# Cardiac Hypertrophy in Copper-Deficient Rats Is Not Attenuated by Angiotensin II Receptor Antagonist L-158,809 (44018)

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*Abstract.* **We tested the hypothesis that cardiac hypertrophy which accompanies copper (Cu) deficiency was mediated by angiotensin II (Ang II). Thirty 17-day-old male Holtzman rats were offered a semipurified low-Cu diet (0.45 mg Cu/kg) for 39 days. Fifteen controls (Cu adequate) were given supplemental Cu (20 μg/ml) in their drinking water, the other 15 (Cu deficient) were given deionized water. Five from each dietary treatment group were chronically infused by using osmotic pumps for 4 weeks with the Ang II receptor antagonist L-158,809, and five from each group were infused with propylene glycol vehicle. Five from each group were not implanted. Blood pressure (BP) changes to injection of Ang II, phenylephrine, and acetylcholine were monitored in cannulated rats. Cu-deficient rats had higher heart weight, left ventricular (LV)/body weight (BW), right ventricular (RV)/BW, lower mean BP, and coronary vascular resistance (CVR) than Cu-adequate rats. L-158-809 did not alter Cu levels or RV/BW in either dietary group, but did lower LV/BW, CVR, and mean BP in both dietary groups. Since Ang II blockage lowered HW/BW and LV/BW in both groups, the stimulus for cardiac hypertrophy in Cu-deficient rats remains unknown.** [P.S.E.B.M. 1996, Vol 212]

Cardiac hypertrophy is one of the most consistent and striking features of copper (Cu) deficiency (1, 2). However, the signals that induce this hypertrophy are not well understood. Since arterial pressures are low in Cu-deficient rats (3–6), pressure overload *per se* does not appear to be a factor in the cardiac growth. Furthermore, although Cu deficiency can induce anemia and a potential volume overload, the anemia is not necessary for hypertrophy to occur (2, 7, 8), nor does the degree of anemia correlate with the degree of hypertrophy (1, 9). Total blood volume, assessed by tracer techniques, was not altered by Cu deficiency (10). Other signs of volume overload (high heart rates and expanded end-diastolic volumes) have not been identified in Cu deficiency.

The purpose of the present study was to test the hypothesis that activation of the renin-angiotensin system (RAS) and an increase in the peptide hormone, angiotensin II (Ang II), may be importantly involved in the cardiac hypertrophy that accompanies Cu deficiency. Recent studies indicate that Ang II may induce cardiac growth independent of influences on cardiac mechanical loading (11–13). In Cu deficiency, an elevated Ang II level might occur as part of a compensatory response to chronic low blood pressure. Previous research on the renin-angiotensin system and Cu deficiency is not conclusive. Plasma angiotensin-converting enzyme (ACE) activity was reportedly lower in Cu-deficient rats (4, 6), lung ACE was not altered (4), and kidney ACE was higher (14). Plasma renin activity was not altered by Cu deficiency (6). An increase in angiotensinogen, the precursor of Ang II, may arise as a result of increased oxygen radical production in Cu deficiency (15). Angiotensinogen gene transcription is enhanced by oxygen radical activation of the DNA

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binding nuclear regulatory factor κ β (NFκβ) (16). In light of these conflicting data, and since measurement of ACE, renin, and angiotensinogen do not necessarily predict changes in Ang II levels or efficacy, we decided to use a more direct way to test the possible causative role of Ang II in the cardiac hypertrophy of Cu deficiency by specifically blocking its receptor.

In the present study, specific blockade of the $AT_1$ Ang II receptor was achieved by chronic 4-week infusion of the potent nonpeptide $AT_1$ antagonist, L-158-809, (5,7-dimethyl-2-ethyl-3-[[2′-($^1$H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-$^3$H-imidazo [4,5-b]pyridine) (17). The Ang II antagonist L-158-809 has been shown to be 50 to 200 times more potent than DuP-753 (losartan), another nonpeptide Ang II receptor antagonist (17). The effects of this drug treatment on both Cu-adequate and Cu-deficient rats were evaluated to determine if prevention of Ang II action influenced the development of cardiac hypertrophy in the Cu-deficient rats.

## Methods and Materials

**Animal Care and Diets.** Thirty 17-day-old male weanling Holtzman rats were purchased commercially (Harlan Sprague-Dawley, Indianapolis, IN) and placed in stainless steel cages. All animals were then placed on a commercial Cu-deficient purified diet (Teklad Laboratories, Madison, WI). They were randomly divided into two dietary groups and assigned either to receive Cu in their drinking water (20 μg/ml Cu as cupric sulfate), or to receive deionized water. Later each dietary group was further divided into three treatment groups for a total of six experimental groups ($n$ = 5 each).

The purified diet followed the American Institute of Nutrition AIN-76A diet (8) 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 0.45 mg Cu/kg and 46 mg iron/kg. Food and drinking water were available *ad libitum*.

