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FEMALE RATS ARE PROTECTED AGAINST THE FRUCTOSE INDUCED MORTALITY OF COPPER DEFICIENCY

MEIRA FIELDS, CHARLES LEWIS, DANIEL J. SCHOLFIELD, ANDREA S. POWELL
ALICE J. ROSE, SHELDON REISER AND JAMES C. SMITH

U.S. Department of Agricuture, ARS
Beltsville Human Nutrition Research Center
Vitamin and Mineral Nutrition Laboratory
Beltsville, Maryland 20705

Abstract Experiments were conducted in copper deficient male and female rats fed diets containing fructose or starch in order to determine whether the same type of interaction between copper status and dietary carbohydrate found in male rats also occurs in the female rat. Mortality occurred only in the male rats fed the fructose diet deficient in copper with 40% of the animals dying during the 8 week study. Only anemia, hypercholesterolemia, increased BUN, heart hypertrophy and reduced body weight were observed in these animals which could be related to their mortality. Despite the increased mortality, plasma ceruloplasmin, erythrocyte SOD and hepatic copper concentrations were reduced to a similar extent in all rats regardless of the sex of the animals or of the type of dietary carbohdrate fed. The results of the present study indicate that although direct measurements of copper status of female rats fed fructose diet deficient in copper are similar to their male counterpart, they are apparently protected from the lethal consequences of the deficiency.

Introduction Fructose feeding but not starch has been shown to exacerbate the symptoms associated with copper deficiency (1). These include: reduced body weight, but increased liver weights, hypercholesterolemia, hypertriglycerdemia, anemia, hypertrophy and histopathological changes of the heart. The most dramatic sign of copper deficiency in rats fed diets high in fructose is sudden death due to the rupture of the heart (1).

A recent study has shown that female rats fed copper deficient diets containing fructose do not die (2). In addition, since human premenopausal females have a lower incidence of heart related abnormalities, it is therefore possible that female rats are protected against the undesirable risk factor effects of fructose copper deficiency. The purpose of the present study was to determine whether the same type of interaction between copper status and dietary carbohydrate found in male rats also occurs in female rats.

Material and Methods Weanling male and female Sprague Dawley rats were randomly divided into one of two copper deficient diets differing in the source of dietary carbohydrate as previously described (3).

Group 1 - fructose copper deficient, male rats (n=40)
Group 2 - corn starch copper deficient male rats (n=20)
Group 3 - fructose copper deficient, female rats (n=6)
Group 4 - corn starch, copper deficient, female rats (n=6)

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The copper deficient diets contained 0.6 ug Cu/g diet as analyzed by atomic absorption spectroscopy.

All rats were maintained in individual stainless steel cages and were fed their respective diets for 8 weeks. They were fed ad libitum and were allowed free access to deionized water. Body weights were recorded weekly. Rats were killed by decapitation after an overnight fast. Hematocrit levels were determined immediately after decapitation. Plasma was obtained upon centrifugation and stored at -20°C. Liver and hearts were weighed. Livers were homogenized in 0.2% Triton-X-100 as previously described (4) and were analyzed for superoxide dismutase (CuSOD) activity (5). The activity of CuSOD was also determined in erythrocytes. Aliquots of plasma were taken for the determination of cholesterol, triglycerides, blood urea nitrogen (BUN) and uric acid by the automated procedure. In addition, plasma was analyzed for ceruloplasmin activity (6). Tissue and diets were digested for mineral determinations by a method combining dry heat and acid digestion (7). To verify accuracy, National Bureau of Standards (NBS) Certified Reference Materials were digested and analyzed along with tissues and diets. The concentrations of copper in the liver and diets were measured by atomic absorption spectroscopy (8). Data were analyzed by ANOVA and Duncan's Multiple Range Test (9). A $P < 0.05$ was considered as statistical significant.

