Pergamon

0891-5849(94)E0072-Q

Original Contribution

WEAK ANTIOXIDANT DEFENSES MAKE THE HEART A TARGET FOR DAMAGE IN COPPER-DEFICIENT RATS

Yan Chen,* Jack T. Saari,† and Y. James Kang*

*Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, and †United States
Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center,
Grand Forks, ND, USA

Abstract—Copper deficiency causes more salient pathologic changes in the heart than in the liver of rats. Although oxidative stress has been implicated in copper deficiency-induced pathogenesis, little is known about the selective toxicity to the heart. Therefore, we examined the relationship between the severity of copper deficiency-induced oxidative damage and the capacity of antioxidant defense in heart and liver to investigate a possible mechanism for the selective cardiotoxicity. Weanling rats were fed a purified diet deficient in copper (0.4 µg/g diet) or one containing adequate copper (6.0 µg/g diet) for 4 weeks. Copper deficiency induced a 2-fold increase in lipid peroxidation in the heart (thiobarbituric assay) but did not alter peroxidation in the liver. The antioxidant enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase were, respectively, 3-, 50- and 1.5-fold lower in the heart than in the liver, although these enzymatic activities were depressed in both organs by copper deficiency. In addition, the activity of glutathione reductase was 4 times lower in the heart than in the liver. The data suggest that a weak antioxidant defense system in the heart is responsible for the relatively high degree of oxidative damage in copper-deficient hearts.

Keywords—Copper-deficiency, Lipid peroxidation, Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione reductase, Heart, Liver, Free radicals

INTRODUCTION

Copper deficiency causes a wide diversity of pathophysiologic consequences in many species, including humans.[1] Studies have shown that severe copper deficiency results in retarded growth and impaired reproduction.[2] Alterations in morphology, function, and biochemical pathways in many organs of copper-deficient animals have also been observed.[3,4] The heart, in particular, is severely affected by copper deficiency, leading to cardiac enlargement and mitochondrial swelling,[5–7] as well as associated reductions in electrical,[8] contractile,[9] and respiratory function.[10] Other organs, including the liver, incur relatively mild changes.

Postulated mechanisms for copper deficiency-induced pathogenesis have focused on functions of copper-dependent enzymes. For example: the reduced contractile force and occasional rupture of copper-deficient hearts have been attributed to a reduction of connective tissue tensile strength caused by the reduced activity of copper-dependent lysyl oxidase[9,11]; attempts have been made to relate altered cardiac morphology and function to the impaired activity of copper-dependent dopamine β hydroxylase[9,12,13]; and reduced mitochondrial respiratory function could in part be attributed to the depressed activity of copper-dependent cytochrome c oxidase.[10] Regarding the latter suggestion, disruption of mitochondrial processes could be especially debilitating to the heart because of its relatively high degree of aerobic metabolism.

Because activities of both copper-dependent and non-copper-containing antioxidant enzymes are reduced in copper deficiency,[4] other studies have pursued the hypothesis that oxidative stress may be involved in the pathophysiology of copper deficiency. This area of research has been recently reviewed in detail.[14] Although, when critically reviewed, the evidence for an oxidative mechanism of copper deficiency appears equivocal,[14] a substantial amount of support for this view exists. This includes inhibition of defects by antioxidants,[15,16] increased susceptibility of mitochondria to in vitro oxidation,[17,18] enhanced tissue damage with oxidative

Address correspondence: to Y. James Kang, Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, P.O. Box 9037, Grand Forks, ND 58202-9037, USA.

stress,[19-21] and enhanced recovery of lipid peroxidation products [19,22-26] in copper-deficient animals.

Two basic free radical defense systems, primary and secondary, have been defined.[27] The primary defense system consists of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, and nonenzymatic components, including glutathione, metallothionein, α-tocopherol, and ascorbate. This system provides the first line of defense against free radicals. The secondary defense system includes enzymes and other mechanisms for repairing free radical-induced cell damage. These defense systems exist in almost all cell types.[28] However, cellular responses to oxidative stress are different from one cell type to another, suggesting that alterations in antioxidant defense, such as differential distribution of the antioxidant enzymes, occur between cell types. Although prior research has indicated that antioxidant enzyme activities are greater in the liver than in the heart,[29,30] the consequent effect of this differential distribution on oxidative responses in the two organs has not been specifically examined. The more profound pathologic changes observed in the heart than in the liver suggest that, if oxidative stress contributes to the damage of copper deficiency, peroxidation should be relatively higher in the heart than in the liver. The purpose of this study was to examine the extent of lipid peroxidation and the status of the enzymatic defense system in the liver and the heart of copper-deficient rats with the aim of determining whether the differential effects of copper deficiency on the heart and liver could be attributed to a difference in antioxidant defenses in the two organs.

MATERIALS AND METHODS

Diets and animals

Diets. A copper-adequate diet was formulated according to Reeves et al. (AIN-93G diet),[31] except that no antioxidant (*tert*-butylhydroquinone) was added. The primary ingredients were cornstarch (53%), casein (20%), sucrose (10%), and soybean oil (7%). Vitamins and minerals provided by the diet included the addition of 6 mg of Cu/kg of diet. A copper-deficient diet was similarly formulated except for the replacement of copper by the corresponding weight of cornstarch. Diet analysis for copper (see later) yielded values of 6.0 mg Cu/kg diet for the copper-adequate diet and 0.4 mg Cu/kg diet for the copper-deficient diet.