Rats were maintained on a 12-h light 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.

**Drug Treatment.** When the animals reached 28 days of age, osmotic pumps (Alzet osmotic pumps), containing either the Ang II receptor antagonist L-158, 809 (1.7 mg/ml), or vehicle (propylene glycol), were implanted subdermally in 20 of the animals. (10 from each diet group, leaving five per group as nonimplanted rats.) L-158,809 was delivered at a rate of 0.12 mg/day resulting in a range of doses that progressed from 1.0 mg/kg body wt to 0.33 mg/kg due to growth during the 4-week infusion period.

One week prior to sacrifice, all animals with osmotic pump implants were placed in metabolic cages to monitor food and water intake, and urine output. The animals were allowed to acclimate for 24 hr, prior to subsequent 24-hr measurements. The urine volume was measured, and a small aliquot was saved and stored at −70°C for catecholamine and sodium analysis. Two Cu-deficient vehicle-treated animals died prior to sacrifice.

**Blood Pressure Measurements.** Rats were sacrificed over a 5-day period when they were between 56 and 60 days of age. The implanted animals were anesthetized (50 mg/kg sodium pentobarbitol, ip) and a femoral vein and artery were exposed and cannulated. The venous cannula was filled with physiological saline solution (PSS), containing the following: 118 m$M$ NaCl, 4.7 m$M$ KCl, 1.2 m$M$ $MgSO_4$, 1.2 m$M$ $KH_2PO_4$, 25 m$M$ $NaHCO_3$, 3 m$M$ $CaCl_2$, and 0.5 m$M$ $Na_2EDTA$. The arterial cannula PSS contained 20 units heparin/ml PSS. A Gilson recorder (Model 5/6H) was used to monitor arterial pressure and heart rate. Animals were challenged with iv injections of 0.1 ml Ang II ($3 \times 10^{-7}$ $M$), phenylephrine ($1 \times 10^{-4}$ $M$), and acetylcholine ($1 \times 10^{-5}$ $M$) to assess drug efficacy and pressor responses. Injections were done in duplicate at 5-min intervals with 0.3 ml PSS given between each drug treatment so that baseline conditions were established prior to each injection. After recordings were completed, each animal was given 0.2 ml heparin (1000 U/ml) i.v. After 2 min, blood was collected in a microfuge tube, and a small aliquot was removed for hemoglobin analysis. The heart and liver were removed from each animal. Approximately 1 g of liver was saved for Cu and iron (Fe) analyses.

The nonimplanted animals were anesthetized (50 mg/kg sodium pentobarbitol, ip) and were given 0.4 ml heparin (1000 U/ml) ip 20 min prior to sacrifice. Hearts and livers were removed from each animal as per the protocol above. Blood samples were collected from the pleural cavity immediately after heart removal.

**Isolated Heart Perfusion for Assessment of Coronary Vascular Resistance.** Hearts from some of the rats in each of the above groups were quickly removed from the animal and placed in an ice-cold modified Krebs-Henseleit solution containing (in m$M$): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; $CaCl_2$, 3.0; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; glucose, 10.0; and $Na_2EDTA$, 0.5. The solution also contained heparin (1000 U/l) and insulin (10 U/l). After trimming and weighing the heart, the aortic root was then attached to a stainless steel cannula and hearts were perfused *via* the aorta (Langendorff fashion) with the solution above (18). Temperature was maintained at 27°C by water jacketing the perfusion lines. Flow was initially set by adjustment of

a peristaltic pump to produce a perfusion pressure of ~45 mm Hg as measured from a side arm at heart level in the perfusion line. A fluid-filled balloon in the left ventricle attached to a pressure transducer was used to monitor beating rates and left ventricular pressures. These measurements were made to assure that the hearts were functioning within normal limits and were not evaluated further for this study.

After approximately 20 min of washout and equilibration time, the coronary flow was reduced to a set minimal pump speed of 2.6 ± 0.1 ml/min and then ramped up in 10 steps, each 2 min in duration, to a final maximum flow of 24.8 ± 0.2 ml/min. Measurements at each step included coronary flow rate and perfusion pressure. The coronary vascular resistance (mm Hg perfusion pressure/ml/min flow/g heart tissue) was determined for each heart at a perfusion pressure of 50 mm Hg and comparisons were made between experimental groups.

**Heart Weight Determinations.** At the end of this procedure, hearts were removed from the perfusion apparatus, trimmed free of nonventricular tissue, and blotted lightly. The right ventricular free wall was cut away from the left ventricle and each ventricular segment was weighed separately. Combined right and left ventricular weights were used to determine the coronary flow rate/g ventricular tissue.