Results Male rats began dying after the fructose diet deficient in copper was fed for 5 weeks. By the 8th week of the study, 40% of these animals died (Table 1). In contrast none of the female copper deficient rats fed fructose and none of the male or female corn

Table 1. Body, liver and heart weights, mortality and metabolic indices in blood of female and male rats fed copper deficient fructose or starch diets*

	Fructose Diet		Starch Diet	
	Female	Male	Female	Male
Body wt (g)	186 ± 4^b	133 ± 3^c	192 ± 3^b	224 ± 10^a
Liver wt g/100 g b.w.	4.4 ± 0.2^b	5.9 ± 0.4^a	2.8 ± 0.07^c	3.2 ± 0.2^c
Heart wt g/100 g b.w.	0.45 ± 0.03^b	0.74 ± 0.03^a	0.38 ± 0.01^b	0.44 ± 0.02^b
Mortality	0	16/40	0	0
Hematocrit %	38 ± 1.6^b	25 ± 1.5^c	44 ± 1.0^a	45 ± 1.0^a
Cholesterol mg/dl	138 ± 10^b	204 ± 30^a	82 ± 10^c	71 ± 8^c
Triglycerides mg/dl	162 ± 27^a	63 ± 1.8^b	52 ± 4.5^b	40 ± 2.3^b
BUN mg/dl	36 ± 5.4^b	52 ± 6.3^a	17 ± 0.7^c	14 ± 0.6^c
Uric acid mg/dl	1.6 ± 0.3^a	1.6 ± 0.08^a	0.7 ± 0.1^b	0.7 ± 0.04^b

ANOVA+	Sex	CHO++	Sex x CHO
Body wt	N.S.	0.0001	0.0001
Liver wt	0.002	0.0001	0.05
Heart wt	0.0001	0.0001	0.0006
Hematocrit	0.0005	0.0001	0.0001
Cholesterol	NS	0.0001	0.03
Triglycerides	0.0007	0.0001	0.005
BUN	NS	0.001	0.03
Uric acid	NS	0.0001	NS

*Each value represents the mean ± SEM of 6 rats per group. Means within a row not sharing the same superscript letter are significant different from each other ($P < 0.05$) as determined by Duncans's Multiple Range Test.

+A 2x2 analysis of variance. Effects and interactions significant ($P < 0.05$) or not significant (NS).

++Carbohydrate.

Table 2. Ceruloplasmin activity, erythrocyte and hepatic SOD, and hepatic copper concentration in female and male rats fed fructose or starch diet*

	Fructose Diet		Starch Diet	
	Female	Male	Female	Male
Ceruloplasmin U/l	ND[1]	ND	ND	ND
Erythrocyte SOD U/ml packed red cells	ND	29 ± 9[a]	114 ± 62[a]	25 ± 7[a]
Hepatic SOD, U/g	609 ± 238[b]	623 ± 102[b]	1518 ± 204[a]	1078 ± 57[ab]
Hepatic copper, ug/g wet wt	1.58 ± 0.30[a]	1.83 ± 0.19[a]	2.23 ± 0.26[a]	2.11 ± 0.22[a]

ANOVA+	Sex	CHO++	Sex x CHO
Erythrocyte SOD	NS	NS	NS
Hepatic SOD	NS	0.001	NS
Hepatic copper	NS	NS	NS

[1]ND - Non detectable.
*Same footnotes as Table 1.

starch-fed rats died. Fructose fed male rats exhibited extreme pallor.

Body and individual tissue weights are also presented in Table 1. Copper-deficient male rats fed fructose weighed significantly less than all other male or female rats. Liver weight was significantly increased in male and female rats fed fructose compared to animals fed starch but was further increased in male rats. The livers were pale and friable only in male rats fed fructose. Heart weight was significantly increased only in male rats fed the fructose diet. In addition, the subcutaneous and internal fat stores were depleted only in copper deficient male rats fed fructose. The pallor noted previously, was marked and extended throughout the internal organs.

Hematocrit, plasma cholesterol, triglycerides, BUN and uric acid concentrations are presented in Table 1. Although hematocrit was reduced by fructose feeding as compared to starch feeding, anemia was observed only in male rats fed the fructose diet. Cholesterol, triglycerides, BUN and uric acid were increased by fructose feeding as compared to starch feeding. However, cholesterol and BUN were further increased in male rats. In contrast, female rats exhibited increased blood triglyceride concentrations when compared with all other groups.

