Low Dietary Iron Prevents Free Radical Formation and Heart Pathology of Copper-Deficient Rats Fed Fructose (43531)

MEIRA FIELDS,*,[1] CHARLES G. LEWIS,† MARK D. LURE,† WILLARD A. BURNS,‡ AND WILLIAM E. ANTHOLINE§

Division of Endocrinology, Georgetown University, Washington, DC 20007; Carbohydrate Nutrition Laboratory,† Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland 20705; Laboratory Services,‡ Veterans Administration Hospital, East Orange, New Jersey 07019; and National Biomedical ESR Center,§ Medical College of Wisconsin, Milwaukee, Wisconsin 53225*

Abstract. **Two studies were conducted to determine whether hepatic iron overload in rats fed fructose plays a role in the exacerbation of the signs associated with copper deficiency. When fed the adequate iron diet (50 μg Fe/g), copper-deficient rats fed either fructose or starch exhibited hepatic iron overload of similar magnitude. However, only livers of copper-deficient rats fed fructose exhibited the presence of high peaks associated with an iron compound detected by electron spin resonance. In addition, only copper-deficient rats fed fructose developed anemia, pancreatic atrophy, and heart hypertrophy with histopathologic changes, and they died prematurely of heart-related abnormalities. Lowering dietary iron from 50 μg/g to 30 μg/g was not sufficient to protect the animals against the pathologic consequences of copper deficiency. In contrast, the consumption of a fructose diet inadequate in both copper (0.6 μg/g) and iron (17 μg/g) resulted in the reduction of hepatic iron, which in turn caused the amelioration of the deficiency, compared with rats fed the adequate iron (50 μg/g) diet. None of these rats developed pancreatic atrophy, none exhibited myocardial lesions, and none died of the deficiency. Electron spin resonance spectra of their livers did not show the presence of free radicals. The data suggest that hepatic iron overload plays a role in the exacerbation of copper deficiency only when fructose diets are consumed.** [P.S.E.B.M. 1993, Vol 202]

Regardless of the type of dietary carbohydrate or the sex of the rat, copper-deficient rats exhibit hepatic iron overload (1–7). Iron under certain conditions has been shown to play a major role in the pathogenesis of numerous diseases (8–11). It generates free radicals and accelerates lipid peroxidation (8–11).

We have shown repeatedly that copper-deficient rats fed either fructose or starch-based diets exhibit hepatic iron accumulation compared with their copper-adequate controls (12, 13). However, only copper-deficient rats fed fructose exhibit anemia and, heart hypertrophy with histopathologic changes, and they die prematurely of heart-related abnormalities. In contrast, copper-deficient rats fed starch are protected against these abnormalities and they survive (14, 15). In a recent study, we demonstrated that it is not the absolute concentration of hepatic iron, but rather it is the redox state of iron, that plays a role in the exacerbation of copper deficiency when fructose is fed. This conclusion was derived from the spectra of electron spin resonance (ESR) of livers of copper-deficient rats fed fructose (13). Although similar levels of hepatic iron were observed in copper-deficient rats fed either fructose or starch, only those fed fructose demonstrated the ESR spectra of a very high peak of an iron compound, which confirmed the presence of free radicals. Such a peak was absent in the livers of the starch-fed, copper-deficient rats (13). These results confirmed our hypothesis that iron played a role in the severity of copper deficiency in rats fed fructose. The purpose of a subsequent study was to reduce hepatic iron accumulation in rats fed fructose. This was achieved by the daily injection of deferoxamine. Deferoxamine is an iron-chelating drug that has been proved to be successful in reducing iron

[1] To whom requests for reprints should be addressed at Carbohydrate Nutrition Laboratory, USDA, Building 307-E, Room 323-E, Beltsville, MD 20705.

Received March 30, 1992. [P.S.E.B.M. 1993, Vol 202]
Accepted September 15, 1992.

0037-9727/93/2022-0225$3.00/0

overload both in experimental animals and human beings (16, 17). Indeed, deferoxamine reduced hepatic iron concentration, which in turn ameliorated the pathology of copper deficiency when fructose-based diets were consumed (13). No pathologic changes could be found in the hearts of copper-deficient rats fed fructose, and the animals survived.

However, a reduction of hepatic iron could also be achieved by simply lowering the intake of dietary iron. This noninvasive approach should result in a reduction of hepatic iron, which in turn should ameliorate the deficiency. This study reports data from two studies. In the first, lowering dietary iron from 50 μg Fe/g diet to 30 μg Fe/g diet resulted in a moderate amelioration of the signs associated with the deficiency in rats fed fructose. In these animals, the levels of dietary iron (30 μg/g) were not sufficient to reduce hepatic iron to the corresponding levels of copper-adequate rats. We concluded that lower concentrations of dietary iron were necessary to further reduce hepatic iron overload in copper-deficient rats fed fructose to prevent the pathologies associated with the deficiency.

Recently, Johnson and Gratzek (4) fed rats copper-deficient and -adequate diets that were also either deficient (8 μg Fe/g) or adequate in iron. The consumption of the low iron diet by the copper-deficient rats resulted in a lower hepatic iron concentration than that in their copper-adequate controls. In addition, copper-adequate rats that were fed the low iron diet were anemic (4). This low dietary iron regimen is too severe for the purpose of our study. Our goals were to prevent hepatic iron overload and to lower hepatic iron concentration in copper-deficient rats fed fructose to the same concentration of hepatic iron that is present in copper-adequate controls. Therefore, a second study was conducted in which 17 μg Fe/g diet was fed to copper-deficient rats fed fructose.

