.1	63.58 ± 6.45	0.46 ± 0.04	164 ± 10
CuA	M	8	287 ± 18	33.2 ± 2.2	74.49 ± 13.37	0.48 ± 0.02	138 ± 6
CuD	F	8	162 ± 12	46.3 ± 3.6	11.65 ± 3.46	0.39 ± 0.04	ND
CuD	M	6	225 ± 23	51.3 ± 6.8	6.62 ± 0.11	0.33 ± 0.04	ND
Source of variation			Analysis of variance				
			P-value				
Cu			0.0001	0.0001	0.0001	0.0001	0.0001
Gender			0.0001	NS	NS	NS	NS
Cu × gender			0.0033	0.0074	0.0012	0.0287	0.0012

Values are means ± SD.
ND—not detectable. NS—not significant ($P > 0.005$).

ceruloplasmin activity was determined with o-dianisidine dihydrochloride as a substrate according to Schosinsky et al.[27]

Fructose-metabolizing enzymes, except SDH, were extracted from livers by the following procedure: 1 part of liver was homogenized with 4 parts (wt/vol) of ice-cold 0.01 mol/L Tris-HCl buffer, pH 7.4, containing 0.15 mol/L KCl, 5 mmol/L ethylenediamine-tetraacetic acid (EDTA), and 10 mmol/L 2-mercaptoethanol. The homogenate was centrifuged at 34,500 × g for 40 min at 4°C. The resulting supernatant served as the source of the enzymes. For the analysis of SDH, enzyme extract was prepared by the following procedure: 1 part of liver was homogenized with 6 parts (wt/vol) of ice-cold 0.25 mol/L sucrose, pH 7.4, containing 20 mmol/L Tris-HCl and 0.2 mmol/L dithiothreitol. The homogenate was centrifuged at 18,000 × g for 10 min at 4°C. The resulting supernatant served as the source of the enzyme.

Protein content of the two supernatants was measured according to the method of Lowry et al.,[28] using bovine serum albumin as a standard.

All auxiliary enzymes and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO USA). All enzymes were determined spectrophotometrically at 340 nm and 25°C.

Activity of SDH (EC 1.1.1.14) was assayed according to the procedure described by Gerlach.[29] Activity of aldehyde dehydrogenase (ALDEH; EC 1.2.1.5) was assayed according to the method described by Bergmeyer et al.[30] Activity of glycerol 3-phosphate dehydrogenase (G3PDH; EC 1.1.1.8) was assayed according to the method described by Bergmeyer et al.[31]. Activity of alcohol dehydrogenase (ADH; EC 1.1.1.2) was assayed following the procedure described by Sillero et al.[15]

Hepatic metabolites were extracted by the following procedure: 1 part of liver was homogenized with 3 parts (wt/vol) of ice-cold 0.6 mol/L perchloric acid at 4°C. The homogenate was centrifuged at 10,800 × g for 15 min at 4°C. The resulting supernatant was collected and brought to pH 7.0 with 2 mol/L KOH at 4°C. Ice-cold water was added to bring the final volume to 8 parts (wt/vol) of the initial hepatic sample weight. The solution was kept tightly closed on ice for 1 hr, centrifuged at 10,800 × g for 15 min at 4°C, and the metabolite concentration determined in the supernatant. In all the metabolite examinations, the blank contained water instead of metabolite extract.

The concentrations of dihydroxyacetone phosphate (DHAP), fructose 1,6-diphosphate (F1,6DP), and glyceraldehyde 3-phosphate (GA3P) were determined colorimetrically at 340 nm according to the procedure described by Michal.[32] F1P was determined according to Eggelston.[33] Sorbitol concentration was determined as described by Bergmeyer et al.[34] Glyceraldehyde (GAD) concentrations were determined as described by Redina and Cleland.[35] Hepatic fructose concentrations were determined colorimetrically as described by Roe.[36]

Statistical analysis was performed on a personal computer using the SAS software.[37] All data were analyzed by two-way analysis of variance designed to study the main and interactive effects of Cu and gender using the general linear models procedure of SAS.

