Atria and Ventricles of Copper-Deficient Rats Exhibit Similar Hypertrophy and Similar Altered Biochemical Characteristics (44147)

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Abstract. **Male Holtzman rats were offered a semipurified low-copper (Cu) diet (0.36 mg Cu/kg) for 5–6 weeks to further characterize cardiac hypertrophy, which accompanies Cu deficiency. Cu-adequate (controls) were given supplemental Cu (20 μg/ml) in their drinking water, and Cu-deficient rats were given deionized water. Cu-deficient rats had lower plasma ceruloplasmin activity, lower hemoglobin levels, higher heart weights, and similar body weights compared with Cu-adequate rats. The relative degree of hypertrophy in the right ventricle of Cu-deficient rats was significantly higher (2.3-fold) than that in the left ventricle and atria (both were 1.9-fold higher than the values in Cu-adequate rats). Edema was not detected. Ventricles and atria of Cu-deficient rats had markedly lower Cu and no significant differences in iron concentrations compared with Cu-adequate rats. Heart protein concentrations were not altered consistently by Cu deficiency. Enzyme activities of the cuproenzymes cytochrome-*c* oxidase (CCO), copper,zinc-superoxide dismutase (SOD), dopamine β-monooxygenase (DBM), peptidylglycine α-amidating monooxygenase (PAM), and the selenoenzyme glutathione peroxidase (GPX) were measured in the atria and ventricles. Cu deficiency resulted in lower specific activities of all cuproenzymes, with the exception of ventricular PAM. GPX was not altered by chamber region or diet. Specific activity of PAM was 200-fold higher in atria than in ventricles in control rats. Catecholamine analyses by HPLC confirmed that, like ventricular tissue, atria of Cu-deficient rats had lower norepinephrine and higher dopamine concentrations, consistent with lower DBM activity. Another experiment detected no differences between the two dietary groups in mean arterial blood pressure, heart rates, or responses after challenge with angiotensin II, phenylepherine, or acetylcholine in cannulated rats. In this Cu-deficient rat model, all chambers of the heart exhibit similar and marked hypertrophy. Biochemical alterations following dietary Cu deficiency were also similar in atria and ventricles. The hypertrophic response appears different from the response to simple pressure or volume overload.** [P.S.E.B.M. 1997, Vol 215]

Deficiencies of various essential trace metals, such as copper (Cu), are known to lead to many pathological events. Cu deficiency is characterized by the consistent development of cardiac hypertrophy (1–3). Stimuli responsible for cardiac hypertrophy following various pathophysiological states have not been identified as causative agents in the Cu-deficient model of hypertrophy. The anemia, which is sometimes induced in Cu-deficient rats, and the corresponding volume overload are not necessary for hypertrophy to occur (2, 4, 5), nor does the degree of anemia correlate with the degree of hypertrophy (1, 6).

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Total blood volume, as assessed by tracer techniques, is not altered by Cu deficiency (7). High heart rates and expanded end-diastolic volumes, signals of volume overload, have not been identified in Cu-deficient rats. Pressure overload does not appear to be a factor in the cardiac growth since arterial pressures are low in weanling Cu-deficient rats (3, 8–10). Furthermore, the hypertrophy observed is not confined to left ventricles, as commonly observed with pressure overload; right ventricles are also hypertrophied (11). The causative agent for the cardiac hypertrophy observed in Cu-deficient rats remains unknown.

Cu deficiency leads to altered activity of many cuproenzymes in various tissues, such as ceruloplasmin (CPL), lysyl oxidase (LOX), copper, zinc-superoxide dismutase (SOD), cytochrome-*c* oxidase (CCO), dopamine β-monooxygenase (DBM), and peptidylglycine α-amidating monooxygenase (PAM) (2, 12). Changes in these enzyme activities during Cu deficiency may be partly responsible for the cardiac hypertrophy or the cardiac myopathy, but this has not yet been established.

One possible cause for the hypertrophy could be related to the antioxidant state in the Cu-deficient rat. We and others have previously reported that iron (Fe) accumulates in the livers of Cu-deficient weanling rats (3, 5, 11). The antioxidant selenoenzyme GPX and cuproenzyme SOD have been shown to be lower in liver following Cu deficiency (12). Both GPX and SOD are believed to be important enzymes that protect cells from oxygen radicals and subsequent lipid peroxidation. Some studies have shown that Cu-deficient rats do indeed exhibit increased lipid peroxidation (13–15). There is no direct evidence that reactive oxygen species (ROS) are responsible for the development of cardiac hypertrophy, yet gene transcription of various peptides and growth factors are enhanced by oxygen radical activation of the DNA binding nuclear regulatory factor kappa beta (NFκB) (16, 17). Thus, elevation in ROS may lead to abnormal growth.

Hypertrophy of the ventricles and atria may not be equivalent. Individual chambers of the Cu-deficient heart have not been studied previously. The purpose of the present experiments was to characterize biochemically the atria and ventricles of the Cu-deficient hypertrophied hearts and compare these with tissue from control rats. A second objective was to determine if the consequences of Cu deficiency were similar in atria and ventricles. Enzymatic, mineral, and limited metabolite analyses were carried out in five similar experiments.