Materials and Methods

Study I. Weanling male Sprague-Dawley rats weighing 40–45 g each were fed copper-deficient (0.6 μg Cu/g diet) or -adequate (6.0 μg Cu/g) diets containing fructose or starch for 5 weeks. The diet consisted of the following ingredients (g/kg): 627 carbohydrate as fructose or corn starch, 200 egg white, 95 corn oil, 30 nonnutritive fiber (cellulose), 35 AIN salt mix (18), prepared in our laboratory and formulated to omit cupric carbonate from the mineral mix, 2.7 choline bitartrate, and 10 vitamin mix AIN 76A (19) supplemented with 2 mg biotin/kg diet. The copper-adequate diets were prepared by adding copper carbonate to the copper-deficient mineral mixture to produce a final concentration of 6.0 μg Cu/g diet. The iron content of the diets was either 50 μg Fe/g or 30 μg Fe/g (low Fe). The study was terminated after the rats had been fed their respective (adequate Fe) diets for 5 weeks due to the mortality of the first rat that consumed the fructose diet deficient in copper.

Statistical analyses. All data were subjected to a 2 × 2 × 2 analysis of variance (20), two levels of Cu, two levels of Fe, and two types of dietary carbohydrate. Main effects and interactions of significance level of $P < 0.05$ were considered statistically significant.

Study II. Fifty weanling male Sprague-Dawley rats weighing approximately 40–45 g each were randomly divided into five dietary groups according to levels of copper and iron and type of carbohydrates. The diets contained 62% carbohydrate as fructose or starch. The composition of the diets was described above (Study I). All diets were either inadequate (0.6 μg Cu/g diet) or adequate in copper (6.0 μg Cu/g).

All rats in Groups 1–4 consumed a diet that was adequate in iron (50 μg Fe/g diet). Rats belonging to the fifth group consumed a diet that was low in iron (17 μg Fe/g) (Group 1: fructose – copper (–Cu) and adequate in iron, 50 μg Fe/g; Group 2: starch – copper (–Cu) and adequate iron; Group 3: fructose + copper (+Cu) and adequate iron; Group 4: starch + copper (+Cu) and adequate iron; and Group 5: fructose – copper (–Cu) and low in iron, 17 μg Fe/g).

There was no justification for feeding the copper-adequate or copper-deficient rats that consumed starch the low iron diet because these animals did not exhibit pathologic changes. When fed a low iron diet, these animals developed anemia (4), but feeding a low iron diet to copper-deficient rats that consume fructose should have ameliorated the anemia and protected the rats against the severity of copper deficiency.

During the fifth week, three rats fed the fructose diet deficient in copper but adequate in iron died. The remaining rats from this dietary group were sick and emaciated. This forced us to terminate the study prematurely. Since none of the copper-deficient rats consuming the low iron diet that contained fructose died during the fifth week of the study, we decided to extend the duration of the study and keep five rats alive for an additional 3 weeks. However, this meant that no fructose-fed, copper-deficient rats consuming the adequate iron diet could be used as a comparison. The copper-deficient group that consumed starch was chosen to serve as a control. This was justified by the consistent results obtained from our numerous studies (12–15). Namely, except for having a reduced copper status, the rats of the starch – copper (–Cu) dietary group behaved like copper-adequate rats. They did not exhibit abnormalities in either biochemical, physiologic, or metabolic functions. They never developed anemia, their hearts did not develop severe pathologic changes, and they survived (12–15).

In both Studies I and II, blood was collected for hematocrit. Livers were removed and weighed, and

portions were taken for the analysis of copper and iron (21).

Histologic studies. Hearts and pancreata were removed, weighed, and fixed in Carson's modified Millonig's phosphate-buffered fomalin. After fixation, a hemisection of the heart and half of the pancreas were processed and embedded in paraffin in the usual manner and stained with hematoxylin and eosin, periodic acid-Schiff, and Gomori's trichrome for light microscopy.

ESR study. Livers and hearts were frozen in dry ice and stored at −70°C. The 9.1-GH_z, 77-K liver and heart samples were prepared by pressing the tissue into precision pore Pyrex molds (i.d., 4 mm; length, 3 cm) freezing the tissue, and warming the mold until the frozen tissue cylinder could be extracted. These specimens were analyzed in a fingertip Dewar. The ESR spectra were obtained on a varian E-9 spectrometer at the National Biomedical ESR Center in Milwaukee, WI.

Statistical analyses. Data from 5 weeks and 8 weeks were separately analyzed. Data for 5 weeks were subjected to (i) a 2 × 2 analysis of variance (20) with two levels of copper (−Cu and +Cu) and two types of dietary carbohydrate (fructose and starch). Data from the 8-week study, were analyzed by one-way analysis of variance with Duncan's use for mean separations comparing starch minus Cu and adequate iron with fructose minus Cu low iron diet.

Results

Study I. All animals belonging to the fructose minus copper dietary group were thin, with roughened, dry, dull coats. The copper-deficient rat fed fructose that died prematurely exhibited clotted blood in the chest cavity. The hemorrhage originated from a rupture at the left ventricular apex, with blood leaking from a torn pericardium into the right thorax.