Results

At the end of the feeding period, two CuD male rats died. Autopsy revealed urinary bladder enlargement and accumulation of blood in the bladder, suggesting that death occurred due to kidney failure. No deaths occurred in any of the other dietary groups.

The effects of dietary Cu and gender on body weight, relative liver size, hepatic Cu concentration, hematocrit, and ceruloplasmin activity are summarized in *Table 1*. Female rats in both dietary Cu regimens gained significantly less body weight than did male rats, and Cu deficiency significantly reduced body weight in both sexes. Male CuD rats had significantly higher relative liver size than did female rats. Hepatic Cu levels in CuD rats were significantly lower than in their CuA counterparts and were significantly lower in CuD male than in female rats. Cu deficiency reduced hematocrits in both sexes, but CuD male rats exhibited the lowest hematocrits. Serum ceruloplasmin activity was not detected in CuD rats of either gender.

The specific activities of the tested enzymes participating in the metabolism of fructose in the liver are presented in *Table 2*. Hepatic SDH activity was not affected by gender, but was lower in CuD rats than in their adequate controls. Regardless of dietary Cu status, the activity of hepatic G3PDH was significantly higher in male than in female rats. The activities of hepatic ALDEH were significantly higher

Table 2 Specific activity of sorbitol dehydrogenase (SDH), glycerol 3-phosphate dehydrogenase (G3PDH), aldehyde dehydrogenase (ALDEH), and alcohol dehydrogenase (ADH) in female (F) and male (M) rats fed high-fructose diets either adequate (CuA) or deficient (CuD) in copper (Cu) for 45 days

Study variables			Fructose-metabolizing enzymes (nmol/(sec • mg protein)			
Cu	Gender	*n*	SDH	G3PDH	ALDEH	ADH
CuA	F	8	1.368 ± 0.217	5.403 ± 0.601	0.341 ± 0.046	0.052 ± 0.003
CuA	M	8	1.384 ± 0.116	6.544 ± 0.374	0.466 ± 0.049	0.044 ± 0.004
CuD	F	8	1.149 ± 0.129	5.226 ± 0.535	0.357 ± 0.043	0.079 ± 0.007
CuD	M	6	0.978 ± 0.169	6.013 ± 0.643	0.374 ± 0.039	0.057 ± 0.008
Source of variation			Analysis of variance			
			P-value			
Cu			0.0001	NS	0.0244	0.0001
Gender			NS	0.0001	0.0001	0.0001
Cu × gender			NS	NS	0.0021	0.0058

Values are means ± SD.
NS—not significant ($P > 0.05$).

in male than in female rats and were reduced by Cu deficiency only in male rats. Specific activities of hepatic ADH were significantly higher in female than in male rats. Cu deficiency elevated ADH activities in both sexes, but to a greater extent in CuD female rats.

The concentrations of the seven metabolites involved in the metabolism of fructose in the liver are summarized in *Table 3*. Hepatic sorbitol levels were higher in male than in female rats. The highest hepatic sorbitol levels were observed in CuD male rats. Hepatic fructose levels were not affected by Cu status or by gender. The concentrations of hepatic F1P were elevated by Cu deficiency in both sexes, and the highest level was observed in CuD male rats. Regardless of gender, hepatic GAD levels were increased by Cu deficiency. Hepatic concentrations of DHAP were decreased by Cu depletion and were found to be higher in female than in male rats. Levels of GA3P were not affected by dietary Cu status, but were found to be higher in female than in male rats. Hepatic F1,6DP levels were higher in female than male rats. The highest F1,6DP levels were observed in CuD female rats.