Materials and Methods

Animal Care and Diets. Male weanling Holtzman rats (purchased commercially, Harlan Sprague-Dawley, Indianapolis, IN) were studied in five experiments. The rats were placed in stainless-steel cages at 19–21 days old. All animals were offered a commercial Cu-deficient purified diet (Teklad Laboratories, Madison, WI). Rats were randomly divided into two dietary groups and given Cu in their drinking water (20 μg/ml Cu as cupric sulfate) or deionized water. Food and drinking water were available *ad libitum* for 5–6 weeks.

The purified diet followed the American Institute of Nutrition AIN-76A diet (5) without added cupric carbonate, and contained the following components (g/kg diet): sucrose, 500; casein, 200; cornstarch, 150; corn oil, 50; cellulose, 50; modified AIN-76 mineral mix, 35; AIN-76A vitamin mix, 10; D,L-methionine, 3; choline bitartrate, 2; and ethoxyquin, 0.01. The purified diet contained 0.36 mg Cu/kg and 43 mg Fe/kg. Rats were maintained on a 12:12-hr light:dark cycle (0700 to 1900 hr) at 24°C with 55% humidity in an AAALAC accredited facility. All protocols were formally approved by the University of Minnesota Animal Care Committee.

Blood Pressure Measurements. Mean arterial pressures (MAP), heart rates, and pressor responses to challenges with iv injections of 0.1 ml angiotensin II (Ang II) (3×10^{-7} *M*), phenylephrine (PE) (1×10^{-4} *M*), and acetylcholine (ACh) (1×10^{-5} *M*) were measured in rats from Experiment 4. The rats were anaesthetized with sodium pentobarbital (NaPB), 50 mg/kg. Heart rate and pressor responses were measured through cannulated femoral arteries as described previously (11). Injections were made through cannulated femoral veins in duplicate at 5-min intervals. Between pressor agents 0.3 ml of phosphate-buffered saline was injected so that baseline conditions were established prior to each agent injection. Maximal pressure changes were determined from the recordings and the average response was calculated for further analyses.

Tissue Sampling. In other experiments, rats were anesthetized with NaPB (50 mg/kg) and killed by exsanguination through the abdominal vena cava. A small aliquot of blood was removed for hemoglobin analysis and the CPL assay. The heart, right gastrocnemius muscle (Experiment 1 only), and liver were removed from each animal. Approximately 1 g of liver was saved for Cu and Fe analyses. The right and left appendages of the atria were trimmed away from the hearts, pooled, and weighed. The right ventricular free wall was cut away from the left ventricle and each ventricular segment weighed separately. The left ventricle weight includes the intraventricular septum. The water content was determined by drying the tissues to constant weight (24 hr at 50°C) and the percentages dry weight calculated.

Chemical Analyses. Liver and diet samples were wet-digested with HNO_3 and analyzed for Cu and Fe by flame atomic absorption spectroscopy (Model 2380; Perkin-Elmer, Norwalk, CT) as described previously (5). The right gastrocnemius muscle, atria, left ventricle, and right ventricle were analyzed for Cu and Fe by flame atomic absorption spectroscopy after dry weights were obtained. Ventricular and atrial catecholamine concentrations were measured using HPLC with electrochemical detection as described previously (18). Hemoglobin (Hb) was deter-

mined spectrophotometrically as metcyanhemoglobin (19). Ceruloplasmin activity was determined by a modification of the method described by Lehman *et al.* (20) measuring the ability of plasma to oxidize o-dianisidine. SOD was measured following the inhibition of pyrogallol autoxidation at 320 nm as described previously (19). One unit of activity is described as the amount of SOD that inhibited autoxidation by 50%. Due to the loss of activity observed with storage, CCO activity was determined only in fresh homogenates according to a method described previously which monitors the loss of ferrocytochrome-*c* (19). GPX activity was quantified by monitoring the loss of NADPH at 340 nm in a coupled-enzyme procedure (21). PAM activity was measured as previously described (5) where the trinitrophenyl-labeled substrate and product were separated by reverse-phase HPLC with the effluent monitored at 350 nm (Kratos 757 UV detector; Applied Biosystems, Foster City, CA). *N*-ethylmaleimide (5 m*M*) was used in the PAM reaction for atria and ventricles. Peak heights were recorded (Omniscribe recorder, Houston Instruments) and picomoles of product were calculated by comparison with trinitrophenyl-D-Tyr-Val-NH_2. DBM activity was determined spectrophotometrically as described previously (5). Protein concentrations were determined by a modified Lowry protein assay using bovine albumin as reference (22).

Data Analysis. Data were analyzed using a personal computer and statistical software (Statview 4.5; Abacus Concepts, Berkeley, CA). Means ± SEM were calculated. Some data were pooled (Experiments 1–3) and analyzed by one-way analysis of variance (ANOVA) to determine statistical significance ($\alpha = 0.05$) since the degree of hypertrophy and Cu status were similar in these three experiments. Variance equality was evaluated by the *F* test. For comparisons between groups with unequal variances the data were first transformed (natural logarithm) prior to further analysis.