Body weight, relative tissue sizes, and hematocrit of rats fed the copper-deficient and -adequate diets containing 50 μg Fe/g and 30 μg Fe/g are summarized in Table I. Copper deficiency resulted in a reduced body weight only in rats fed fructose. The consumption of the copper-deficient diet containing fructose and low in iron resulted in a greater body weight compared with rats fed the adequate iron diet. Heart size was increased only in copper-deficient rats fed fructose. Liver size was larger in fructose-fed rats than in starch-fed rats, and it was further increased by copper deficiency. Copper deficiency reduced epididymal fat pad size. The lowest epididymal fat pad size was found in copper-deficient rats fed fructose. Low dietary iron resulted in an increased epididymal fat pad in rats fed fructose.

Pancreatic atrophy occurred only in copper-deficient rats fed fructose. The consumption of the low iron diet inadequate in copper increased the size of the pancreas compared with copper-deficient rats that consumed the adequate iron diet.

Hepatic copper and iron concentrations and hematocrits are presented in Table II. All copper-deficient rats had a reduced concentration of hepatic copper compared with copper-adequate controls. Except for copper-deficient rats fed fructose, the consumption of a low iron diet resulted in an increased concentration of hepatic copper. All copper-deficient rats exhibited a significant increase in hepatic iron concentration when compared with copper-adequate rats. Copper-deficient rats fed starch had the highest concentration of hepatic iron. Copper deficiency in combination with low iron intake resulted in a reduced hepatic iron concentration. Only copper-deficient rats fed fructose were anemic. The anemia was ameliorated by the consumption of the low iron diet. All other rats, when fed the low iron diet, exhibited low hematocrits.

Study II. Three rats fed the fructose diet deficient in copper and adequate in iron died during the fifth week of the study. In contrast, none of the copper-deficient rats that had been fed fructose with low iron content died during the study. Likewise, no mortality occurred in the starch minus copper (−Cu) group.

Body weights, relative organ sizes, hematocrit, and mortality of rats fed their respective diets for 5 weeks are presented in Table III. In iron-adequate rats, fructose feeding in combination with low copper intake resulted in lower body weights as compared with all other rats. Hematocrit was reduced by copper deficiency in rats fed fructose. Liver and heart sizes were larger, but the pancreas was smaller compared with all other copper-deficient and -adequate rats.

The reduced intake of dietary iron (17 μg Fe/g) in copper deficiency resulted in higher body weights and smaller livers and hearts, but larger pancreata compared with copper-deficient rats fed fructose and adequate in iron. The anemia was ameliorated by the low dietary iron.

Hepatic copper and iron concentrations are summarized in Table IV. Copper deficiency was verified by the reduced hepatic copper concentration compared with copper-adequate controls. Fructose-fed, copper-deficient rats had a reduced hepatic copper concentration compared with copper-deficient, starch-fed rats. All copper-deficient rats exhibited hepatic iron overload, compared with copper-adequate rats. The reduction in the consumption of dietary iron resulted in an increase of hepatic copper but a decrease in hepatic iron when compared with copper-deficient rats fed an iron-adequate diet containing fructose.

Data from the 8-week study are presented in Table V. Rats fed starch weighed more than rats fed fructose. No difference in liver, heart, or pancreas size could be found between the two groups of rats. Hematocrit was lower in rats fed fructose than in rats fed starch. None

Table I. Body Weight and Relative Tissue Sizes of Male Rats Fed Adequate (50 μg Fe/g) and Low (30 μg Fe/g) Iron Diets[a]

	Body wt (g)	Relative organ sizes[b]			
		Heart (g/100 g)	Liver (g/100 g)	Epididymal fat pad (g/100 g)	Pancreas (g/100 g)
FR–Cu	206 ± 8	0.52 ± 0.02	4.07 ± 0.26	0.60 ± 0.05	0.35 ± 0.06
FR–Cu low Fe	244 ± 8	0.45 ± 0.05	3.92 ± 0.19	0.66 ± 0.08	0.41 ± 0.04
FR+Cu	263 ± 5	0.35 ± 0.01	3.16 ± 0.06	1.16 ± 0.15	0.68 ± 0.02
FR+Cu low Fe	260 ± 8	0.35 ± 0.01	3.06 ± 0.06	1.04 ± 0.11	0.65 ± 0.03
ST–Cu	275 ± 3	0.35 ± 0.05	2.72 ± 0.39	0.96 ± 0.08	0.54 ± 0.02
ST–Cu low Fe	268 ± 4	0.42 ± 0.01	2.86 ± 0.08	0.90 ± 0.06	0.57 ± 0.02
ST+Cu	289 ± 10	0.36 ± 0.01	2.98 ± 0.03	1.22 ± 0.12	0.56 ± 0.03
ST+Cu low Fe	269 ± 5	0.37 ± 0.01	2.68 ± 0.08	0.91 ± 0.04	0.55 ± 0.03
Analysis of variance					
Fe	S	NS	NS	NS	NS
Cu	S	S	S	S	S
CHO	S	S	S	NS	S
Cu × CHO	S	S	S	S	S
Cu × Fe	S	NS	NS	S	S
Cu × Fe × CHO	NS	NS	NS	NS	S

[a] Abbreviation used in table: FR, fructose; ST, starch; S, significant; NS, not significant; CHO, carbohydrate.
[b] Mean ± SE of eight observations/group except for fructose minus Cu, which had seven.