Discussion

In agreement with previous studies, the results of the present investigation demonstrated that Cu deficiency combined with fructose feeding caused a reduction in body weight gain, anemia, enlarged liver, reduced hepatic Cu stores in both sexes, and mortality among male rats.[4,7,18,38]

In male rats, sucrose and fructose as compared with starch caused elevation in the activity of several enzymes of fructose metabolism[24] and the pentose phosphate shunt.[39] In the present study we focused on four more enzymes and relevant metabolites involved in the hepatic fructose metab-

Table 3. Hepatic sorbitol, fructose, fructose 1-phosphate (F1P), glyceraldehyde (GAD), dihy-droxyacetone phosphate (DHAP), glyceraldehydes 3-P (GA3P), and fructose 1,6-diphosphate (F1,6DP) in female (F) and male (M) rats fed high-fructose diets either adequate: (CuA) or deficient (CuD) in copper (Cu) for 45 days

Study variables			Metabolites (μ*mol/g wet wt*)						
Cu	Gender	*n*	Sorbitol	Fructose	F1P	GAD	DHAP	GA3P	F1,6DP
CuA	F	8	0.202 ± 0.026	8.40 ± 2.50	0.867 ± 0.182	2.98 ± 0.58	0.040 ± 0.012	0.014 ± 0.002	0.055 ± 0.018
CuA	M	8	0.215 ± 0.025	8.06 ± 1.98	0.832 ± 0.123	2.61 ± 0.29	0.034 ± 0.008	0.008 ± 0.001	0.041 ± 0.009
CuD	F	8	0.187 ± 0.033	8.99 ± 2.71	0.926 ± 0.080	3.36 ± 0.33	0.035 ± 0.008	0.010 ± 0.003	0.064 ± 0.010
CuD	M	6	0.258 ± 0.030	7.06 ± 2.66	1.126 ± 0.133	3.31 ± 0.85	0.024 ± 0.007	0.010 ± 0.002	0.039 ± 0.007
Source of variation			Analysis of variance						
			P-value						
Cu			NS	NS	0.0028	0.0415	0.0385	NS	NS
Gender			0.0037	NS	NS	NS	0.0271	0.0203	0.0005
Cu × gender			0.0306	NS	0.0362	NS	NS	0.0046	NS

Values are means ± SD.
NS—not significant ($P > 0.05$).

olism in both sexes. Hepatic fructose metabolism is a complex mechanism composed of sequential events involving several enzymes that may be affected by allosteric effectors caused by variations in metabolites (*Figure 1*). However, measuring the specific activity of fructose-metabolizing enzymes in liver extracts in vitro does not necessarily reflect the activity of these enzymes in situ. Furthermore, although the enzymes could be the targets, it is the metabolites' deranged patterns that are probably the agents of destruction. Therefore, to establish credibility of the enzyme's impact, we tried to relate specific activities with metabolite concentration. Because hepatic fructose metabolism in Cu deficiency deals with a series of enzymes with altered specific activities, we expect those enzymes altered early in the pathway to repeal the influence of the later ones. Therefore, if a lesion site exists, the enzymes beyond this point may not contribute or would have only a secondary impact on the pathology. However, one should keep in mind that non-rate-limiting enzymes operating at less than full capacity may still maintain a normal metabolic pathway.

Low activities of hepatic SDH were observed in CuD, fructose-fed male rats. However, no evidence exists in the literature suggesting that SDH requires Cu for optimal activity. SDH is found mainly in liver, kidney, and prostate gland, located both in the cytoplasm and mitochondria. No specific activators for SDH are known, and most of the familiar inhibitors are not present naturally. Although SDH activity in CuD male rats is lower than that in female rats (*Table 2*), they demonstrate the highest hepatic sorbitol concentration (*Table 3*). This discrepancy is probably due to the fact that both glucose and fructose can be reduced to sorbitol by aldose reductase and SDH, respectively. Thus, although hepatic fructose was not altered in our study, glucose as a precursor of sorbitol cannot be ruled out. Sorbitol is a strong chelator of Cu,[40] and the increased levels of sorbitol may chelate the limited amounts of Cu in the liver of CuD male rats, making the chelated Cu unavailable for utilization.