Differences in selected characteristics between atria and ventricle were analyzed in Cu-adequate rats using one-way ANOVA, $\alpha = 0.05$. Effects of dietary treatment between groups and within a given chamber were analyzed by one-way ANOVA, $\alpha = 0.05$. To evaluate the potential differential effect of Cu-deficiency between chambers, data from Cu-deficient rats was converted to a percentage of the mean value for Cu-adequate rats for a given characteristic. This data (% Cu-adequate) was analyzed by one-way ANOVA and Fisher's PLSD test, $\alpha = 0.05$.

Results

Effect of Copper-Deficient Diet on Copper Status. Animals from five similar experiments provided tissue for the analyses reported (Table I). Rats fed the Cu-deficient diet and drinking deionized water had significant cardiac hypertrophy as determined by their heart weight to body weight (HW/BW) ratios compared with rats drinking Cu-supplemented water. The increased ratio was a result of an absolute increase in heart mass since body weights of the rats in the two diet groups were not different.

The Cu-deficient rats exhibited characteristics consistent with severe Cu deficiency such as lower hemoglobin levels (three of five experiments) and lower liver Cu concentrations and higher liver Fe concentrations compared with control rats. Ceruloplasmin activity was nearly eliminated in the Cu-deficient rats, which also confirmed the altered Cu status between the two dietary treatment groups.

Table I. Effect of a Copper-Deficient Diet on Characteristics of Experimental Rats

Characteristic	Diet	Experiment				
		1	2	3	4	5
Age (days)	+Cu	63	55	58	55	54
	–Cu	63	55	58	55	54
Sample (*n*)	+Cu	6	5	3	4	6
	–Cu	5	4	3	4	6
Body weight (g)	+Cu	356 ± 4.3	304 ± 6.2	324 ± 3.8	300 ± 13.2	317 ± 6.5
	–Cu	353 ± 17.9	296 ± 11.8	328 ± 6.0	300 ± 10.2	298 ± 13.1
Heart/BW (mg/g)	+Cu	3.38 ± 0.13	3.22 ± 0.14	3.19 ± 0.02	3.65 ± 0.07	3.57 ± 0.08
	–Cu	6.76 ± 0.49*	6.24 ± 0.50*	6.09 ± 0.61*	5.14 ± 0.30*	6.16 ± 0.61*
Hemoglobin (g/100 ml)	+Cu	13.9 ± 0.38	13.6 ± 0.72	14.5 ± 0.24	14.6 ± 0.31	14.0 ± 0.50
	–Cu	9.69 ± 0.76*	11.9 ± 1.1*	11.1 ± 1.24	13.3 ± 1.27	10.5 ± 1.1*
Liver Cu (μg/g)	+Cu	4.03 ± 0.11	4.69 ± 0.22	5.15 ± 0.64	5.09 ± 0.36	4.01 ± 0.11
	–Cu	0.49 ± 0.07*	0.63 ± 0.05*	0.56 ± 0.05*	1.02 ± 0.18*	0.60 ± 0.07*
Liver Fe (μg/g)	+Cu	91.2 ± 10.5	85.8 ± 10.0	56.3 ± 5.41	77.4 ± 10.9	65.5 ± 6.8
	–Cu	159 ± 23.1*	253 ± 29.5*	113 ± 3.89*	150 ± 12.1*	110 ± 8.23*
Ceruloplasmin (units/liter)	+Cu	97.0 ± 8.0	149 ± 9.8	159 ± 9.1	144 ± 13.4	132 ± 5.5
	–Cu	0.5 ± 0.1*	0.7 ± 0.1*	0.1 ± 0.1*	<0.1*	0.3 ± 0.1*

Note. Values of rat characteristics are means ± SEM. Data were analyzed by one-way ANOVA for a given experiment. Some data were transformed (natural logarithm) prior to analyses because of unequal variances.
* A significant difference ($P < 0.05$).

Effect of Copper-Deficient Diet on Hearts. The hypertrophy measured in the Cu-deficient rats was observed in all chambers of the heart. The left and right atria to body weight ratios were both higher in tissue from Cu-deficient rats (data not shown). Representative data pooled from experiments one and two demonstrated that the combined (left and right) atria-to-body weight ratio was 1.9-fold higher in Cu-deficient rats than in Cu-adequate rats (Fig. 1). The same phenomenon was seen in the ventricles. The left ventricle-to-body weight (LV/BW) ratio was also 1.9-fold higher in Cu-deficient than in Cu-adequate rats. The right ventricle-to-body weight (RV/BW) ratio was 2.3-fold higher in Cu-deficient than in Cu-adequate rats. One-way ANOVA analysis of the data from Cu-deficient rats indicated that hypertrophy (as a percentage of the mean Cu-adequate value) was significantly greater in the RV than the LV or atria ($P < 0.05$).