**Chemical Analyses.** Liver, right ventricle, 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 (8). Urine was diluted 2500-fold in deionized water, and analyzed for sodium (Na) by flame atomic absorption spectroscopy. Urinary creatinine was determined spectrophotometrically using an alkaline-picric acid procedure (kit no. 555A; Sigma Chemical Co., St. Louis, MO) (19). Urinary and left ventricle catecholamines were measured as described previously by HPLC with electrochemical detection (20). Hemoglobin (Hb) was determined spectrophotometrically as metcyanhemoglobin (21). Ceruloplasmin activity was determined by a modification of the method described by Lehman *et al.* (22), measuring the ability of plasma to oxidize *o*-dianisidine.

**Data Analysis.** Data were analyzed by factorial analysis of variance (2 × 2 ANOVA), $\alpha = 0.05$, using a personal computer and statistical software (Statview 4.0; Abacus Concepts, Berkeley, CA). Data are reported as mean ± SEM. Any significant interactive terms were further analyzed by Fisher's PLSD test to determine statistical significance ($P < 0.05$). Variance equality was evaluated by the $F$ test. For any comparisons between groups with unequal variances the data were first transformed (natural logarithm) prior to further ANOVA analysis.

## Results

**Effect of Copper Deficient Diet.** Compared with rats drinking Cu-supplemented water, 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 (Table I). This difference in ratio was a result of an absolute increase in heart mass since body weights of the two diet groups were not different. There was hypertrophy of both ventricles. For example, the left ventricle/BW (mean ± SEM) was 4.48 ± 0.27 mg/g for Cu-deficient nonimplanted rats compared with 2.41 ± 0.07 for Cu-adequate controls ($P < 0.01$). For the right ventricle/BW Cu-deficient rats averaged 1.42 ± 0.12 mg/g compared with 0.71 ± 0.04 mg/g for Cu-adequate rats ($P < 0.01$).

The Cu-deficient rats had lower liver and right ventricular Cu concentrations, hemoglobin levels, and left ventricle norepinephrine (NE) concentrations than their Cu-adequate counterparts whereas liver Fe and left ventricular dopamine (DA) concentrations were higher in the Cu-deficient rats compared with the Cu-

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

| | BW (g) | HW/BW (mg/g) | Hb (g/100 ml) | Liver Cu (μg/g) | Liver Fe (μg/g) | RV Cu (μg/g) | RV Fe (μg/g) | LV NE (ng/g) | LVDA[a] (ng/g) |
|---|---|---|---|---|---|---|---|---|---|
| +Cu N ($n$ = 4) | 348 ± 5 | 3.12 ± 0.07 | 15.3 ± 0.68 | 4.53 ± 0.16 | 77.6 ± 5.9 | 5.58 ± 0.20 | 53.2 ± 5.8 | 658 ± 47 | 7.6 ± 2.0 |
| −Cu N ($n$ = 5) | 343 ± 11 | 5.91 ± 0.35 | 13.0 ± 0.15 | 1.04 ± 0.11 | 144 ± 7.1 | 0.91 ± 0.10 | 44.1 ± 2.2 | 261 ± 19 | 35.8 ± 8.5 |
| +Cu V ($n$ = 5) | 348 ± 14 | 3.34 ± 0.17 | 16.2 ± 0.49 | 4.71 ± 0.51 | 66.7 ± 4.2 | 5.50 ± 0.27 | 51.1 ± 8.9 | 643 ± 27 | 6.0 ± 0.77 |
| −Cu V ($n$ = 3) | 326 ± 11 | 6.28 ± 0.40 | 13.3 ± 0.32 | 1.19 ± 0.10 | 133 ± 17.2 | 1.17 ± 0.26 | 47.4 ± 2.8 | 191 ± 30 | 20.1 ± 2.8 |
| ANOVA | | | | | | | | | |
| Treatment | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Diet | NS | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | NS | 0.0001 | 0.0001 |
| Treatment*diet | NS | NS | NS | NS | NS | NS | NS | NS | NS |

*Note.* Values expressed as mean ± SEM. Data were analyzed by Factorial ANOVA (2 × 2). NS, $P > 0.05$; +Cu N, copper-adequate nonimplanted; −Cu N, copper-deficient nonimplanted; +Cu V, copper-adequate vehicle-implanted; −Cu V, copper-deficient vehicle-implanted; RV, right ventricle; LV, left ventricle; NE, norepinephrine; DA, dopamine.
[a] ANOVA was performed on transformed data (ln) because of variance inequality among groups.

adequate rats. The difference in Cu status between the dietary groups was also reflected by ceruloplasmin activity which was nearly eliminated in the Cu-deficient rats (Cu-adequate, 170 ± 39 units/l [mean ± SEM], $n = 4$; Cu-deficient, 0.01 ± 0.10, $n = 5$). Right ventricle iron levels were not different between the groups.