In our recent study[24] we observed that Cu deficiency significantly reduced hepatic FK activity in both sexes. Though FK has a high affinity for fructose, with a K_m of approximately 0.5 mM, it is not specific for fructose and phosphorylates other ketoses such as sorbose, tagatose, xylulose, and galactoheptulose.[41] Because hexokinase activity is essentially absent in the liver,[42] phosphorylation of fructose to fructose 6-phosphate by hexokinase (*Figure 1*) is unlikely to occur. FK catalyzes the phosphorylation of fructose using adenosine triphosphate (ATP) and the resultant two major metabolites F1P and adenosine diphosphate (ADP).[43] Another source of ADP in the fructose metabolic pathway comes via the phosphorylation of GAD to GA3P. The formation of F1P and GA3P may lead to the depletion of ATP and inorganic phosphate, accumulation of ADP, breakdown of adenine nucleotides, and in turn increased formation of uric acid.[44] Indeed, ATP depletion,[45] ADP accumulation,[46] and hyperuricemia[24] were previously observed in CuD male rats. In the present study we demonstrate that hepatic F1P levels in CuA rats remain unchanged by gender during the consumption of a high-fructose diet, whereas levels in CuD animals are elevated (*Table 3*). Two enzymes, FK and aldolase-B, influence the concentration of F1P in the liver. We previously demonstrated that in both CuA and CuD dietary regimens, specific activity of FK is higher than aldolase-B[24] and thus, the depletion of hepatic F1P after fructose intake is mainly controlled by aldolase-B. The higher hepatic levels of F1P in CuD male than in female rats are probably the result of higher FK and lower aldolase-B activities.[24] Gomez et al.[47] showed that insulin enhanced the transcription of the aldolase-B gene. Likewise, Munnich et al.[48] showed that transcription of aldolase-B in the liver of fructose-fed rats is enhanced by thyroid hormones. On the other hand, it was also documented that CuD, fructose-fed rats accumulate insulin in the pancreas and secretion of the hormone into the blood circulation is retarded.[49] In addition, Fields et al.[50] showed that plasma levels of triiodothyronine in CuD, fructose-fed rats are reduced. Thus, low plasma levels of these two hormones in CuD, fructose-fed rats may reduce hepatic aldolase-B gene expression and interfere with aldolase-B activity.

In the present study, hepatic GAD levels were higher in CuD rats (*Table 3*), but no gender effect was observed. GAD has been shown to generate free radicals that in turn can cause damage to tissues.[51] Several enzymes mediate the concentrations of GAD and DHAP (*Figure 1*). However, the elevated hepatic levels of GAD observed in CuD rats is somewhat confusing because the activity of its generating enzyme, aldolase-B, was reduced by Cu deficiency,[24] whereas the activity of its degrading enzymes ADH, ALDEH, and TK were elevated, reduced, and unchanged, respectively.[24] Among the three degrading metabolic pathways of GAD (*Figure 1*), the conversion to GA3P by TK is the favored route because the affinity of TK to GAD is higher than that of ADH and ALDEH.[15] Thus, we cannot rule out the possibility that ADH and ALDEH generate GAD, under CuD regimen, from glycerol and glycerate, respectively.

Significant gender differences in ALDEH, ADH, and G3PDH specific activities were observed (*Table 2*). Enzymatic activity measurements were performed when the rats were 66 days old and may have reached sexual maturity, suggesting that these enzymes may be influenced by sex hormones. ALDEH oxidizes a variety of aldehydes and possesses a loose substrate specificity and ubiquitous subcellular distribution. This may suggest that ALDEH plays a vital role in protecting the liver against the harmful effect of exogenous or metabolic-generated aldehydes.[52] The specific activity of ADH was elevated by Cu deficiency in both sexes and was higher in CuD female than in male rats (*Table 2*). It is difficult to rationalize the deficiency-induced increase in hepatic ADH activity, and its activity is unlikely to depend on substrate supplementation by aldolase-B because no differences in GAD were observed (*Table 3*). However, Lewis et al.[53] showed that CuD rats have increased plasma levels of glucocorticoids known to enhance transcription of the growth hormone gene[54] and may subsequently increase ADH mRNA and its activity.[55] In addition, Cu deficiency shifts the oxidative reduction balance toward the reduced stage by increasing the activity of hepatic glucose 6-phosphate dehydrogenase and producing reduced glutathione.[56] Because the activity of ADH depends on free reduced sulfur (SH) residues in its active domain, high levels of reduced glutathione may protect the

viable SH residues and maintain ADH activity. Reduction of GAD to glycerol by ADH facilitates lipogenesis (*Figure 1*), and thus higher ADH activities in CuD female than in male rats may explain the more severe hypertriglyceridemia observed in female rats.[19]