Rats from Experiment 4 were used to evaluate cardiac characteristics. The mean arterial blood pressure of cannulated, anesthetized Cu-deficient rats was 105 ± 9 mm Hg compared with 112 ± 6 mm Hg in the Cu-adequate rats. The difference in MAP between groups was not significant ($P > 0.05$). We were unable to detect differences in the mean heart rates between groups. Changes in blood pressure in response to challenges with Ang II, PE, and ACh in the Cu-deficient rats were (mean ± SEM) 39 ± 4 mm Hg, 49 ± 6 mm Hg, and −39 ± 3 mm Hg, respectively, which were not different from the responses of Cu-adequate rats, of 31 ± 4 mm Hg, 47 ± 4 mm Hg, and −37 ± 7 mm Hg, respectively. Subsequent postmortem analyses indicated that the Cu-deficient rats did exhibit cardiac hypertrophy and altered Cu status (Table I).

Effect of Copper-Deficient Diet on Heart Water and Metals. Some of the dissected heart tissue from rats from Experiments 1–3 and 5 was dried to constant weight and analyzed for mineral content. We failed to find a difference in the percentage dry weight of the left ventricle, right ventricle, or atria between the two dietary treatment groups (Table II). The metal concentrations were expressed on the basis of fresh weight. Mineral analyses were performed on tissues from rats perfused briefly to eliminate blood. In preliminary work we found this had a major im-

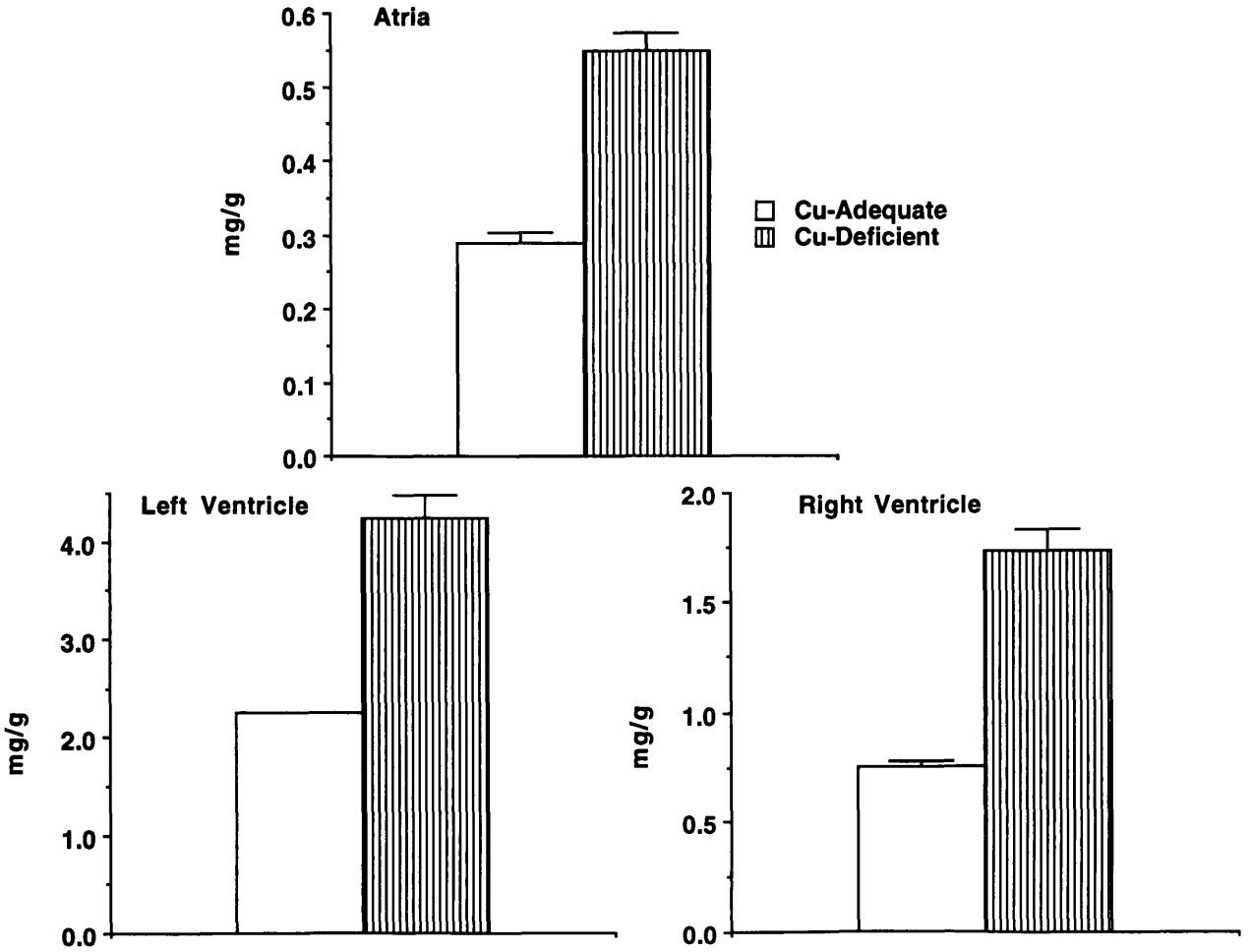

Figure 1. Left ventricular weight–to–body weight, right ventricular weight–to–body weight, and atria weight–to–body weight ratios of Cu-adequate and Cu-deficient rats. Bars, mean ± SEM (n = 11 Cu^+, n = 9 Cu^-) for rats from Experiments 1 and 2. One-way ANOVA indicated significant effects of diet for LV/BW, RV/BW, and atria/BW ($P < 0.05$). One-way ANOVA in Cu-deficient rats indicated that the hypertrophy (as a percentage of the Cu-adequate mean value) was significantly greater in the RV than the LV or atria ($P < 0.05$).