**Effect of Implantation.** The effect of the presence of the osmotic pump implant and the infusion of the vehicle, proplyene glycol, upon the Cu-adequate and Cu-deficient rats was evaluated by comparing vehicle-treated rats with nonimplanted rats in each dietary group (Table I). Within each dietary group, the pump implant and vehicle infusion had no significant effect on any of the measured variables (no significant treatment effects by ANOVA).

**Effect of Angiotensin II Receptor Antagonist.** Figure 1 compares the heart weight–to–body weight ratios of the vehicle- and L-158,809–treated rats in both the Cu-adequate and Cu-deficient groups. The heart weight–to–body weight ratios were significantly decreased by drug treatment in both dietary groups. This decrease reflects a reduction in the absolute heart weight that was a result of a decrease in the left ventricular mass. Left ventricle–to–body weight ratios of the drug treated rats were significantly lower than those of the vehicle-treated rats in both dietary groups, whereas there was no significant influence of drug treatment on the right ventricle–to–body weight ratio in either dietary group.

A comparison of the characteristics of the vehicle-treated rats with those of the L-158,809–treated rats is indicated in Table II. As can be seen, treatment with the Ang II receptor antagonist did not influence overall growth of the rats in either group since body weights were similar at the time of sacrifice. Drug treatment lowered hemoglobin concentration in the Cu-adequate group but did not evoke any further lowering of hemoglobin in the Cu-deficient group (Fisher's PLSD). Characteristics that were not influenced significantly by treatment with L-158,809 included liver Cu and Fe concentrations, right ventricular Cu and Fe concentrations, and left ventricular NE and DA. Thus, treatment with L-158,809 did not alter the Cu or Fe status or catecholamine levels in either of the treatment groups.

The influence of diet and drug treatment on urinary variables is indicated in Table III. The Cu-deficient rats had increased levels of urinary NE/creatinine (Cr) compared with Cu-adequate rats. However, dietary Cu deficiency did not alter urine volume, DA/Cr, or Na/Cr output. Drug treatment did not affect any of the measured urinary variables.

**Effect of Dietary and Drug Treatments on Arterial Pressures.** The effects of Cu deficiency and Ang II receptor antagonism on heart rates and blood pressures of anesthetized rats are shown in Figure 2.

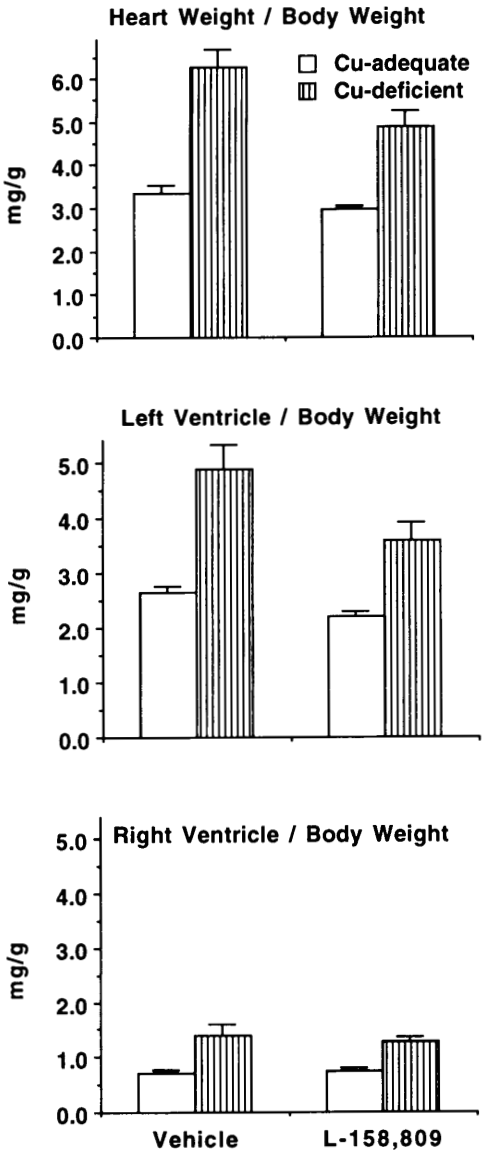


**Figure 1.** Heart weight–to–body weight, left ventricular weight–to–body weight, and right ventricular weight–to–body weight ratios of Cu-adequate and Cu-deficient rats receiving a chronic 4-week infusion of either an Ang II receptor antagonist (L-158, 809) or vehicle (propylene glycol). Factorial ANOVA indicated significant main effects of diet for HW/BW, LV/BW, and RV/BW ($P = 0.0001$), and significant drug effects for HW/BW ($P = 0.007$) and for LV/BW ($P = 0.003$) but not for RV/BW ($P > 0.05$). No significant interaction terms were detected, $P > 0.05$.