Table II. Hepatic Copper and Iron Concentrations and Hematocrit in Rats Fed Adequate (50 μg Fe/g) or Low (30 μg Fe/g) Iron Diets[a]

	Copper (μg/g wet wt)	Iron (μg/g wet wt)	Hematocrit (%)
FR–Cu	1.10 ± 0.14	134 ± 6.7	28 ± 2
FR–Cu low Fe	0.79 ± 0.10	119 ± 9.6	34 ± 2
FR+Cu	4.17 ± 0.15	79 ± 4.8	46 ± 1
FR+Cu low Fe	4.83 ± 0.19	50 ± 2.6	42 ± 1
ST–Cu	1.47 ± 0.10	171 ± 11.2	44 ± 1
ST–Cu low Fe	1.71 ± 0.15	89 ± 2.6	42 ± 1
ST+Cu	4.54 ± 0.14	94 ± 4.4	44 ± 1
ST+Cu low Fe	5.82 ± 0.80	44 ± 3.2	42 ± 1
Analysis of variance			
Fe	S	S	NS
Cu	S	S	S
CHO	S	NS	S
Cu × CHO	NS	NS	S
Cu × Fe	S	NS	S
CHO × Fe	NS	NS	S
Cu × Fe × CHO	NS	S	S

[a] Data are mean ± SE of eight observations/group except for fructose minus Cu, which had seven. Abbrevation used in table: FR, fructose; ST, starch; S, significant; NS, not significant; CHO, carbohydrate.

of the animals died during this period. Hepatic copper concentration was not affected by the type of dietary carbohydrate. Hepatic iron was lower in rats fed the low iron diet compared with those fed the copper-deficient diet that was adequate in iron.

The pancreas of the copper-deficient rats fed fructose was atrophied, with destruction of acini accompanied by a mild inflammatory response and early fibrosis (Fig. 1). Acinar cells were depleted and the number of zymogen granules was reduced. Ducts were not obstructed and the islets of Langerhans were not affected. Copper-adequate animals showed no pancreatic pathology. Likewise, the consumption of a fructose diet that was low in both copper and iron prevented pancreatic atrophy and pancreatic pathology (Fig. 2).

Heart hypertrophy was evident in all copper-deficient rats. However, the greatest heart size was found in copper-deficient rats fed fructose. Autopsy of the dead rats revealed clotted blood in the chest cavity due to ruptured hearts in the area of the apex. All other rats belonging to this dietary group exhibited heart hypertrophy with gross pathologic changes. The hearts showed mild to severe myocardial inflammation, degeneration, and fibrosis (Fig. 3). Aneurysms of the left ventricle and pericarditis were also common. Myocardial vessels did not demonstrate any atherosclerotic changes or vasculitis. Although hearts from copper-deficient rats fed starch and from copper-deficient rats fed the fructose diet low in iron exhibited heart hypertrophy of moderate magnitude compared with copper-adequate rats, no myocardial lesions could be demonstrated (Fig. 4).

Only in those rats that consumed the fructose diet deficient in copper but adequate in iron did the ESR spectra of the liver show the presence of high peaks in the area of $g = 2.03$ (Fig. 5a). This signal has an iron center (22). None of the other rats exhibited high peaks in this region. The reduced intake of iron in copper-deficient rats fed fructose prevented the presence of this high peak (Fig. 5e). ESR of heart tissue revealed similar

Table III. Body Weight, Relative Organ Sizes, Hematocrit, and Mortality in Rats Fed Copper-Deficient or -Adequate Diets Containing Fructose or Starch for 5 Weeks[a]

	Body wt (g)	Hematocrit (%)	Relative organ sizes			Mortality (%)
			Liver (g/100 g)	Heart (g/100 g)	Pancreas (g/100 g)	
FR–Cu	191 ± 2	24 ± 2.5	4.7 ± 0.2	0.63 ± 0.04	0.24 ± 0.02	30
ST–Cu	277 ± 5	44 ± 1.4	3.0 ± 0.2	0.46 ± 0.03	0.51 ± 0.02	0
FR+Cu	267 ± 5	44 ± 1.5	3.8 ± 0.1	0.31 ± 0.01	0.53 ± 0.02	0
ST+Cu	285 ± 3	45 ± 1.2	2.8 ± 0.02	0.35 ± 0.01	0.53 ± 0.01	0
FR–Cu Fe inadequate (17 μg Fe/g)	218 ± 4[b]	30 ± 1.5[b]	3.7 ± 0.1[b]	0.51 ± 0.02[b]	0.33 ± 0.05[b]	0
Analysis of variance						
Cu	S	S	S	S	S	
CHO	S	S	S	S	S	
Cu × CHO	S	S	S	S	S	

[a] Data are expressed as mean ± SE. Abbreviations used in table: FR, fructose; ST, starch; S, significant; CHO, carbohydrate. All rats belonging to the FR–Cu, ST–Cu, FR+Cu, and ST+Cu dietary groups consumed an adequate iron diet (50 μg Fe/g). Rats belonging to the FR–Cu low Fe group consumed a diet containing 17 μg Fe/g.
[b] FR–Cu versus FR–Cu low Fe.