Table II. Effect of Copper-Deficient Diet on Characteristics of Cardiac Tissue from Rats

	Left ventricle		Right ventricle		Atria	
	Cu-adequate	Cu-deficient	Cu-adequate	Cu-deficient	Cu-adequate	Cu-deficient
No. rats	6	5	5	4	5	5
Dry weight (%)	22.7 ± 0.3	21.9 ± 0.6	22.5 ± 0.2	22.6 ± 0.8	16.2 ± 0.9	18.2 ± 0.7
Cu (μg/g)	5.33 ± 0.09	0.68 ± 0.03*	5.58 ± 0.11	0.75 ± 0.10*	2.86 ± 0.13	0.52 ± 0.11*
Fe (μg/g)	34.0 ± 2.2	37.2 ± 2.3	36.9 ± 1.9	37.8 ± 1.2	28.0 ± 2.6	30.5 ± 1.3
Protein (mg/g)	176 ± 2.7	162 ± 5.9*	161 ± 3.7	161 ± 2.9	105 ± 3.2	118 ± 3.9*

Note. Values are means ± SEM. Data within a heart chamber were analyzed by one-way ANOVA. Left ventricle data came from rats in Experiment 1, right ventricle data from Experiment 2, and atrial data from Experiments 3 and 5.
* A significant difference ($P < 0.05$).

pact on Fe analyses. Originally we detected an 18% lower Fe concentration in both the LV and RV of unperfused Cu-deficient rats compared with controls (data not shown). Anemia was likely responsible for this apparent difference since Fe data from perfused hearts rats indicated tissues from Cu-deficient rats exhibited no difference in Fe concentration compared with Cu-adequate rats (Table II). Analyses, however, indicated that the effect of Cu deficiency was similar and profound in the atria and ventricles regarding Cu concentration. The mean values in tissues from Cu-deficient rats were 13%, 13%, and 18% of the values from Cu-adequate rats for the left ventricle, right ventricle, and atria, respectively. Protein concentration was not altered in a consistent manner in the heart chambers from Cu-deficient rats.

To compare the effects of Cu deficiency between cardiac muscle and skeletal muscle, the right gastrocnemius was dissected from Cu-deficient ($n = 5$) and Cu-adequate ($n = 6$) rats in Experiment 1. Dietary Cu deficiency did not alter the gastrocnemius muscle-to-body weight ratio, $P > 0.05$. Mean muscle weight of Cu-deficient rats was 6.8 ± 0.4 mg/g compared with 5.7 ± 0.5 mg/g for the Cu-adequate rats. The percentages dry weight were also similar between dietary groups. The Cu-deficient rat gastrocnemius muscle percentage dry weight was 22.7 ± 0.3 compared with 21.9 ± 0.6 for muscle from Cu-adequate rats. Gastrocnemius muscle of the Cu-deficient rats had a markedly lower Cu concentration (0.23 ± 0.00 μg/g) than Cu-adequate rats (1.17 ± 0.03 μg/g), $P < 0.01$. Gastrocnemius muscle Fe concentrations of Cu-deficient and Cu-adequate rats were not different, at 9.55 ± 0.6 μg/g and 10.6 ± 0.1 μg/g, respectively.

Effect of Copper-Deficient Diet on Heart Enzymes. Whole heart homogenate enzyme activities of Cu-deficient and Cu-adequate rats from Experiment 4 were compared (Table III). Compared with the specific activities from Cu-adequate rats, there were significantly lower activities of all four cuproenzymes (CCO, DBM, PAM, and SOD) in the Cu-deficient rats. Values for Cu-deficient rats were 20%, 25%, 14%, and 42%, respectively, of the mean for Cu-adequate rats. No difference in GPX activity was observed between the two dietary groups. Heart protein concentration of Cu-deficient rats was 9% higher than that of Cu-adequate rats.

Enzyme activities were also determined for both ventricles and atria on other rats of similar Cu status from Experiments 1 and 2 (Table I). Experiment 1 utilized RV and atria and experiment two LV and atria. Values for RV and LV were similar. Representative data from experiment two (LV and atria) are illustrated (Fig. 2). Specific activities of DBM and PAM were significantly higher in atria than ventricles of both dietary treatment groups (ANOVA, $P < 0.01$) (Fig. 2). Atria of Cu-adequate rats had 185-fold higher specific activity of PAM than ventricles of Cu-adequate rats. Specific activity of SOD was also modestly higher in atria than ventricles, but only in the Cu-adequate rats (Fishers PLSD, $P < 0.01$). CCO activity was moderately higher in ventricles of both dietary groups than atria (ANOVA, $P < 0.05$). No difference in specific activity of GPX was observed between atria and ventricles.