ANOVA analysis of the data indicated that, compared with Cu-adequate rats, Cu-deficient rats had significantly lower mean arterial pressure. L-158,809 treatment significantly lowered mean arterial pressure in both Cu-deficient rats and in Cu-adequate rats compared with vehicle-implanted rats. Heart rates were significantly increased by L-158,809 in both Cu-adequate and Cu-deficient rats.

The effects of Cu-deficiency and Ang II receptor antagonism on the pressure responses to challenges with vasoactive agents are shown in Figure 3. Bolus injections of Ang II and phenylephrine resulted in tran-

**Table II.** Effect of an Ang II Receptor Antagonist and a Copper-Deficient Diet on Characteristics of Experimental Rats

| | BW (g) | Hb (g/100 ml) | Liver Cu (μg/g) | Liver Fe (μg/g) | RV Cu (μg/g) | RV Fe (μg/g) | LV NE (ng/g) | LVDA[a] (ng/g) |
|---|---|---|---|---|---|---|---|---|
| +Cu V ($n$ = 5) | 348 ± 14 | 16.2 ± 0.49[b] | 4.71 ± 0.51 | 66.7 ± 4.2 | 5.50 ± 0.27 | 51.1 ± 8.9 | 643 ± 27.4 | 6.0 ± 0.77 |
| −Cu V ($n$ = 3) | 326 ± 11 | 13.3 ± 0.32[c] | 1.19 ± 0.10 | 133 ± 17.2 | 1.17 ± 0.26 | 47.4 ± 2.8 | 191 ± 29.9 | 20.1 ± 2.77 |
| +Cu D ($N$ = 5) | 368 ± 16 | 14.1 ± 0.31[c] | 5.30 ± 0.34 | 68.6 ± 7.0 | 4.95 ± 0.45 | 41.8 ± 3.7 | 729 ± 103 | 11.9 ± 2.69 |
| −Cu D ($n$ = 5) | 311 ± 9 | 14.0 ± 0.63[c] | 1.55 ± 0.34 | 138 ± 12.3 | 0.84 ± 0.10 | 41.7 ± 3.6 | 439 ± 112 | 28.0 ± 10.3 |
| ANOVA | | | | | | | | |
| Drug | NS | NS | NS | NS | NS | NS | NS | NS |
| Diet | 0.01 | 0.009 | 0.0001 | 0.0001 | 0.0001 | NS | 0.001 | 0.003 |
| Drug × diet | NS | 0.02 | NS | NS | NS | NS | NS | NS |

*Note.* Values are expressed as mean ± SEM. Data were analyzed by Factorial ANOVA (2 × 2). NS, $P < 0.05$; +Cu V, copper-adequate vehicle-implanted; −Cu V, copper-deficient vehicle-implanted; +Cu D, copper-adequate L-158,809–implanted; −Cu D, copper-deficient L-158,809–implanted; RV, right ventricle; LV, left ventricle; NE, norepinephrine; DA, dopamine. Significant interactions were further analyzed by Fisher's PLSD.

[a] ANOVA was performed on transformed data (ln) because of variance inequality among groups.

[b,c] Means not sharing a common superscript letter were significantly different ($P < 0.05$).

sient increases in arterial pressure, whereas injections of acetylcholine resulted in a transient fall in arterial pressure. In both the Cu-adequate and Cu-deficient vehicle-treated groups, Ang II injections produced substantial increases in blood pressure, while these responses were significantly blunted in the groups treated with L-158,809. This verifies the efficacy of the chronic drug treatment to block the physiological pressor response to Ang II.

Blood pressure responses to the α-agonist, phenylephrine, were equivalent in all four implanted groups with a mean rise in pressure of ~50 mm Hg. Thus, the presence of the Ang II receptor antagonist did not significantly influence pressor responses to α adrenergic receptor challenge. Challenge with acetylcholine transiently lowered the mean arterial pressure in all groups; however, the pressure reduction was attenuated in the rats treated with the Ang II antagonist.

**Effect of Dietary and Drug Treatments on Coronary Vascular Resistance of Isolated Perfused Hearts.** The effects of Cu deficiency and Ang II receptor antagonism on the coronary vascular resistance of the isolated perfused rat hearts perfused at 50 mm Hg are indicated in Figure 4. Cu deficiency was associated with a decrease in coronary vascular resistance. Treatment of the rats with L-158,809 resulted in a significant reduction in coronary vascular resistance in the Cu-adequate group so that no difference between dietary groups existed.