Table IV. Hepatic Copper and Iron Concentrations in Rats Fed the Copper-Deficient or -Adequate Diets Containing Fructose or Starch for 5 Weeks[a]

	Copper (μg/g wet wt)	Iron (μg/g wet wt)
FR–Cu	0.75 ± 0.09	150 ± 14
ST–Cu	0.98 ± 0.13	155 ± 16
FR+Cu	4.72 ± 0.23	80 ± 3
ST+Cu	4.62 ± 0.25	85 ± 4
FR–Cu Fe inadequate (17 μg/g)	0.94 ± 0.10[b]	75 ± 8[b]
Analysis of variance		
Cu	S	S
CHO	S	NS
Cu × CHO	S	NS

[a] For details, see footnotes to Table III.
[b] FR–Cu versus FR–Cu low Fe.

Table V. Body Weight, Relative Organ Sizes, Hematocrit, and Hepatic Copper and Iron Concentrations in Rats Fed Copper-Deficient Diets for 8 Weeks[a]

	Starch–Cu Fe adequate (50 μg/g)	Fructose–Cu Fe inadequate (17 μg/g)
Body wt (g)	327 ± 10[b]	251 ± 4
Relative organ sizes (g/100)		
Liver	2.99 ± 0.11	3.42 ± 0.2
Heart	0.43 ± 0.01	0.47 ± 0.04
Pancreas	0.49 ± 0.01	0.52 ± 0.03
Hematocrit (%)	45 ± 1.1[b]	36 ± 0.3
Hepatic copper (μg/g wet wt)	1.71 ± 0.29	1.48 ± 0.11
Hepatic iron (μg/g wet wt)	149 ± 19[b]	91 ± 5
Mortality	0	0

[a] Data are expressed as mean ± SE.
[b] Significantly different starch minus Cu versus fructose minus Cu low Fe.

spectra of the same magnitude for all animals (data not shown). ESR of heart tissue from copper-deficient rats fed fructose was not different from any of the other dietary groups.

Discussion

As reported previously (14, 15), data from the two studies reported here show that the type of dietary carbohydrate greatly affected the severity of copper deficiency. Starch feeding ameliorated the signs associated with the deficiency, but fructose aggravated them. Only copper-deficient rats fed fructose were severely anemic. Their pancreata were atrophied, and their hearts exhibited gross pathologic changes. In addition, only rats fed the fructose diet deficient in copper died prematurely during both studies.

The reduced intake of dietary iron from 50 μg Fe/g diet to 30 μg Fe had only a moderate effect. Unfortunately, in that study, hepatic iron concentrations were not sufficiently reduced to the levels of hepatic iron of copper-adequate controls. However, once dietary iron was further reduced, by feeding the copper-deficient rats a fructose diet that contained 17 μg Fe/g, the signs associated with the deficiency were significantly ameliorated. The most dramatic effect was on rat survival. None of the rats died during the study. This allowed us to continue the experiment for an additional period of 3 weeks. By the eight week of the study, the anemia was ameliorated, the pancreas was not atrophied, and both the pancreas and the heart were devoid of pathol-

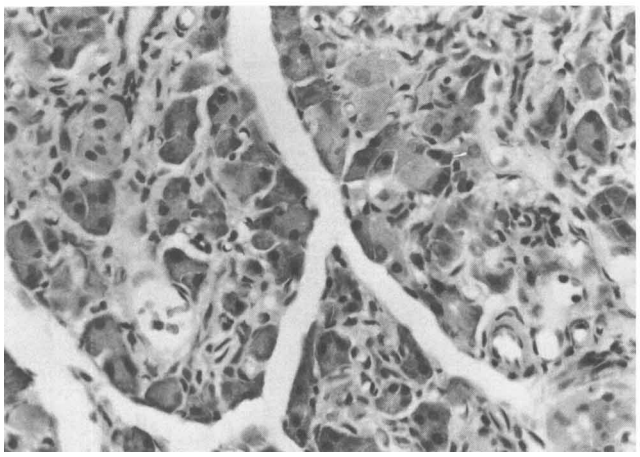

Figure 1. Pancreatic atrophy in fructose-fed, copper-deficient rat.

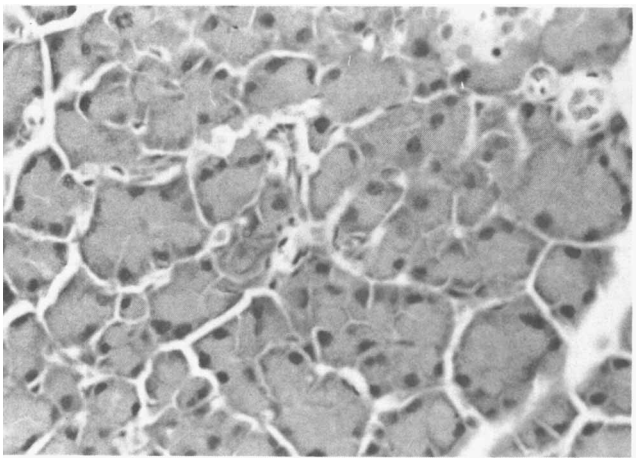

Figure 2. Normal pancreas in fructose-fed, copper-deficient rat that consumed the low iron (17 μg/g) diet.