Animals. Male, weanling Sprague–Dawley rats (46–57 g; Sasco, Lincoln, NE, USA) were housed in quarters maintained at 22–24°C with a 12-h light/dark cycle. They were divided into three weight-matched groups having average weights of 52 g each. One group

was given free access to the copper-adequate diet; a second group was given free access to the copper-deficient diet; a third group was provided the copper-adequate diet but was pair-fed to the intake of the copper-deficient group. Rats had free access to deionized water. These experiments were conducted in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals.*[32]

Tissue sample preparation

After 4 weeks on their respective diets and an overnight fast, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg body weight, Vet Labs, Lenexa, KS). Blood was withdrawn from the inferior vena cava for erythrocyte counting and plasma assays. The heart and liver were removed, flushed with cold 0.9% NaCl via their major vessels, and divided for subsequent assays. Tissue samples for mineral assays were stored at −20°C, and samples for enzyme assays were placed in liquid nitrogen and then stored at −80°C.

Blood analysis

Hematocrit and hemoglobin content were determined on a Coulter Counter (Model S Plus 4, Hialeah, FL). A Cobas Fara automated analyzer (Roche Diagnostic Systems, Nutley, NJ) was used to determine serum ceruloplasmin[33] and total cholesterol.[34]

Analysis of minerals

Trace element contents of organs were determined by inductively coupled argon plasma emission spectroscopy (Jarrell-Ash, Model 1140, Waltham, MA) after lyophilization and digestion of organs with nitric acid and hydrogen peroxide.[35] Assay of dietary copper content was performed by a dry washing procedure,[36] dissolution in aqua regia, and measurement by atomic absorption spectrophotometry (Perkin Elmer, Model 503, Norwalk, CT). Mineral contents of National Institute of Standards and Technology (NIST) reference samples (#1577a, bovine liver for organs; #1572, citrus leaves for diets) were within the specified ranges by NIST, thus validating our assay procedure.

Lipid peroxidation

Estimates of lipid peroxidation on whole tissue homogenates of heart and liver were obtained by spectrophotometric determination (Beckman, Model DU-70, Fullerton, CA) of the concentration of thiobarbituric acid reactive substances by the method of Ohkawa et al.[37] Malonaldehyde tetraethyl acetal (Eastman Kodak,

Rochester, NY) was used as a standard for this method, thus results are expressed in units of nmol of malonaldehyde/mg wet weight of tissue.

Analysis of enzymes

Cu,Zn-superoxide dismutase. For measurement of Cu,Zn-superoxide dismutase (Cu, Zn-SOD) activity, tissue homogenates were treated with 0.4 volumes of a solution of ethanol and chloroform (25:15) to inactivate Mn-SOD.[38] This solution was mixed well and centrifuged at 5,000 × g for 15 min. An aliquot of the clear supernatant was dialyzed against deionized water for 12 h (4°C, 12,000 MW exclusion membrane) and then used to measure Cu,Zn-SOD activity. Enzyme activity was measured spectrophotometrically (Beckman, Model DU-70, Fullerton, CA) by using the Bioxytech SOD-525 Method, which is available in a kit (Cayman Chemical, Ann Arbor, MI). The method is based on the ability of SOD to accelerate autoxidation of a proprietary reagent to a visible chromophore. A unit of activity is defined as that amount of enzyme required to double the rate of autoxidation.

Catalase. Tissue samples from liver and heart were homogenized in 0.1% Triton X-100. The homogenates were centrifuged at 10,000 rpm for 20 min at 4°C. Supernatant was diluted with phosphate buffer, pH 7.0 (liver, 1:500; heart, 1:50) prior to analysis. Catalase was assayed by the method of Aebi.[39] The activity was calculated from the first order rate constant, that is, $K = \log (S_o/S_t) \times 2.3/t$, where S_o is the initial substrate concentration, S_t is the final substrate concentration, and t is the reaction time (1 min). It was expressed as units that represent the calculated rate constant (K) per milligram protein.

Glutathione peroxidase (GSH-Px). Tissue samples were homogenized in 0.1 M potassium phosphate buffer and 1 mM EDTA (1:29), pH 7.0 at 4°C. The homogenate was centrifuged at 10,000 rpm for 60 min, and the supernatant was assayed for GSH-Px activity using H_2O_2 as substrate. The GSH-Px activity was assayed by the method of Flohe et al.[40] The 1-ml reaction mixture contained 500 μl 0.1 M potassium phosphate buffer containing 1 mM EDTA and 2 mM sodium azide, 100 μl 2.4 U/g glutathione reductase, 100 μl 10 mM GSH, 100 μl enzyme sample, and 100 μl 1.5 mM NADPH. The enzymatic reaction was initiated by adding 100 μl 1.5 mM hydrogen peroxide. A sample blank contained all reagents except the enzyme preparation for which 100 μl of phosphate buffer was substituted. The enzyme activity was expressed as μmol of NADPH oxidized to NADP per milligram of protein

per minute using the extinction coefficient for NADPH of 6.22×10^{-3} mol^{-1} cm^{-1}.