Direct indicators of copper status in blood and tissues are presented in Table 2. All copper deficient rats had undetectable levels of ceruloplasmin activity. Erythrocyte SOD, hepatic copper concentration and hepatic SOD were not affected by the sex of the animal. Hepatic SOD was reduced by fructose feeding as compared to starch feeding.

Discussion In agreement with our previous studies (3), male rats fed the fructose diet but not those fed the starch diet were emaciated, sick and lost weight. In addition they were pale, anemic, had hypertrophied hearts and numerous deaths (40%) occurred only in this dietary group. None of the other copper deficient rats died. The risk factor metabolites traditionally associated with heart disease such as; blood cholesterol, triglycerides and uric acid concentrations were increased by fructose feeding in both male and female rats. However, although triglyceride concentrations were further increased in female rats compared to male rats, they were protected from the fructose induced heart hypertrophy and mortality of copper deficient male animals. This protection against the exacerbated symptoms of copper deficiency in female rats fed fructose could not be due to copper status since except for hepatic SOD, which was re-

duced to the same levels by fructose, irrespective of sex, all rats fed the copper deficient diets exhibited similar change in indices of copper status.

The reason for the protection against the fructose copper interaction in copper deficient females is unclear. It has been shown that estrogen secretion associated with the estrous cycle, as well as exogenous estrogen administration, alters the subcellular distribution of copper in the liver (10,11) and increases plasma copper by inducing synthesis of ceruloplasmin (12). If fructose feeding alters the hepatic subcellular distribution of copper and reduces its availability to the general circulation, then less copper will be available for utilization by extrahepatic tissues. The blood of copper supplemented rats fed fructose contains less copper and ceruloplasmin than those fed starch (3) and the hearts of copper deficient rats fed fructose exhibit hypertrophy and histopathological changes (1), and they contain less copper than the hearts of rats fed starch (13,14).

Only male rats fed fructose were anemic. The anemia could not be due only to the reduced copper status since except for hepatic SOD all other rats, female and male, were as copper deficient but they did not develop anemia. In addition, when copper deficient rats fed fructose were switched to copper deficient diets containing starch, the anemia disappeared (15). Thus, the anemia in copper deficient male rats is manifested only upon fructose feeding. The anemia in male rats fed fructose can result in hypertrophy of the heart (16). Female rats were not anemic. If estrogen increases the activity of lysyl oxidase (17) a copper metalloenzyme important in collagen and elastin formation, then female rats can be protected against the characteristic heart related abnormalities observed upon fructose feeding in the copper deficient male rat.

Hepatic SOD activity was significantly reduced by fructose as compared to starch feeding in both male and female rats. It is well established that fructose feeding is lipogenic (18) and its metabolites generate free radicals (19,20). Thus, fructose metabolism compared to glucose metabolism would be expected to require greater protection against harmful peroxidation. Fructose as compared to starch feeding to male rats has been shown to reduce hepatic SOD and glutathione peroxidase activity (GSH-Px) and produce greater peroxidation of the lipid membranes (4). However, since SOD activity was reduced to the same magnitude by dietary fructose in male and female rats, but female rats were neither emaciated, nor did they die, it is suggested that either fructose is metabolized differently in the female so that fewer free radicals are generated, enabling the low levels of hepatic SOD to provide sufficient protection against peroxidation, or the reduced SOD may not be a major factor in the cause of mortality of male rats fed fructose.

Although the female rats used in the present study were fed from weaning the copper deficient diets for only 8 weeks, a length of time which may not be sufficient for the expression of the deficiency, it has been shown that feeding females a copper deficient diet containing high-fructose corn syrup for 12 months did not affect their body weight and did not cause any deaths (2). It is still unclear whether postmenopausal female rats are also protected against this enhanced mortality observed in male rats fed fructose. The role of estrogens in copper homeostasis and its possible physiological relevance warrants further attention.