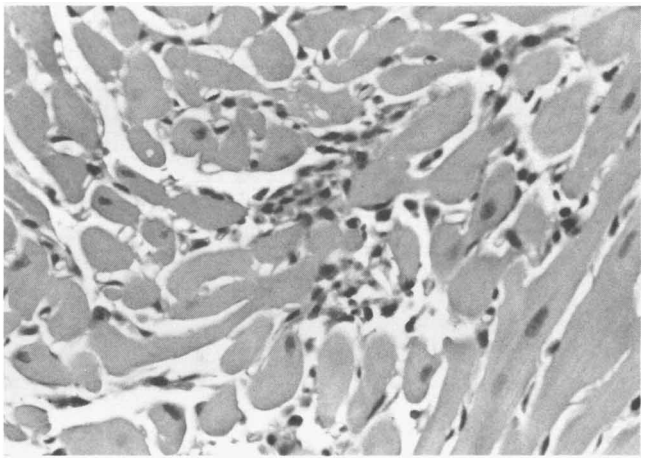

Figure 3. Focal lesions in the myocardium of fructose-fed, copper-deficient rat.

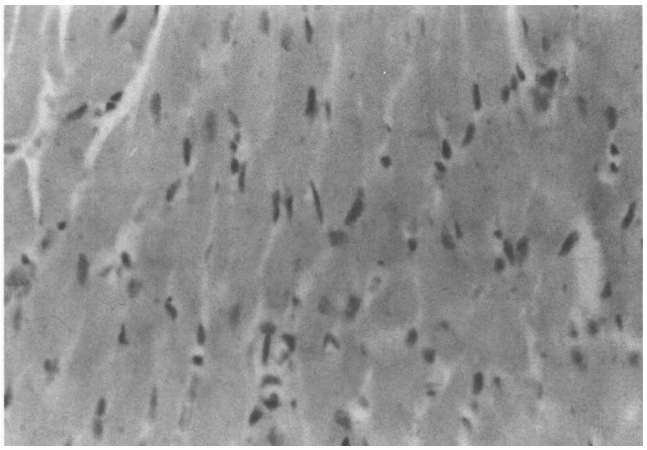

Figure 4. Normal-appearing myocardium in fructose-fed, copper-deficient rat that consumed the low iron (17 μg/g) diet.

ogy. These findings prove that iron plays a role in the exacerbation of copper deficiency when fructose is fed. However, it is not simply the absolute concentration of iron that induces pathologic changes. Copper-deficient female rats exhibit a higher concentration of hepatic iron than males. Their livers do not generate free radicals and they are protected from the lethal consequences of copper deficiency (23). As in our previous publication (13), a high peak at g = 2.03 was present only in the livers of rats fed the copper-deficient diet containing fructose. The redox state of the g = 2.03 signal, which has an iron center (22), is the signal that is changing due to the presence of a high concentration of hepatic iron in copper-deficient rats fed fructose. The magnitude of the peaks at g = 2.03 in copper-deficient male rats consuming fructose were between 6- and 9-fold the magnitude of all other peaks at g = 2.03 obtained from rats belonging to other dietary groups, including the low-iron-fed rats. A change in the redox state of iron may result in the generation of free radicals. By either consuming a starch-based diet or by lowering dietary intake of iron, this high peak was absent (Fig. 5, b and e). Once hepatic iron overload was prevented, and free radicals were absent, iron became more available for utilization. Indeed, the anemia was ameliorated, which in turn prevented heart hypertrophy. Once the anemia was prevented, no heart pathology could be demonstrated in copper-deficient rats fed fructose (12). In addition, histologic examinations of the hearts could not reveal any lesions once the levels of dietary iron were reduced. Likewise, by reducing hepatic iron with deferoxamine in copper-deficient rats fed fructose, the severity of the deficiency was ameliorated, the anemia was prevented, no heart pathology could be demonstrated, and the animals survived (13).

The intake of low dietary iron in combination with copper deficiency in rats fed fructose resulted in the prevention of hepatic iron overload. Hepatic iron overload is a constant feature of copper deficiency (1–7). Indeed, copper-deficient rats fed starch also exhibited hepatic iron overload. However, iron by itself is harm-