Glutathione reductase. Cardiac and hepatic tissues were homogenized with 0.2 M potassium phosphate buffer and 2 mM EDTA (1:19), pH 7.0 at 4°C, then centrifuged at 10,000 rpm for 60 min at 4°C. The enzyme activity was determined by the assay described by Carlberg.[41] The 1-ml reaction mixture contained 500 μl 0.2 M potassium phosphate buffer and 2 mM EDTA, pH 7.0, 100 μl 2 mM NADPH with 10 mM Tris-HCl (pH 7.0), 100 μl 20 mM GSSG, 200 μl dd H_2O. The reaction was initiated by addition of 100 μl enzyme sample to the reaction mixture, and the decrease in absorbance at 340 nm was followed. A sample blank contained all reagents except the enzyme preparation for which 100 μl phosphate buffer was substituted. The enzyme activity was expressed as μmol of NADPH oxidized to NADP per milligram of protein per minute.

Statistical analysis

Data were initially analyzed by one-way ANOVA. Scheffe's test was then applied to analyze differences between treatments further. All experiments were repeated at least three times, with an exception of Cu,Zn-SOD determinations (two experiments). The results obtained were in agreement with each other experiment. The data (all tables and figures) were presented from the same representative study, as the value of mean ± SD for each determination from 10 replicate animals for each treatment. Differences between treatments were considered significant at $p < 0.05$.

RESULTS

In Table 1, characteristics of rats fed a copper-deficient diet are compared to those of rats fed a copper-adequate diet. Feeding of a copper-deficient diet depressed plasma copper concentration, reduced activity of the copper-dependent enzyme ceruloplasmin, and caused cardiac enlargement and anemia; the latter was indicated by reduced hematocrit and hemoglobin concentration. All of these changes have been observed previously and are indicative of severe copper deficiency.[1-7]

The status of several minerals in the heart and liver was also markedly affected by the copper deficiency. As summarized in Table 2, Cu concentrations decreased 6 and 10 times in the heart and liver, respectively. Zinc (Zn) concentrations also significantly decreased in both the heart and the liver, whereas iron (Fe) concentrations decreased in the heart but increased in the liver. Among the minerals analyzed in this study,

Table 1. Characteristics of Rats Given Free Access to Copper-Adequate
(CuA) and Copper-Deficient (CuD) Diets and of Rats Fed a CuA Diet but
Pair-Fed to CuD Rats (CuA-PF)

	CuA	CuD	CuA-PF
Plasma Cu, $\mu g/mL$	0.53 ± 0.07	ND*	0.55 ± 0.04
Body weight, g	278 ± 26	259 ± 26	251 ± 15
Heart weight, g/kg body wt	3.8 ± 0.3	$6.2 \pm 0.8*$	3.9 ± 0.2
Hematocrit, %	38 ± 2	$27 \pm 3*$	39 ± 2
Hemoglobin, g/L	132 ± 5	$90 \pm 11*$	134 ± 6

Values are means $\pm$ SD, $n = 10$ for each dietary group.
ND: Indicates that variable is not detectable.
* Indicates that value for CuD rats is significantly different from those for
CuA and CuA-PF rats ($p < 0.05$).

Zn and Cu are involved in superoxide dismutase (Cu,Zn-SOD) activity, Cu is also closely associated with cytochrome c oxidase, and Fe modulates catalase activity. These enzymes are actively involved in the antioxidant defense system (Cu,Zn-SOD and catalase) and the generation of oxygen free radicals (cytochrome c oxidase). Changes in the status of these minerals may thus result in an imbalance between the generation of oxygen free radicals and the antioxidant defense system. Therefore, the extent of lipid peroxidation in the heart and liver was examined to determine whether oxidative stress-induced damage occurred in either or both of the organs. As shown in Figure 1, copper deficiency increased the amount of lipid peroxidation products in the heart, but not in the liver.

To investigate a possible mechanism for the copper deficiency-induced selective cardiac damage relative to the liver, the antioxidant enzymatic activities in the heart and liver, including Cu,Zn-SOD, catalase, and glutathione peroxidase (GSH-Px), and their changes in response to copper-deficiency were determined. Cu,Zn-SOD is a cytosolic enzyme that catalyzes the reaction converting superoxide anion ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2). This enzyme plays a predominant role in superoxide dismutation, although this reaction also occurs nonenzymatically. As shown in Figure 2, the Cu,Zn-SOD activities in the heart and liver were 17 and 50 units $\cdot$ h^{-1} $\cdot$ g wet weight^{-1}, respectively, that is, the activity of Cu,Zn-SOD was three times lower in the heart than in the liver. This enzymatic

activity was, however, significantly depressed ($p < 0.01$) in both organs resulting from the copper deficiency.

Catalase and GSH-Px are the major enzymes involved in the reduction of H_2O_2. The relative contribution of the two enzymes to the elimination of intracellular-generated H_2O_2 appears to be related to their kinetic properties and spatial compartmentation. Therefore, to evaluate the capacity of the cellular defense against H_2O_2, the activities of both enzymes need to be determined. The catalase activities in the heart and liver are shown in Figure 3. This enzyme activity was 50 times lower in the heart (0.317 K $\cdot$ min^{-1} $\cdot$ mg protein^{-1}) than in the liver (15.97 K $\cdot$ min^{-1} $\cdot$ mg protein^{-1}), and copper deficiency caused a decrease but not a significant change ($p > 0.05$) in the enzymatic activity in the two organs. The GSH-Px activities are shown in Figure 4. This enzyme was also significantly lower in the heart (0.272 μmol NADPH $\cdot$ min^{-1} $\cdot$ mg protein^{-1}) relative to in the liver (0.405 μmol NADPH $\cdot$ min^{-1} $\cdot$ mg protein^{-1}), but the enzymatic activities in both the heart and the liver were significantly depressed ($p < 0.05$) by copper deficiency.