## Discussion

As expected from previous studies (1–3, 7–10), the Cu-deficient rats of this study developed significant cardiac hypertrophy. In both dietary treatment groups, infusion with the Ang II receptor antagonist reduced the cardiac heart weight–to–body weight ratio, with no influence on body weight. Therefore, L-158,809 did not specifically attenuate the hypertrophy associated with dietary Cu deficiency. The drug-induced decrease in cardiac hypertrophy was also accompanied by a lowering of the arterial pressure. Thus, the cardiac hypertrophy that develops as a re-

**Table III.** Effect of an Ang II Receptor Antagonist and a Copper-Deficient Diet on Urinary Characteristics of Experimental Rats

| | Urine volume (ml/24 hr) | Ur NE (ng/mg Cr) | Ur DA (ng/mg Cr) | Ur Na (mg/mg Cr) |
|---|---|---|---|---|
| +Cu V ($n$ = 5) | 14.4 ± 3.1 | 87.4 ± 8.8 | 169 ± 25.5 | 1.95 ± 0.22 |
| −Cu V ($n$ = 3) | 10.8 ± 3.5 | 112 ± 18.5 | 202 ± 14.4 | 1.98 ± 0.13 |
| +Cu D $n$ = 5) | 11.2 ± 1.7 | 104 ± 16.6 | 138 ± 21.7 | 2.01 ± 0.51 |
| −Cu D $n$ = 5) | 13.1 ± 3.1 | 149 ± 15.6 | 152 ± 24.7 | 2.37 ± 0.36 |
| ANOVA | | | | |
| Drug | NS | NS | NS | NS |
| Diet | NS | 0.04 | NS | NS |
| Drug × diet | NS | NS | NS | NS |

*Note.* Values are means ± SEM. Data were analyzed by Factorial ANOVA (2 × 2). NS, $P > 0.05$; +Cu V, copper-adequate vehicle-implanted; −Cu V, copper-deficient vehicle-implanted; +Cu D, copper-adequate L-158,809–implanted; −Cu D, copper-deficient L-158,809–implanted; Ur, urinary; Cr, creatinine; NE, norepinephrine; DA, dopamine.

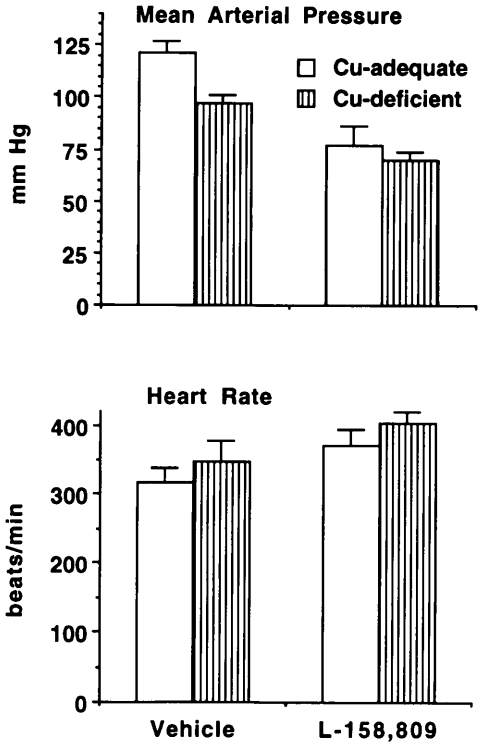


**Figure 2.** Mean arterial pressure (MAP) and heart rates (HR) of Cu-adequate and Cu-deficient rats receiving a chronic 4-week infusion of either Ang II receptor antagonist (L-158,809) or vehicle (propylene glycol). Factorial ANOVA indicated significant main effects of diet for MAP ($P = 0.04$) but not HR ($P > 0.05$), and significant drug effects for MAP ($P = 0.0002$) and for HR ($P = 0.03$). No significant interaction terms were detected; $P > 0.05$.