Atria of Cu-deficient rats had lower specific activities of CCO, DBM, PAM, and SOD. Activities were 13%, 84%, 51%, and 20%, respectively, of the atria from Cu-adequate

Table III. Effect of a Copper-Deficient Diet on Heart Enzymes of Experiment Four Rats

	Copper-adequate	Copper-deficient	Cu-deficient/Cu-adequate (%)
CCO (μmol/[min · mg])	2.07 ± 0.06	0.41 ± 0.07*	20 ± 3.2
DBM (nmol/[h · mg])	0.75 ± 0.03	0.19 ± 0.03*	25 ± 4.4
GPX (μmol/[min · mg])	0.52 ± 0.02	0.44 ± 0.03	
PAM (pmol/[hr · mg])	298 ± 28.61	41.2 ± 15.3*	14 ± 5.0
SOD (units/mg)	79.2 ± 6.1	33.2 ± 2.2*	42 ± 2.8
Protein (mg/g)	133 ± 1.8	146 ± 2.1*	109 ± 1.6

Note. Values are means ± SEM ($n = 4$). Data were analyzed by one-way ANOVA.
* A significant difference ($P < 0.05$).

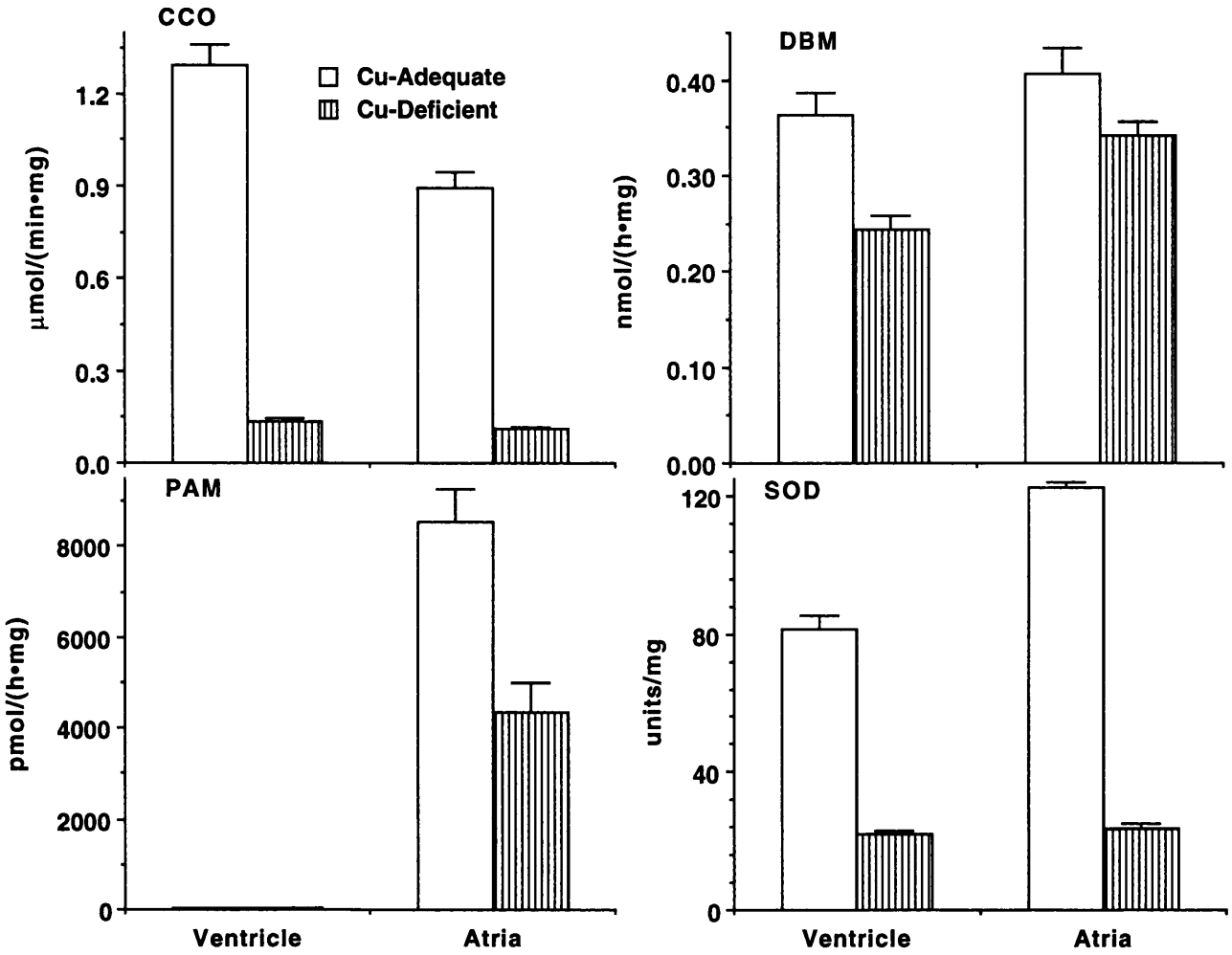

Figure 2. Enzyme specific activities of CCO, DBM, PAM, and SOD of Cu-adequate and Cu-deficient rat atria and ventricles. Bars, mean ± SEM ($n = 5$ Cu^+, $n = 4$ Cu^-) for rats from Experiments 2. One-way ANOVA indicated a significant effect of diet for all four atrial cuproenzymes ($P < 0.05$). Significant diet effects were detected for ventricular CCO, DBM, and SOD ($P < 0.05$) but not PAM ($P > 0.05$). One-way ANOVA indicated that in Cu-adequate rats atria had significantly lower CCO and higher SOD and PAM specific activities than ventricles ($P < 0.05$). No difference in activity was observed for DBM between atria and ventricles of Cu-adequate rats ($P > 0.05$).