Another important enzyme, GSH reductase (GR), which is not directly involved in the interaction with active oxygen free radicals but provides the reductant glutathione to support GSH-Px function, was also evaluated. As shown in Figure 5, this enzyme activity was four times lower in the heart (0.012 μmol NADPH $\cdot$ min^{-1} $\cdot$ mg protein^{-1}) than in the liver (0.051 μmol

Table 2. Status of Elements in the Liver and Heart From Copper-Adequate (CuA), Copper-Deficient (CuD)
and Copper-Adequate, Pair-Fed (CuA-PF) Rats

Minerals	Liver (μg/g dry wt)			Heart (μg/g dry wt)		
	CuA	CuD	CuA-PF	CuA	CuD	CuA-PF
Cu	9.6 ± 0.4	$1.1 \pm 0.3*$	10.7 ± 0.9	19.0 ± 0.57	$3.2 \pm 0.4*$	19.0 ± 0.3
Zn	75 ± 5	$69 \pm 6*$	83 ± 6	73 ± 2	$63 \pm 3*$	72 ± 2
Fe	102 ± 22	$190 \pm 34*$	155 ± 62	159 ± 6	$139 \pm 5*$	155 ± 5

Values are means $\pm$ SD, $n = 10$ for each dietary group.
* Indicates that value for CuD rats is significantly different from those for CuA and CuA-PF rats ($p < 0.05$).
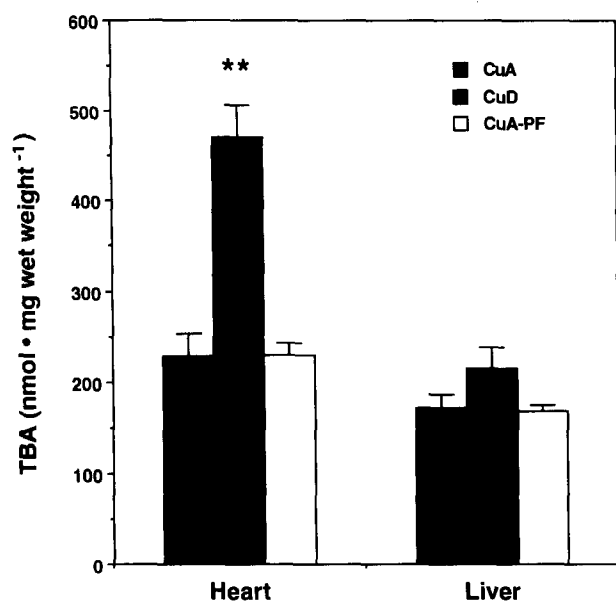
Fig. 1. Comparison of lipid peroxidation in the liver and heart from CuA, CuD, and CuA-PF rats. Lipid peroxidation was measured by the thiobarbituric acid reaction as described in Materials and Methods. Mean values $\pm$ SD from 10 animals were determined for each treatment group. The difference observed in the heart between CuD and others was highly significant by Scheffe's test, $p < 0.01$. There are no significant differences between treatments in the liver ($p > 0.05$).

NADPH $\cdot$ min^{-1} $\cdot$ mg protein^{-1}). Copper deficiency caused a slight, but not significant ($p > 0.05$), increase in this enzyme activity in both organs.

DISCUSSION

The development of selective severe damage to the heart resulting from dietary copper deficiency has long been recognized.[5–7] Although a variety of mechanisms have been proposed, many studies have focused on the effect of copper deficiency on the antioxidant defense system as a possible mechanism by which copper deficiency causes tissue damage. Although these studies have indicated that oxygen free radicals may play a role in the copper deficiency-induced tissue damage, few studies have been directed at determining the mechanisms for the selective cardiotoxicity of copper deficiency.

In this study, copper deficiency-induced lipid peroxidation and the status of the antioxidant defense system in the heart and liver were evaluated. It was aimed at elucidating a possible mechanism for the copper deficiency-induced selective damage to the heart relative to the liver. The results obtained showed that copper deficiency induced a well-described pathologic change in the heart, that is, cardiac hypertrophy. More importantly, copper deficiency enhanced lipid peroxidation in the heart but did not cause such pathologic

change in the liver. Although other peroxidation assay techniques (breath ethane, induced peroxidation of subcellular organelles) consistently show enhanced peroxidation in copper deficiency, the finding of enhanced lipid peroxidation products in homogenates of tissues taken directly from copper-deficient animals has not been universal, as pointed out by Johnson et al.[14] Prior studies that have failed to show increased TBARS in copper-deficient tissues could be explained by the use of diets with different peroxidation potential, the use of different assay procedures,[30,42] or by the use of animals that were less copper-deficient.[43,44] In our laboratory, the currently used technique has shown consistent enhancement of TBARS in uninduced copper-deficient tissues in recent studies[45,46] and is supportive of studies from other laboratories that have seen enhancement of peroxidation products in uninduced cells or tissues.[22,23,25,26]