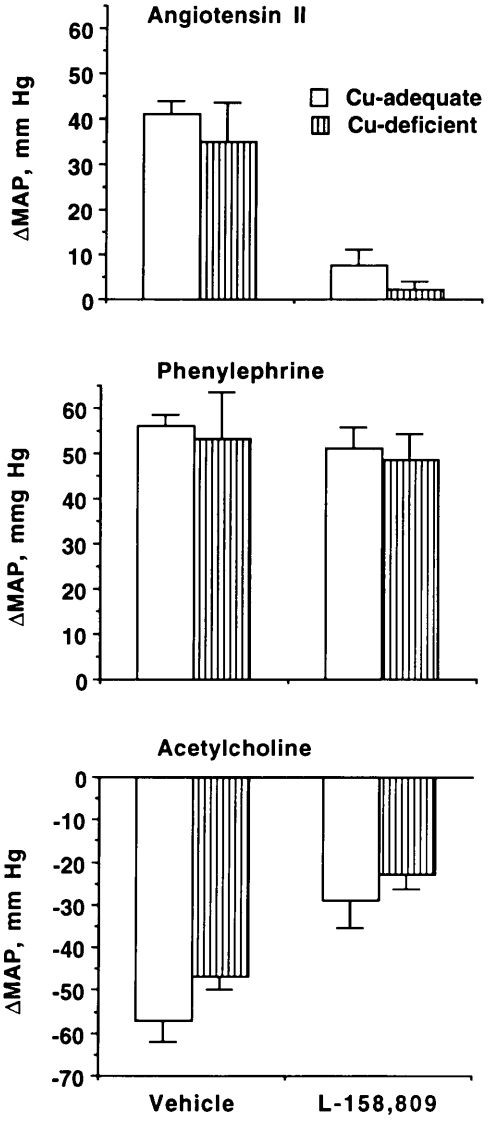


**Figure 3.** Mean blood pressure changes of Cu-adequate and Cu-deficient rats receiving a chronic 4-week infusion of either an Ang II receptor antagonist (L-158,809) or vehicle (propylene glycol) in response to challenges with bolus injections of Ang II, phenylephrine, and acetylcholine. Factorial ANOVA indicated no significant diet effects for any of the three pressors ($P > 0.05$), but significant drug effects were detected for Ang II ($P = 0.0001$) and for acetylcholine ($P = 0.0002$) but not phenylephrine ($P > 0.05$). No significant interaction terms were detected; $P > 0.05$.

sult of Cu deficiency is not necessarily due to an Ang II–mediated mechanism acting directly on the heart. It is likely that the reduction in cardiac hypertrophy that accompanied Ang II receptor antagonism may reflect the drug-induced decrease in cardiac afterload.

The hypotension that was observed in the Cu-deficient rats in this study has been observed previously (3–6). It is interesting that the left ventricles are so enlarged in these rats that are somewhat hypotensive. The fact that treatment with the Ang II receptor antagonist was able to reduce further the arterial pressure in both the Cu-deficient and Cu-adequate rats indicates that the renin-angiotensin system may be playing an important role in arterial pressure maintenance in this post-weanling male Holtzman rat model. That only the left, and not the right, ventricular hypertrophy was influenced by the drug treatment further suggests that Ang II does not have a direct cardiac growth effect but rather indirectly influences cardiac growth by altering arterial pressure. Indeed, the differences between the dietary groups in coronary vascular resistance and the changes evoked by the drug treatment closely parallel the differences and drug-induced changes in mean arterial pressure. The increased heart rate observed with Ang II receptor blockade is most likely a consequence of the lowered MAP as a compensatory mechanism to maintain cardiac output.

Results of these experiments clearly show that the dietary paradigm produced rats that exhibit characteristics of Cu deficiency consistent with characteristics observed by others, including decreased liver Cu concentration, elevated liver iron concentration, and decreased plasma ceruloplasmin activity. Treatment with the Ang II receptor antagonist L-158,809 did not alter any of these indices of Cu status, validating any potential changes in the attenuation of cardiac hyper-

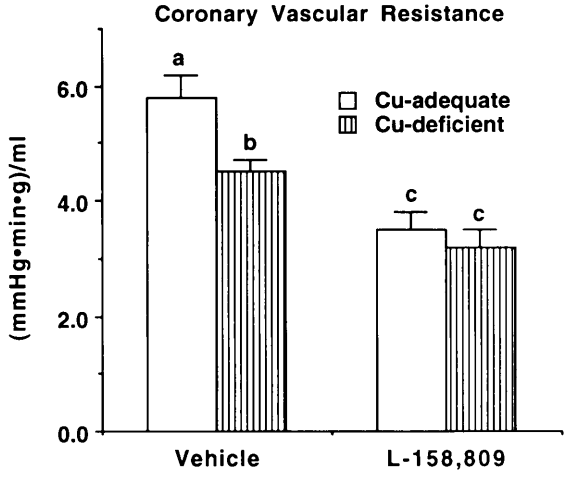


**Figure 4.** Coronary vascular resistance determined at a perfusion pressure of 50 mm Hg of isolated perfused hearts of rats fed either a Cu-adequate or a Cu-deficient diet and receiving a chronic 4-week infusion of either an Ang II receptor antagonist (L-158,809) or the vehicle (propylene glycol). Factorial ANOVA indicated a significant diet ($P = 0.02$) and a significant drug effect ($P = 0.0001$), and a significant interaction ($P = 0.04$). Bars with different superscript letters were significantly different ($P < 0.05$, Fisher's PLSD test).

trophy as being direct cardiovascular effects rather than secondary effects due to enhanced Cu mobilization.

Many of the biological roles of Cu are believed to be expressed by its presence in a number of specific Cu-binding proteins and cuproenzymes. Examples of these cuproenzymes include ceruloplasmin, superoxide dismutase, cytochrome c oxidase, dopamine β-monooxygenase, peptidylglycine α-amidating monooxygenase, and lysyl oxidase (2, 15). Cu deficiency leads to altered activity levels of many cuproenzymes in various tissues. To what extent these enzyme changes are responsible for the pathophysiological consequences of Cu deficiency, such as cardiac hypertrophy, is not clear. Several hypotheses have been proposed previously to explain the cardiac hypertrophy that accompanies Cu deficiency.