Regardless of sex, starch feeding compared to fructose feeding ameliorates the deficiency in male rats. This was evident by the lack of anemia, heart hypertrophy and mortality and the low levels of risk factor metabolites in blood. Thus, in addition to the type of dietary carbohydrate, the sex of the animal also determines the degree of the severity of copper deficiency. It is clear from the studies reported here as well as our previous study (3) that the conventional indices used to assess copper status in rats do not accurately reflect the greater degree of deficiency or mortality in male rats fed the fructose diet deficient in copper.

References

1. Reiser S., Ferretti RJ, Fields M, Smith JC. Role of dietary fructose in the enhancement of mortality and biochemical changes associated with copper deficiency in rats. Am J Clin Nutr 38:214-22, 1983.
2. Strause LG, Hegenauer J, Saltman P, Cone R, Resnick D. Effect of long-term dietary manganese and copper deficiency on rat skeleton. J Nutr 116:135-42, 1986.
3. Fields M, Holbrook J, Scholfield S, Rose A, Smith JC, Reiser S. Development of copper deficiency in rats fed fructose or starch; weekly measurements of copper indices in blood. Proc Soc Expt Biol Med 191: 120-24, 1986;
4. Fields M, Ferretti RJ, Smith JC, Reiser S. Interaction between dietary carbohydrate and copper nutrition on lipid peroxidation in rat tissues. Biol Trace Element Res 6:379-91,1984.
5. Misra HP, Fridovich I. Superoxide dismutase: A photochemical augmentation assay. Arch Bioch Biophys 181:308-12, 1977.
6. Schosinsky KH, Lehmann HP, Beeler MF. Measurement of ceruloplasmin and its oxidase activity in serum by use of o-dianisidine dihydrochloride. Clin Chem 20:1556-63, 1974.
7. Patterson KY, Hill AD, Veillon C, Morris ER. Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. Fed Anal Chem Spectrosc Soc 10:393 1983 (Abstract).
8. Anonymous. Analytical methods for atomic absorption spectrophotometry. Perkin Elmer Inc., Norwalk, CT, 1976.
9. SAS Institute, Inc. SAS User's Guide: Statistics, Version 5 edition, Cary NC., 1985.
10. Russanov E, Banskalieva V, Ljutakova S. Influence of sex hormones on the subcellular distribution of copper in sheep liver. Res Vet Sci 30:233-5, 1981.
11. Saylor WW, Downer JV. Copper and zinc distribution in the liver and oviduct of estrogen and testrosterone treated hens. Nutr Res 181-90, 1986.
12. Carruthers ME, Hobbs CB, Warren RL. Raised serum copper and ceruloplasmin levels in subjects taking oral contraceptives. J Clin Path 19:498-504, 1966.
13. Holbrook J, Fields M, Smith, JC. Reiser S, Los Alamos Medical Research Group. Tissue distribution and excretion of copper-67 intraperitoneally administered to rats fed fructose or starch. J Nutr 116:831-838, 1986.
14. Cornatzer WE, Klevay LM. Fructose lowers cardiac copper in rats. Fed Proc 45(3): 357, 1986 (abstract).
15. Fields M, Ferretti RJ, Reiser S, Smith JC. The severity of copper deficiency in rats is determined by the type of dietary carbohydrates. Proc Soc Expt Biol Med 275:530-37, 1984.
16. Kelly WA, Kesterson JW, Carlton WW. Myocardial lesions in the offspring of female rats fed a copper deficient diet. Exp Mol Path 20:40-50, 1974.
17. Ozasa H, Tominaga T, Nishimura T, Takeda T. Lysyl oxidse activity in the mouse uterine cervix is physiologically regulated by estrogen. Endocrinology 109:618-21, 1981.
18. Blakley S, Hallfrisch J, Reiser S, Prather E. Long-term effects of moderate fructose feeding on lipogenic parameters in Wistar rats. Nutr Rep Int 25: 674-85, 1982.
19. Thomalley P, Wolff S, Crabbe J, Stern A. the autoxidations of glyceraldehyde and other simple monosaccharides under physiological conditions catalyzed by buffer ions. Biochim Biophys Acta 797:276-87, 1984.
20. Narins R, Weisberg JS, Meyers AR. Effects of carbohydrates on uric acid metabolism. Metabolism 23: 455-459, 1974.

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