A dramatic difference that exists between the heart and the liver even prior to their responses to copper deficiency is a considerably weaker antioxidant system in the heart. The role of antioxidant enzymes including Cu,Zn-SOD, catalase, and GSH-Px in the detoxification of oxygen free radicals has been well defined.[27] These enzymes function sequentially as well as cooperatively in the detoxification process of oxygen free radicals. As discussed by Gianni and Myers,[47] under normal conditions, one enzyme activity is lower; another may compensate by an increase in its activity.
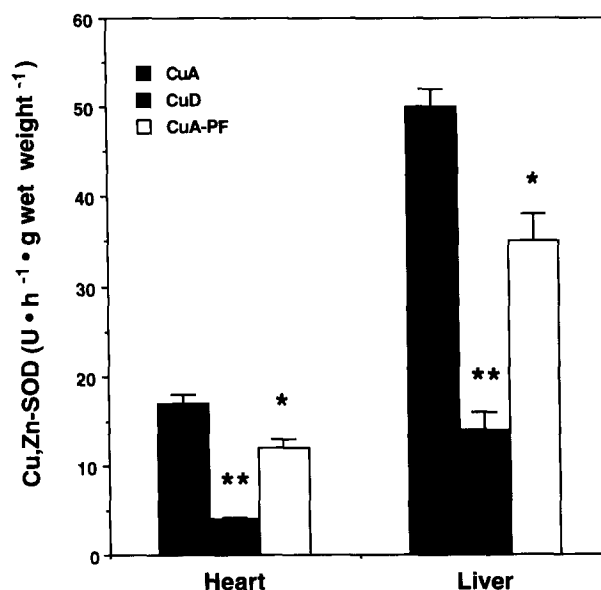

Fig. 2. Comparison of Cu,Zn-superoxide dismutase activity in the liver and heart from CuA, CuD, and CuA-PF rats. This enzyme activity in the liver (50 units $\cdot$ h^{-1} $\cdot$ g wet weight^{-1}) was three times higher than in the heart (17 units $\cdot$ h^{-1} $\cdot$ g wet weight^{-1}). Cu deficiency caused a highly significant decrease in the enzyme activity in both the heart and liver ($p < 0.01$). Pair feeding also decreased the enzyme activity in both organs ($p < 0.05$).
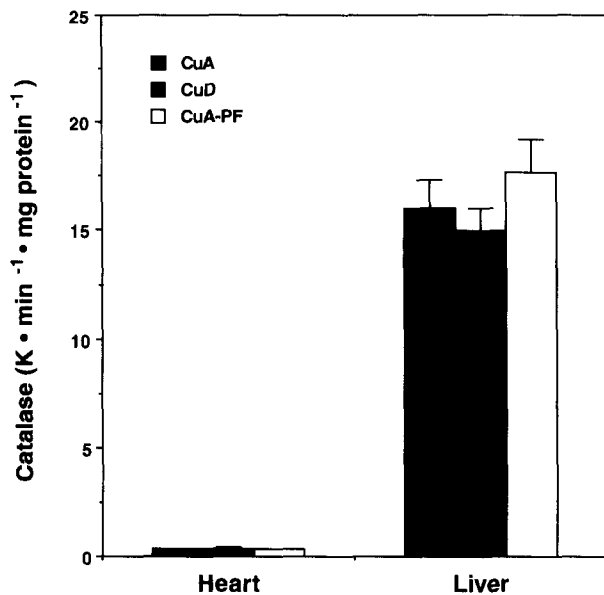
Fig. 3. Comparison of catalase activity in the liver and heart from CuA, CuD, and CuA-PF rats. This enzyme activity in the heart (0.32 K · min^{-1} · mg protein^{-1}) was 50 times lower than in the liver (15.97 K · min^{-1} · mg protein^{-1}). Neither Cu deficiency nor pair feeding caused significant change in the enzyme activity in either organ ($p > 0.05$), although a slight decrease in the enzyme activity was observed.
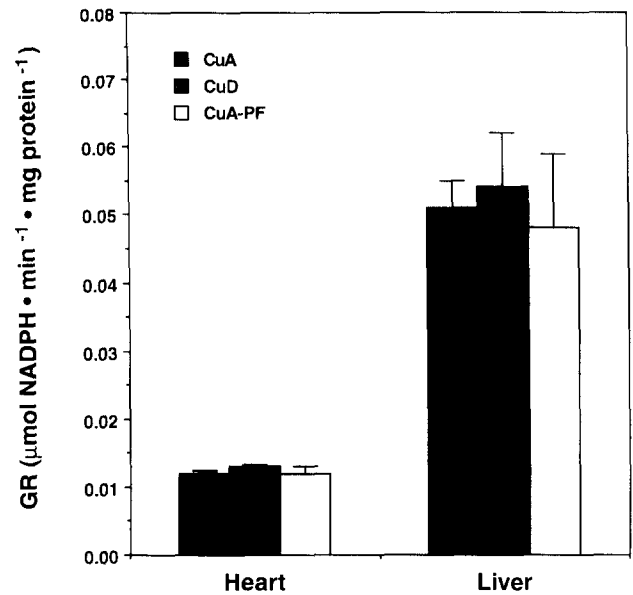

Fig. 5. Comparison of glutathione reductase in the liver and heart from CuA, CuD, and CuA-PF rats. This enzyme activity in the heart (0.012 μmol NADPH · min^{-1} · mg protein^{-1}) was four times lower than in the liver (0.051 μmol NADPH · min^{-1} · mg protein^{-1}). Cu deficiency caused a slight, but not significant ($p > 0.05$), increase in the enzyme activity in both liver and heart.