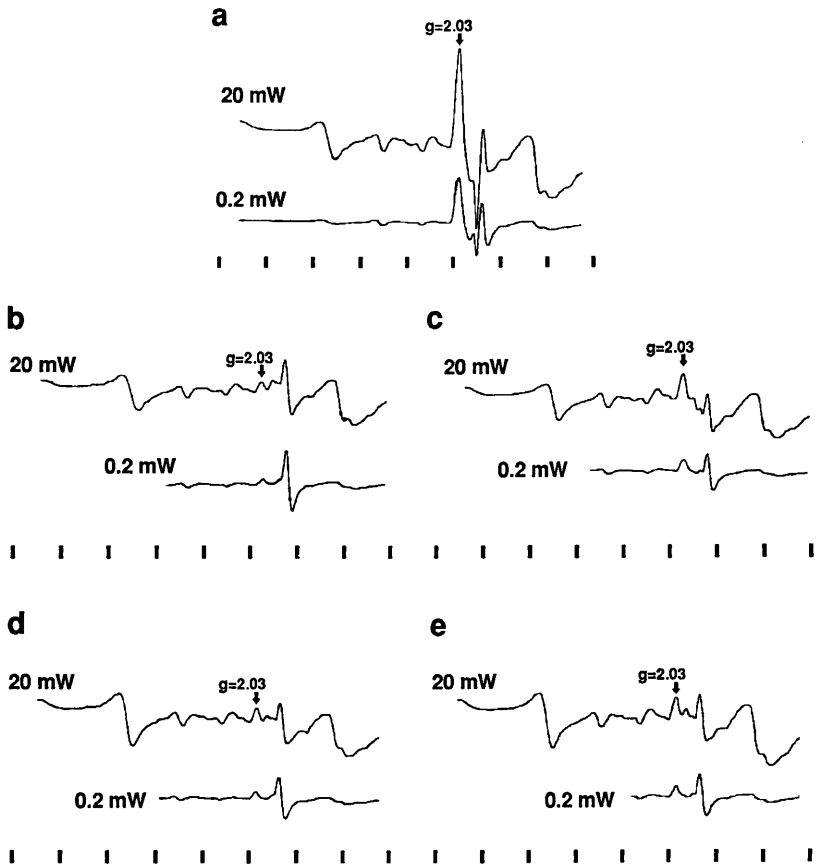

Figure 5. (a) Typical ESR spectra of liver from a copper-deficient rat fed fructose diet deficient in copper. (b) Typical ESR spectra of liver from a copper-deficient rat fed the starch diet deficient in copper. (c) Typical ESR spectra of liver from a copper-deficient rat fed the fructose diet adequate in copper. (d) Typical ESR spectra of liver from a copper-deficient rat fed the starch diet adequate in copper. (e) Typical ESR spectra of liver from a copper-deficient rat fed the fructose diet deficient in copper and low in iron.

less, but it becomes toxic under certain redox environments (8–11). When copper-deficient rats are fed starch, the concentration of hepatic iron is also elevated compared with copper-adequate rats. However, the redox state of iron that is stored in the livers of copper-deficient rats fed starch does not generate a high ESR signal, and their iron can be utilized. Indeed the starch-fed, copper-deficient animals do not develop anemia. However, if fructose metabolism, which occurs primarily in the liver, creates a different redox state than starch, iron overload will generate free radicals that are capable of causing tissue injury (9–11). In support of this contention is the finding that fructose-fed copper-deficient rats exhibited a greater degree of lipid peroxidation than copper-deficient rats fed starch (24). Once hepatic iron concentrations in copper deficiency can be reduced to the same concentration of hepatic iron as in copper-adequate controls, the copper-deficient rats fed fructose should be protected against the toxicity of copper deficiency.

Pancreatic atrophy is a constant feature of copper deficiency in rats fed fructose-based diets (25–28). The reduction of dietary iron prevented iron accumulation in the pancreas, which in turn prevented pancreatic pathology.

It is interesting to note that although heart pathology is a constant feature of copper deficiency in rats fed fructose, this pathology is not caused by free radicals. Free radicals were absent from the hearts of rats fed the copper-deficient diet containing fructose. In addition, ESR of the myocardium was similar in the hearts of copper-deficient rats fed fructose with adequate iron (50 μg/g) and in those fed the low iron diet (17μ), although heart pathology was prevented in the latter group. In contrast, once dietary iron was lowered, hepatic free radicals were prevented, heart tissue was devoid of pathology, and the animals survived. Since the metabolism of fructose occurs mainly in the liver, the liver and not the heart should be sensitive to changes in the redox state of iron. Although an increasing number of reports describe the role of active oxygen species in the development or exacerbation of various types of diseases (10, 11), free radicals do not seem to play a role in heart pathology in copper deficiency. It is suggested that the reduction of cardiac hypertrophy in copper-deficient rats fed fructose-containing diets by the antioxidant compound *t*-butylhydroquinone (29) was due to the ameliorating effect of the anemia rather than to its effect as an antioxidant. The administration

of the spin trap agent *N-tert*-butyl-α-phenylnitrone to copper-deficient rats fed fructose did not have any effect (data not shown).

In contrast to most researchers, the study by Weisenberg *et al.* (30) showed that copper-deficient rats exhibited lower liver iron than their copper-adequate controls. The administration of iron prevented the anemia in these animals (30). However, some researchers claim that high dietary iron does not reverse the anemia in rats (31, 32), cattle (33), and humans (34–37). This controversial subject requires additional consideration and research.