The concentrations of hepatic DHAP in CuD female rats were higher than those in CuD male rats (*Table 2*). These differences are probably due to the net effect of enzymes controlling DHAP metabolism (*Figure 1*). Within the formation route, specific activity of aldolase-B was higher in CuD female than in male rats, whereas no differences were found in the degradation route.[24] In contrast, CuD male rats demonstrated higher TIM[24] and G3PDH specific activities than did female rats. These results agree with those obtained in a previous study by Fields et al.[57] Elevated G3PDH in male rats may explain the enhanced levels of glycerol 3-phosphate observed by Kopp et al.[46] in CuD male rats. However, although the male rat demonstrates elevated G3PDH activity, it is the CuD female rat that develops hypertriglyceridemia.[19] GA3P is the common metabolite of both GAD and DHAP (*Figure 1*), and by comparing the specific activities of TIM with those of TK,[24] we may assume that GA3P is produced several times faster from DHAP than from GAD.

The concentration of hepatic F1,6DP in male rats was not affected by dietary Cu status (*Table 3*). Similar results were obtained in male rats fed high-sucrose diets.[46] In the present study, however, significantly higher levels of F1,6DP were observed in the livers of CuD females than in those of CuD males. The activities of aldolase-B observed in our previous study[24] are not sufficient to explain the differences in hepatic F1,6DP levels between CuD male and female rats. At this point it is interesting to mention that according to Gopher et al.[58] F1,6DP may also be the outcome of direct phosphorylation of F1P by 1-phosphofructokinase. Previous studies have shown that administration of F1,6DP efficiently improves cardiac output and ATP levels in acute myocardial ischemia,[59] restores hemodynamic and electrocardiographic properties to patients with acute myocardial infarction,[60] prevents cardiac oxidative damage induced by subchronic doxorubicin treatment,[61] protects the liver during CCl_4 hepatotoxicity,[62] prevents hepatocytes necrosis and prominent portal and parenchymal inflammation imposed by an intraperitoneal injection of galactosamine,[63] and reverses uricemia induced by an intravenous fructose load.[64] The protective properties of F1,6DP are probably due to its ability to cross the cell membrane and accelerate ATP production via the glycolytic pathway not only as a metabolite regulator but also as a substrate, and in turn to facilitate the recovery of the damaged tissue. Lesions such as reduced cardiac ATP,[46] heart necrosis,[65] mitochondrial membrane oxidation,[45] and hepatic free radical formation[66] were observed in experimental dietary Cu deficiency and were more pronounced in male than in female rats. The gradual increase in hepatic F1,6DP in CuD female rats accompanied by a decrease in F1P, as opposed to male rats, may propose an improvement in hepatic energy balance that allows organelle and cell recovery from the damage caused by Cu deficiency. Furthermore, because F1,6DP may cross the cell membrane, transport of the accumulated metabolite from the liver to the heart and amelioration in heart performance in female rats cannot be ruled out.

Though most of the examined hepatic fructose-metabolizing enzymes and metabolites showed great differences between rats fed diets either adequate or deficient in Cu, it is the activity of FK and aldolase-B, and the concentrations of their common metabolites F1P and notably F1,6DP, that could be in part responsible for differences in the severity of pathologies associated with Cu deficiency observed between female and male rats. Because F1,6DP might play a protective role, further studies are in progress to investigate whether exogenously attenuated F1,6DP will affect fructose metabolism in this CuD experimental model.

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