For instance, if catalase activity is lower in a particular tissue, the GSH-Px activity may be higher. Results obtained from this study, however, revealed that all of
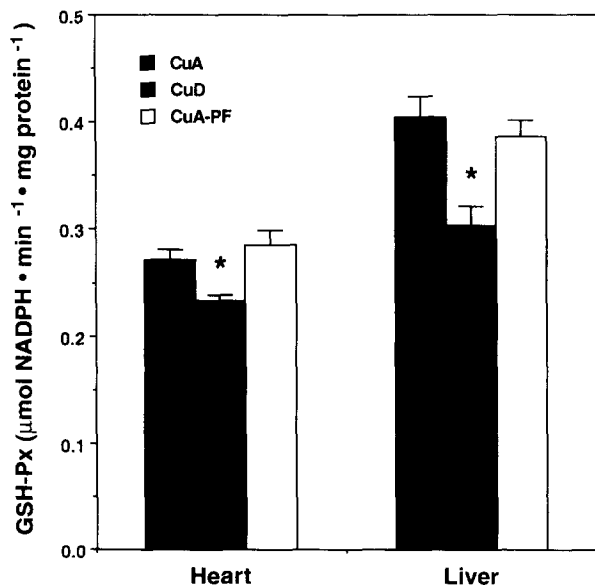

Fig. 4. Comparison of glutathione peroxidase (GSH-Px) in the liver and heart from CuA, CuD, and CuA-PF rats. This enzyme activity in the heart (0.27 μmol NADPH · min^{-1} · mg protein^{-1}) was significantly lower than in the liver (0.41 μmol NADPH · min^{-1} · mg protein^{-1}). Cu deficiency, but not pair feeding, induced a significant decrease in the enzyme activity in both liver and heart ($p < 0.05$).

the measured antioxidant enzyme activities in the heart were lower than in the liver. The catalase activity in the heart should even be considered too low to properly function. Because both catalase and GSH-Px are actively involved in converting H_2O_2 to H_2O, the lack of an efficient catalase activity makes GSH-Px the major enzymatic component of H_2O_2 detoxification in the heart, although this enzyme activity was found to be much lower in the heart than in the liver. Under such conditions of depressed antioxidant enzyme activity and/or increased accumulation of H_2O_2, the H_2O_2 would be converted to hydroxyl radical (·OH), resulting in lipid peroxidation.

The Cu,Zn-SOD activity was also far lower in the heart than in the liver. This enzyme converts superoxide anion to H_2O_2, so the lower Cu,Zn-SOD activity in the heart would result in more superoxide anion accumulation. Thus, the heart would be much more vulnerable to the toxic effect of this oxygen free radical species, although there was no indication whether the severe lipid peroxidation in the heart was caused by a heightened superoxide anion or an increased amount of hydroxyl radical. It is likely, however, that the cardiac lipid peroxidation resulted from a combination of accumulated superoxide anion and over-produced hydroxyl radical.

Glutathione reductase (GR) activity was also determined in the heart and liver. This enzyme catalyzes the reaction that regenerates reduced GSH from its

oxidized form. The reduction of GSSG to GSH is an essential step for maintaining reducing equivalents of the GSH-Px reaction. Under conditions of oxidative stress, oxidation of GSH to GSSG would be enhanced both enzymatically, by the reaction catalyzed by GSH-Px, and nonenzymatically. The GR activity was about four times higher in the liver than in the heart, indicating that the rate of regeneration of reduced GSH from GSSG would be faster in the liver. A slight, but not significant, increase in the enzyme activity was observed in both the liver and the heart of the copper-deficient rats, which suggests that a compensatory response of the enzyme activity to the increased GSSG level may exist.

In this study, decreased enzyme activities of Cu,Zn-SOD and GSH-Px were observed in both the heart and the liver of the copper-deficient rats. It is most likely that the decrease in the Cu,Zn-SOD activity resulted from the depressed availability of Cu and Zn. The possible mechanism for GSH-Px depression by copper deficiency is unknown. Nevertheless, the depression of the GSH-Px activity may not be responsible for the difference in lipid peroxidation between the heart and the liver. This is based on the facts that (1) the GSH-Px activities were decreased both in the heart and liver, but only the heart showed severe lipid peroxidation; and (2) although GSH-Px activity was significantly depressed in the liver, catalase activity remained the same, suggesting an efficient antioxidant system still existed in the liver. These results indicate that the pre-existing lack of an efficient antioxidant system, rather than selective removal of antioxidant defenses, makes the heart preferentially vulnerable to lipid peroxidation in copper deficiency.