rats. Cu deficiency also lowered the specific activities of CCO, DBM, and SOD in ventricles. Means for Cu-deficient rats were 11%, 67%, and 37%, respectively, of the values from Cu-adequate rats. Specific activity of PAM in ventricles was not altered by dietary Cu deficiency. No difference in GPX activity was observed between the two dietary treatment groups for atria or ventricles (data not shown).

Dopamine and norepinephrine were determined by HPLC to extend previous work on whole heart and confirm DBM activity studies. Cu deficiency altered the concentration of catecholamines in both atria and combined ventricles. These tissues were analyzed from a separate sample of rats (n = 4/group) from Experiment 5 (Table I). Norepinephrine concentration in atria of Cu-deficient rats (942 ± 130 ng/g) was 42% of that of Cu-adequate rats (2260 ± 75 ng/g). Atria from Cu-deficient rats contained almost 3-fold higher dopamine concentrations (343 ± 51 ng/g) than the atria of Cu-adequate rats (119 ± 13 ng/g). The same pattern was found for ventricle catecholamines. Norepinephrine concentration in the ventricles of Cu-deficient rats (475 ± 93 ng/g) was 32% of that of Cu-adequate rats (1492 ± 80 ng/g). Dopamine concentrations were 4-fold higher in ventricles of Cu deficient rats (232 ± 59 ng/g) than Cu-adequate rats (58 ± 20 ng/g).

Atrial and Ventricular Differences in Cu-Adequate Rats. Comparisons between atrial and ventricular tissue were made in Cu-adequate rats to determine if there were changes in the biochemical characteristics of the different chambers. Metal concentrations and percentages dry tissue weight are shown in Table II. The percentage dry weight was 33% lower in atria than ventricles ($P < 0.05$). A similar trend for protein concentration was observed. Cu concentrations were significantly lower (48%) in atria than ventricles ($P < 0.05$), whereas Fe concentrations were similar. Differences in enzyme specific activities between atria and ventricles are shown in Figure 2. Calculation of the ratio of mean atrial/ventricular activity for CCO, GPX, PAM, and SOD yielded 0.7, 1.2, 185, and 1.5, respectively, indicating modest regional differences for CCO, GPX, and SOD, and

a striking enrichment of PAM in the atria. No significant differences in DBM activity were found between atria and ventricles ($P > 0.05$).

Discussion

The Cu-deficient rats in this study developed signs of Cu deficiency that were consistent with previous studies (1–3, 5), including significant cardiac hypertrophy. Copper concentration was significantly lower in the livers and heart chambers of Cu-deficient rats compared with their Cu-adequate counterparts. Differences in weight ratios between dietary groups was a result of increased tissue mass in the Cu-deficient rats since the body weights were not altered by diet. The 2-fold hypertrophy observed in the Cu-deficient rats was evident in all four chambers of the heart. In our Cu-deficient rat model, the hypertrophy observed was not restricted to the left ventricle but included the right ventricle and atria. In fact, the degree of hypertrophy of the right ventricle exceeded both the left ventricle and atria. The significance of this observation will require further research. Medeiros *et al.* have reviewed the unique features of cardiac hypertrophy in the Cu-deficient rat (2). Documentation of the symmetrical hypertrophy in these studies adds to those unique features.

Although most of Cu-deficient rats in this study (except experiment four) did develop modest anemia, it has been shown previously that the cardiac hypertrophy observed preceded the development of anemia (23). We are unaware of studies which show that volume overload causes atrial hypertrophy. The hypertrophy observed was not due to edema as water content of the tissues was not altered by Cu deficiency. Some previous studies have observed a statistically significant increase in heart water content (24, 25), but the increase was relatively small and could not account for the degree of hypertrophy observed in Cu-deficient rats. Others have not observed any increase in water content of the heart (26).

We failed to find a change in MAP following Cu deficiency in the anesthetized and cannulated rats that were studied in Experiment 4; however, we and others have previously reported a decrease in MAP in post-weanling male rats following Cu deficiency (2, 8, 9, 11). Thus, elevated systolic pressure is not a mechanism that drives cardiac hypertrophy in this nutritional model. Mean arterial pressure responses of the Cu-deficient rats were equivalent to responses in control rats when challenged with Ang II, PE, and ACh, in agreement with data reported previously from our laboratory (11). While we observed that the Cu-deficient rats' global MAP responses to two vasopressors and a vasodialator were not significantly different from the Cu-adequate rats', others have reported a significant decrease in response to challenges with ACh in the cremaster muscle, an isolated vascular bed (27). It is possible that the intact rat does not respond the same way that an isolated cremaster muscle preparation does.