One hypothesis is that the hypertrophy observed in this model is the result of increased pumping requirements on the heart; a type of volume overload such that cardiac output must be chronically elevated to balance a chronically decreased peripheral resistance. This decrease in resistance could be due to altered structural characteristics of the vascular wall resulting from defective cross-linking in collagen and elastin because of reductions in the activity of the Cu-dependent enzyme, lysyl oxidase. However, studies by others have shown that treatment with the lysyl oxidase inhibitor β-amino proprionitrile (BAPN) did not result in ventricular hypertrophy, though collagen content and myocardial stiffness decreased significantly (23). Results obtained in the current experiments with the isolated perfused heart support the hypothesis that lysyl oxidase activity is impaired and that the tone of the vascular system is impacted by dietary Cu deficiency. Further experiments along these lines are required to confirm this supposition.

A second hypothesis is based on the observation that severe Cu deficiency is often associated with significant anemia which is known to be a strong hypertrophic stimulus (3, 7, 10, 24). However, the prerequisite for anemia to elicit cardiac hypertrophy in Cu deficiency has been questioned previously (1, 2, 7). Indeed, it has been shown that cardiac hypertrophy preceded the characteristic anemia in Cu-deficient rats (25). Clearly, the Cu-deficient model of hypertrophy must be different than the volume overload induced hypertrophy elicited by severe anemia of Fe deficiency (1, 9). Cu-deficient animals in this study were characterized by only a mild anemia which was not affected by drug treatment. These findings make it unlikely that anemia played a major role in the development of cardiac hypertrophy.

A third hypothesis involves the mitochondrial cuproenzyme cytochrome c oxidase. Limitation in cytochrome c oxidase activity, whether caused by dietary Cu deficiency or genetic abberations, is accompanied by enlarged and abnormal mitochondria. In hearts of Cu-deficient rats this increased mitochondrial volume density is very marked (1, 2, 9, 25). Medeiros *et al.* (2) speculate that alteration in cytochrome c oxidase results in increased mitochondrial volume density and is part of the overall heart failure in Cu-deficient rats. Genetic disorders of mitochondrial oxidative phosphorylation are often characterized by cardiomyopathy that is usually hypertrophic with diminished contractile function (26).

A fourth hypothesis concerns the involvement of reactive oxygen species (ROS) and development of cardiac hypertrophy. There are several studies involving the free radical scavengers dimethyl sulfoxide (27), and *t*-butylhydroquinone (28), the iron chelator deferoxamine (29), and the antioxidant vitamin E (30). Dimethyl sulfoxide, *t*-butyl-hydroquinone, and deferoxamine administration did attenuate the cardiac hypertrophy associated with Cu deficiency, whereas vitamin E 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 NFκβ, might occur and lead to abnormal growth.

A fifth hypothesis is that the growth stimulus to the cardiac tissue in the Cu-deficient rat is due to aberrant levels of the trophic factor norepinephrine (NE) (31). The present study in rats and a previous one using a similar paradigm observed an elevated output of

urinary NE (19). A similar study in Cu-deficient mice suggested that the elevation was due to enhanced NE turnover even under limited biosynthesis because of reduced activity of dopamine β-monooxygenase, a Cu-dependent enzyme (20). However, previous work by Seidel and Failla indicated that there was no enhanced turnover of NE in the Cu-deficient rat heart (32). We suggest that the elevated urinary NE could be a result of the failing heart in this experimental model rather than the cause of cardiac hypertrophy. An experiment involving reserpine, which depletes catecholamine stores, was inconclusive because reserpine interfered with normal growth of the rats, and altered Cu status in the heart (33). Saari *et al.* have shown that restricted food intake alone results in the attenuation of the cardiac hypertrophy (34). Therefore, it is difficult to conclude whether reserpine was acting as a catecholamine antagonist or as a growth inhibitor. Thus, the role of altered catecholamine status upon development of cardiac hypertrophy is still not fully resolved.

A recent sixth hypothesis suggests that the cardiac hypertrophy is related to the degree of nonenzymatic glycation which accompanies Cu deficiency (35). Reduction in protein glycation by aminoguanidine attenuated the cardiac hypertrophy of Cu deficiency. In a second study, it was shown that hemoglobin glycation was correlated, positively, to the heart weight of rats (36).

A number of the above hypotheses may be interrelated since, in addition to dietary Cu, dietary carbohydrate source, dietary iron, level of food intake can influence the degree of anemia, glycation, peroxidation, and cardiac hypertrophy (36). The stimulus for the hypertrophied hearts in Cu-deficient rats remains unknown. In the current experiment, we have been able to rule out the hypothesis that Ang II, acting as a growth factor, is the primary stimulus for the cardiac hypertrophy induced by Cu deficiency in rats.

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