Although this study has focused on oxidative damage as a general mechanism of damage in copper deficiency, it is important to recognize that other pathologic mechanisms may come into play. For example, Saari and Medeiros[42] found that, though a powerful antioxidant could inhibit the cardiac enlargement and other effects of copper deficiency, mitochondrial swelling persisted, suggesting an as yet unknown nonperoxidative pathology. Two alternative generalized mechanisms of pathology that have been associated with copper deficiency are sorbitol accumulation[48] and nonenzymatic glycosylation.[46]

In summary, this study examined the status of the antioxidant system in the heart and liver and its manipulation by copper deficiency. The aim of the study was to determine a possible mechanism for the copper deficiency-induced selective damage to the heart relative to the liver. The results obtained demonstrated that copper deficiency caused severe oxidative damage to the heart, but not to the liver. This is consistent with the observation that the heart has a less efficient antiox-

idant system than the liver and suggests that this deficit contributes to the copper deficiency-induced selective oxidative damage to the heart. The information generated from this study is not only valuable for the future study on the mechanism for copper deficiency-induced pathogenesis, but also useful for other investigations related to prooxidant and antioxidant responses.

REFERENCES

1. Davis, G. K.; Mertz, W. Copper. In: Mertz, W. ed. *Trace elements in human and animal nutrition.* New York: Academic Press; 1987:301–364.
2. Dutt, B.; Mills, C. F. Reproduction failure in the rats due to copper deficiency. *J. Comp. Pathol.* **70**:120–125; 1960.
3. Fell, B. F. The pathology of copper deficiency in animals. In: Howell, J. M.; Gawthorne, J. M., eds. *Copper in animals and man.* Boca Raton, FL: CRC Press Inc.; 1987:1–28.
4. Prohaska, J. R. Biochemical changes in copper deficiency. *J. Nutr. Biochem.* **1**:452–461; 1990.
5. Goodman, J. R.; Warshaw, J. B.; Dallman, P. R. Cardiac hypertrophy in rats with iron and copper deficiency: Quantitative contribution of mitochondrial enlargement. *Pediatr. Res.* **4**:244–256; 1970.
6. Medeiros, D. M.; Bagby, G. O.; McCormick, R. Myofibrillar, mitochondrial and valvular morphological alterations in cardiac hypertrophy among copper-deficiency rats. *J. Nutr.* **121**:815–824; 1991.
7. Kopp, S. T.; Klevay, L. M.; Feliksik, J. M. Physiologic and metabolic characterization of cardiomyopathy induced by chronic copper deficiency. *Am. J. Physiol.* **245**:4855–4866; 1983.
8. Viestenz, K. E.; Klevay, L. M. A randomized trial of copper therapy in rats with electrocardiographic abnormalities due to copper deficiency. *Am. J. Clin. Nutr.* **35**:258–266; 1982.
9. Prohaska, J. R.; Heller, L. J. Mechanical properties of the copper-deficient rat heart. *J. Nutr.* **112**:2142–2150; 1982.
10. Bode, A. M.; Miller, L. A.; Faber, J.; Saari, J. T. Mitochondrial respiration in heart, liver, and kidney of copper-deficient rats. *J. Nutr. Biochem.* **3**:668–672; 1992.
11. Allen, K. G. D.; Klevay, L. M. Cholesterolemia and cardiovascular abnormalities in rats caused by copper deficiency. *Atherosclerosis* **29**:81–93; 1978.
12. Prohaska, J. R.; Bailey, W. R.; Gross, A. M.; Korte, J. J. Effect of dietary copper deficiency on the distribution of dopamine and norepinephrine in mice and rats. *J. Nutr. Biochem.* **1**:149–154; 1990.
13. Seidel, K. E.; Failla, M. L.; Rosebrough, R. W. Cardiac catecholamine metabolism in copper-deficient rats. *J. Nutr.* **121**:474–483; 1991.
14. Johnson, M. A.; Fischer, J. G.; Kays, S. E. Is copper an antioxidant nutrient? *Crit. Rev. Food Sci. Nutr.* **32**:1–31; 1992.
15. Saari, J. T. Chronic treatment with dimethyl sulfoxide protects against cardiovascular defects of copper deficiency. *Proc. Soc. Exp. Biol. Med.* **190**:121–124; 1989.
16. Johnson, W. T.; Saari, J. T. Dietary supplementation with t-butylhydroquinone reduces cardiac hypertrophy and anemia associated with copper deficiency in rats. *Nutr. Res.* **9**:467–477; 1990.
17. Paynter, D. I. The role of dietary copper, manganese, selenium, and vitamin E in lipid peroxidation in tissues of the rat. *Biol. Trace Elem. Res.* **2**:121–135; 1980.
18. Fields, M.; Ferretti, R. J.; Smith, J. C.; Reiser, S. Interaction

between dietary carbohydrate and copper nutriture on lipid peroxidation in rat tissues. *Biol. Trace Elem. Res.* **6**:379–391; 1984.

19. Lawrence, R. A.; Jenkinson, S. G. Effects of copper deficiency on carbon tetrachloride-induced lipid peroxidation. *J. Lab. Clin. Med.* **109**:134–140; 1987.

20. Jenkinson, S. G.; Lawrence, R. A.; Grafton, W. D.; Gregory, P. E.; McKinney, M. A. Enhanced pulmonary toxicity in copper-deficient rats exposed to hyperoxia. *Fund. Appl. Toxicol.* **4**:170–177; 1984.