The biological role of Cu is believed to be expressed by its presence in a number of specific Cu-binding proteins and cuproenzymes such as CCO, CPL, DBM, LOX, PAM, and SOD (2, 12). We observed differences in the specific activities of CCO, DBM, PAM, and SOD between the atria and ventricles of normal Holtzman male rats. Atria had significantly higher specific activities of DBM, PAM, and SOD, but lower CCO than ventricles. The most pronounced regional difference observed was in the specific activity of PAM, which was nearly 200-fold higher in the atria of Cu-adequate rats than in the ventricles. It has been observed previously in both rats and sheep that PAM is enriched in the atria (28). PAM is responsible for the α-amidation of many neuropeptides, two atrial candidates are calcitonin gene-related peptide (CGRP) and parathyroid hormone-related protein (PTHrP). Both peptides have a stimulatory effect on heart rate and contractility (29, 30). Whether the reduction in the activity of PAM in the atria of Cu-deficient rats has any physiological consequence is unknown. Heart rate was not altered in these studies or in our previous work (11).

Cu deficiency lowered the specific activity of SOD in both ventricular and atrial tissue. GPX activity was not altered in the same tissues. The Fe content of the heart was not changed significantly following dietary Cu deficiency. Thus, the antioxidant capacity (SOD, GPX, and Fe) of rat ventricles and atria following Cu deficiency may not be greatly altered. Following the same nutritional paradigm, livers of Cu-deficient rats, in contrast, exhibit lower SOD, lower GPX, and higher Fe, consistent with diminished antioxidant capacity (21). There is no direct evidence that reactive oxygen species (ROS) are responsible for the development of cardiac hypertrophy. However, there are several studies involving the free radical scavengers dimethyl sulfoxide (31), *t*-butylhydroquinone (25), the iron chelator deferoxamine (32), and the antioxidant vitamin E (33). Dimethyl sulfoxide, *t*-butylhydroquinone, and deferoxamine administration attenuated cardiac hypertrophy associated with Cu deficiency, while vitamin E administration did not. While these experiments were not conclusive, they do provide evidence that ROS may somehow be involved in the cardiac hypertrophy associated with Cu deficiency. If the state of Cu deficiency is associated with enhanced ROS, then activation of certain gene transcripts dependent on ROS, mediated by NFκB, might occur and lead to abnormal growth. There is evidence that the increase in mass exists in all four chambers of the heart. It is possible that the cardiac hypertrophy of Cu-deficient rats is due to a growth factor. Since significant hypertrophy of skeletal gastrocnemius muscle was not observed, it is unlikely that a global growth factor is released.

DBM activity and catecholamine content were similar in atria and ventricles although atria had somewhat higher concentrations of both dopamine and norepinephrine than ventricles. Cu deficiency altered catecholamine concentrations of both ventricles and atria in a manner similar to earlier work on whole heart (34). Cu-deficient rats had

lower norepinephrine and higher dopamine concentrations than Cu-adequate rats suggesting that DBM activity is rate-limiting in Cu deficiency. It is unknown if the changes in catecholamine concentrations have any physiological consequences. Since norepinephrine has been shown to increase protein synthesis and promote growth (35), there has been some speculation as to whether norepinephrine can be a causative agent in the Cu deficiency induced cardiac hypertrophy. Increased turnover of norepinephrine in the heart has been shown in Cu-deficient mice (18). Seidel *et al.* reported that changes in cardiac catecholamines in Cu-deficient rats preceded development of hypertrophy, and they found no difference in turnover rates (36). Furthermore, a study on norepinephrine-induced cardiac hypertrophy reported that norepinephrine infusion caused selective LV hypertrophy, and failed to find any increase in atrial or RV mass (35). The hypertrophy observed in the Cu-deficient rats was evident in all four chambers of the heart; therefore, it is unlikely that altered DBM activity or altered catecholamine metabolism is a causative agent in the Cu-deficient rat cardiac hypertrophy.

Whether other enzyme activity changes are ultimately responsible for the pathophysiological consequences of Cu deficiency, such as cardiac hypertrophy, is not clear. Medeiros *et al.* (2) have hypothesized that limiting CCO may play a role in the hypertrophy process. Perhaps limitation in lysyl oxidase–dependent crosslinking is a causative factor in the hypertrophy. Further experiments are required to distinguish between these hypotheses. While the exact stimuli responsible for the hypertrophy of the hearts in Cu-deficient rats remain unknown, this study has provided valuable new information. Clearly, the atria of Cu-deficient rats exhibit hypertrophy similar to ventricles, and are susceptible to Cu deficiency similar to ventricles. The nearly 200-fold higher activity of PAM in the atria compared with the ventricles was the most pronounced regional difference observed. Whether alterations in the activity of PAM has any physiological consequence or is related to the cardiomyopathy observed with Cu deficiency remains unknown.

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