1. Williams DM, Kennedy FS, Green BG. Hepatic iron accumulation in copper-deficient rats. Br J Nutr **50**:653–660, 1983.
2. Williams DM, Kennedy FS, Green BG. The effect of iron substrate on mitochondrial haem synthesis in copper deficiency. Br J Nutr **53**:131–136, 1985.
3. Marston HR, Allen SH, Swaby SL. Iron metabolism in copper-deficient rats. Br J Nutr **25**:15–30, 1971.
4. Johnson MA, Gratzek JM. Influence of sucrose and starch on the development of anemia in copper and iron deficient rats. J Nutr **116**:2443–2452, 1986.
5. Evans JL, Abraham AA. Anemia, iron storage and ceruloplasmin in copper nutrition in the growing rat. J Nutr **103**:196–201, 1973.
6. Cohen NL, Keen CL, Lonnerdal B, Hurley L. Effects of varying dietary iron on the expression of copper deficiency in the growing rat: Anemia, ferroxidase I and II, tissue trace elements, ascorbic acid and xanthine dehydrogenase. J Nutr **115**:633–649, 1985.
7. Cohen NL, Keen CL, Hurley LS, Lonnerdal B. Determinants of copper deficiency anemia in rats. J Nutr **115**:710–726, 1985.
8. Wills ED. Mechanisms of lipid peroxide formation in tissues. Role of metal and hematin proteins in the catalysis of the oxidation of unsaturated fatty acids. Biochim Biophys Acta **98**:238–242, 1965.
9. Rowley D, Halliwell B. Superoxide-dependent formation of hydroxyl radicals from NADH and NADPH in the presence of iron salts. FEBS Lett **142**:39–41, 1982.
10. Editorial. Metal chelation therapy, oxygen radicals and human disease. Lancet **1**:143–145, 1985.
11. Diamzani MU. The role of free radicals in liver damage. Proc Nutr Soc **46**:43–52, 1987.
12. Fields M, Lewis CG, Lure MD. Anemia plays a major role in myocardial hypertrophy of copper deficiency. Metabolism **40**:1–3, 1991.
13. Fields M, Lewis CG, Lure MD, Burns WA, Antholine WE. The severity of copper deficiency can be ameliorated by deferoxamine. Metabolism **40**:105–109, 1991.
14. Fields M, Ferretti RJ, Reiser S, Smith JC. The severity of copper deficiency in rats is determined by the type of dietary carbohydrate. Proc Soc Exp Biol Med **175**:530–537, 1984.
15. Redman RS, Fields M, Reiser S, Smith JC. Dietary fructose exacerbates the cardiac abnormalities of copper deficiency in rats. Atherosclerosis **74**:203–214, 1988.
16. Halliwell B. Ascorbic acid, iron overload and desferrioxamine. Br Med J **285**:296–298, 1982.
17. Modell B, Letsky EA, Flynn DM. Survival and deferoxamine in thalassemia major. Br Med J **284**:1081–1084, 1982.
18. American Institute of Nutrition. Report of the AIN ad hoc committee on standards for nutritional studies. J Nutr **107**:1340–1348, 1977.
19. American Institute of Nutrition. Second report of the AIN ad hoc committee on standards for nutritional studies. J Nutr **110**:1726, 1980.
20. SAS Institute. SAS user's guide: Statistics, 5th Ed. Cary, NC: SAS Institute, 1985.
21. Hill AD, Patterson KY, Veillon C, Morris E. Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. Anal Chem **58**:2340–2342, 1986.
22. Beinert H. Insights gained by EPR spectroscopy into the composition and function of the mitochondrial respiratory chain. In: Martonosi AN, Ed. Membranes and Transport. New York: Plenum, Vol **1**: pp389–396, 1983.
23. Fields M, Lewis CG, Lure M, Antholine WE. The influence of gender on developing copper deficiency and on free radical generation of rats fed a fructose diet. Metabolism **41**:989–994, 1992.
24. Fields M, Ferretti RJ, Smith JC, Reiser S. Interaction between dietary carbohydrate and copper nutriture on lipid peroxidation in rat tissues. Biol Trace Elem Res **6**:379–391, 1984.
25. Muller HB. Der Einfluss Kupferarmer Kost auf das pankreas. Liehtmicroscopische untersuchungen am exockrinen teil der Bauchspercheldrusen weisser ratten. Virchows Arch [A] **350**:353–367, 1970.
26. Fell BF, King TP, Davies NT. Pancreatic atrophy of copper-deficient rats: Histochemical and ultrastructural evidence of a selective effect on acinar cells. Histochem J **14**:665–680, 1982.
27. Smith PA, Sunter JP, Case RM. Progressive atrophy of pancreatic acinar tissues in rats fed a copper-deficient diet supplemented with D-penicillamine or triethylene tetramine: Morphological and physiological studies. Digestion **23**:16–20, 1982.
28. Lewis CG, Fields M, Craft N, Yang C-Y, Reiser S. Alteration of pancreatic enzyme activities in small intestine of rats fed a high fructose, low copper diet. J Nutr **117**:1447–1452, 1987.
29. Johnson WT, Saari JT. Dietary supplementation with t-butylhydroquinone reduced cardiac hypertrophy and anemia associated with copper deficiency in rats. Nutr Res **9**:1355–1362, 1989.
30. Weisenberg E, Halbreich A, Mager J. Biochemical lesions of copper-deficient rats caused by secondary iron deficiency. Biochem J **188**:633–641, 1980.
31. Johnson MA, Lee Murphy C. Adverse effects of high dietary iron and ascorbic acid on copper status in copper-deficient and copper-adequate rats. Am J Clin Nutr **47**:96–101, 1988.
32. Johnson MA, Hove SS. Development of anemia in copper-deficient rats fed high levels of dietary iron and sucrose. J Nutr **116**:1225–1238, 1986.
33. Humphrey WR, Brenner I, Phillipo M. The influence of dietary iron on copper metabolism in the calf. In: Mills CF, Brenner I, Chesters JK, Eds. Trace Elements in Man and Animals—TEMA 5, pp371–373, 1985.
34. Al Rashid RA, Spangler J. Neonatal copper deficiency. N Engl J Med **285**:841–843, 1971.
35. Seely JR, Humphrey GB, Matter BJ. Copper deficiency in a premature infant fed an iron-fortified formula. N Engl J Med **286**:109–110, 1972.
36. Ashkenazi A, Lewin S, Djaldetti M, Fishel E, Benvenisti D. The syndrome of neonatal copper deficiency. Pediatrics **52**:525–533, 1973.
37. Carpel JT, Peden VH. Copper deficiency in long-term parenteral nutrition. J Pediatr **30**:32–36, 1972.