21. Akers, T. K.; Saari, J. T. Hyperbaric hyperoxia exaggerates respiratory membrane defects in the copper-deficient rat lung. *Biol. Trace Elem. Res.* **38**:149–163; 1993.

22. Nelson, S. K.; Huang, C.-J.; Mathias, M. M.; Allen, K. G. D. Copper-marginal and copper-deficient diets decrease aortic prostacyclin production and copper-dependent superoxide dismutase activity, and increase aortic lipid peroxidation in rats. *J. Nutr.* **122**:2101–2108; 1992.

23. Balevska, P. S.; Russanov, E. M; Kassabova, T. A. Studies on lipid peroxidation in rat liver by copper deficiency. *Int. J. Biochem.* **13**:489–493; 1981.

24. Saari, J. T.; Dickerson, F. D.; Habib, M. P. Ethane production in copper-deficient rats. *Proc. Soc. Exp. Biol. Med.* **195**:30–33; 1990.

25. Russanov, E. M.; Kassabova, T. A. Enzymes of oxygen metabolism and lipid peroxidation in erythrocytes from copper-deficient rats. *Int. J. Biochem.* **14**:321–325; 1982.

26. Jain, S. K.; Williams, D. M. Copper deficiency anemia: Altered red blood cell lipids and viscosity in rats. *Am. J. Clin. Nutr.* **48**:637–640; 1988.

27. Sies, H., ed. *Oxidative stress.* New York: Academic Press; 1985.

28. Jones, T. W.; Thor, H.; Orrenius, S. Cellular defense mechanisms against toxic substances. *Arch. Toxicol. Suppl.* **9**:259–271; 1986.

29. Prohaska, J. R. Changes in Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase and glutathione transferase activities in copper deficient mice and rats. *J. Nutr.* **121**:355–363;1991.

30. Fischer, J. G.; Tackett, R. L.; Howerth, E. W.; Johnson, M. A. Copper deficient rat heart can compensate for doxorubicin-induced oxidant stress. *Biol. Trace Elem. Res.* **37**:233–251; 1993.

31. Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123**:1939–1951; 1993.

32. National Research Council. *Guide for the care and use of laboratory animals.* Publication no. 85–23 (rev). Bethesda, MD: National Institutes of Health; 1985.

33. Sunderman, F. W.; Nomoto, S. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. *Clin. Chem.* **16**:903–910; 1970.

34. Allain, C. A.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Fu, P. C. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**:470–475; 1974.

35. Nielsen, F. H.; Zimmerman, T. J.; Shuler, T. R. Interactions among nickel, copper and iron in rats: Liver and plasma contents of lipids and trace elements. *Biol. Trace Elem. Res.* **4**:125–143; 1982.

36. Gorsuch, T. T., ed. *The destruction of organic matter.* Elmsford, NY: Pergamon Press; 1970:28–39.

37. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**:351–358; 1979.

38. Paynter, D. E.; Moir, R. J.; Underwood, E. J. Changes in activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rate with changes in dietary copper. *J. Nutr.* **109**: 1570–1576; 1979.

39. Aebi, H. Catalase in vitro. *Methods Enzymol.* **105**:121–127; 1984.

40. Flohe, L.; Gunzler, W. A. Assay of glutathione peroxidase. *Methods Enzymol.* **105**:115–121; 1984.

41. Carlberg, I.; Mannervik, B. Glutathione reductase. *Methods Enzymol.* **113**: 484–490;1985.

42. Saari, J. T.; Medeiros, D. M. Effect of dimethyl sulfoxide on enlarged hearts of copper-deficient rats. *Biol. Trace Elem. Res.* **31**:249–263; 1991.

43. Lynch, S. M.; Strain, J. J. Effects of copper deficiency on hepatic and cardiac antioxidant enzyme activities in lactose- and sucrose-fed rats. *Br. J. Nutr.* **61**:345–354; 1989.

44. Brown, J. C. W.; Strain, J. J. Effect of dietary homocysteine on copper status in rats. *J. Nutr.* **120**:1068–1074; 1990.

45. Kato, N; Saari, J. T.; Schelkoph, G. M. Cystine feeding enhances defects of dietary copper deficiency by a mechanism not involving oxidative stress. *J. Nutr. Biochem.* **5**:99–105; 1994.

46. Saari, J. T.; Bode, A. M. Does nonenzymatic glycosylation contribute to defects associated with dietary copper deficiency? *FASEB J.* **7**:A410 Abstr.; 1993.

47. Gianni, L; Myers, C. E. The role of free radical formation in the cardiotoxicity of anthracycline. In Muggia, F. M.; Green, M. D.; Speyer, J. L., eds. *Cancer treatment and the heart.* Baltimore and London: The Johns Hopkins University Press; 1992:9–46.

48. Fields, M; Lewis, C. G.; Beal, T. Accumulation of sorbitol in copper deficiency: Dependency on gender and type of dietary carbohydrate. *Metabolism* **38**:371–375; 1989.

ABBREVIATIONS

CuA—copper adequate

CuD—copper deficient

CuA-PF—copper adequate pair-fed control

Cu,Zn-SOD—Cu,Zn-superoxide dismutase

DMSO—dimethyl sulfoxide

GR—glutathione reductase

GSH—glutathione

GSH-Px—glutathione peroxidase

GSSG—oxidized glutathione

H_2O_2—hydrogen peroxide