Dietary Iron Deficiency Results in Cardiac Eccentric Hypertrophy in Rats (44306)

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Abstract. **This study reports the presence of eccentric cardiac hypertrophy in rats made anemic by feeding an iron-deficient diet. Male weanling Sprague-Dawley rats were provided free access to diets either adequate ($n = 9$) or inadequate in iron ($n = 8$) for a period of 7 weeks from weanling or until 10 weeks of age. At that time, blood was obtained for hematocrit and hemoglobin determination, and liver and hearts were collected for further analysis. Liver non-heme iron levels confirmed that the rats were iron-deficient, and the very low hematocrit and hemoglobin values revealed the presence of physiological anemia. Despite the lighter body weights in the iron-deficient rats, this group had greater absolute heart weights and heart:body weight, clearly demonstrating the presence of cardiac hypertrophy. Iron-deficient rats had elevated heart rates but lower norepinephrine levels than control rats. Sagittal sectioning of all hearts allowed for the measurements of the wall thicknesses, lumen volume, and width dimensions. Results revealed significantly greater left ventricular lesser diameter, apical thickness, and left ventricular volume in hearts from iron-deficient rats compared to iron-adequate rats. The hypertrophy pattern present in iron-deficiency anemia is in contrast to other nutritional models of hypertrophy, such as copper-deficiency, where a concentric hypertrophy occurs both in the presence and absence of anemia.** [P.S.E.B.M. 1998, Vol 218]

Cardiac hypertrophy is regarded as an adaptation to allow the heart to compensate for overload conditions (1). While this is an adaptation, it is not normal, and heart failure will occur eventually if the overload conditions are not corrected. Three phases of hypertrophy have been described: 1) compensatory hypertrophy following acute and chronic overload; 2) stable hyperfunction with decreased cardiac reserve capacity; and 3) overcompensation or hyperadaptation leading to heart failure (2). Cardiac function may fail due to inadequate venous emptying or reduced venous ejection fraction against arterial pressure (1). Hypertension and aortic stenosis are illustrations of conditions leading to this failure, and the type of hypertrophy that often results here is termed concentric hypertrophy where there is increased ventricular wall thickness. On the other hand, eccentric hypertrophy is characterized by increased left ventricular chamber volume and a relatively thin wall. Volume overload is observed under such conditions as aortic insufficiency, ischemia, mitral valve stenosis, and anemia.

Nutritional deficiencies can lead to hypertrophy. An early known example is the enlarged heart associated with beri-beri or thiamine deficiency. Recently, interests in the role of the trace elements upon heart disease have received some notice and include both essential and toxic elements as copper, selenium, iron, and cadmium, to name a few. Copper and iron deficiencies can lead to cardiac hypertrophy. Petering *et al.* (3) and Stemmer *et al.* (4) reported histological pathology in the hypertrophied hearts of copper-deficient rats. Goodman *et al.* (5) was one of the first reporting the ultrastructural characteristics of hearts made hypertrophic by feeding rodents diets deficient in either copper or iron. In both situations, they reported that mitochondria increased significantly, but the copper-deficient rats had significantly greater heart enlargement as compared to the iron deficient rats. Both groups of rats were made anemic by the restriction of the dietary trace elements. In pigs, Shields *et al.* (6) reported that cardiac hypertrophy in iron-deficient

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pigs was not as great as in copper-deficient pigs despite a greater level of anemia in the iron-deficient pigs. The type of hypertrophy present was not reported. Additionally, some studies have demonstrated that the hypertrophy of copper deficiency has anemia occurring following the presence of the heart enlargement (5, 7), and some have reported cardiac hypertrophy in the absence of anemia in copper-deficiency (8). The issue is further clouded by the fact that rats fed a copper-deficient diet develop a concentric hypertrophy, which is inconsistent with the volume overload of anemia (9). However, the large amounts of mitochondria observed in copper-deficient hearts could cause the heart enlargement in a concentric fashion since they are laid down in parallel arrays along the fibers. Surprisingly, there is an apparent paucity of experimental studies with iron-deficiency induced anemia and the type of hypertrophy in rodent models.

This paper reports on the morphological character and measures of rats that were fed diets deficient in iron and that have become anemic. We predicted that rats fed iron-deficient diets should develop an eccentric hypertrophy where the chamber sizes become enlarged. Additional data on cardiac function such as cardiac rate and blood pressure are provided.

Methods and Materials

Diet and Experimental Design. Male weanling Sprague-Dawley rats were obtained at 21 days of age from a commercial supplier (Harlan-Sprague Dawley, Indianapolis, IN) and housed individually in stainless steel cages with a light:dark cycle (0600–1800 hr) at a room temperature of 24 ± 2°C (mean ± range). Rats were fed nutritionally complete AIN-76 diets (10) with inadequate amounts of iron (3–5 mg Fe/kg diet, $n = 8$) or adequate amounts of iron (35 mg Fe/kg diet added as ferrous sulfate, $n = 9$). Iron content of the diets was verified by analysis *via* flame atomic absorption spectrophotometry. Diets were adequate in all other nutrients as defined by the AIN-76 formulation (10). Corn starch rather than sucrose was the dietary carbohydrate source as recommended by the American Institute of Nutrition for studies in which sucrose may affect the dependent variable (11). Non-nutritive cellulose was deleted from both diets because of its variable iron content. Rats were given free access to feed and distilled deionized water. All procedures were approved by the Pennsylvania State University Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

Tissues Collected and Variables Determined. Animals were allowed to consume their respective diets for 7 weeks or until they were 10 weeks of age. Some rats had blood pressure recorded by cannulating the left carotid artery and the right jugular vein to a transducer as described below. At the end of the study, rats were sacrificed by decapitation. Blood was either collected from the trunk or taken from catheters in those rats that had them. The liver was removed and frozen at –20°C until subsequent analysis described below. Hearts were rinsed briefly in saline, placed in 10% formalin, and shipped to The Ohio State University for morphometric analysis.

Hematocrit was determined with a micro hematocrit centrifuge and reader. Hemoglobin was determined as cyanomethemoglobin using Drabkin's reagent. Liver was measured for nonheme iron after acid hydrolysis as previously described (12) except for the use of ferrozine in place of bathophenanthroline sulfonate.

Plasma transferrin saturation was determined as described previously (11) where plasma total iron concentration and total iron binding capacity using a Ferrochem II analyzer (ESA, Bedford, MA) were determined followed by calculation of the percent saturation.

Heart norepinephrine was determined by homogenizing the heart tissue (1:10 wt/v) in cold 400 mmol/l perchloric acid with 5 mmol/l reduced glutathione using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY). After centrifugation at 1350*g* for 15 min, the supernatant containing the norepinephrine was adsorbed onto 50 mg of acid-washed alumina, at pH = 8.6, and eluted from the alumina with 100 mmol/l perchloric acid. Norepinephrine was quantified using HPLC with electrochemical detection on a reverse-phase column (250 × 4 mm, Biophase II, Bioanalytical Systems, West Lafayette, IN) at +650 mV vs Ag-AgCl reference electrode (13–15).

Blood Pressure and Heart Rate Determination. Three days prior to measurements, PE20 catheters were surgically placed according to the methods as described by Borel *et al.* (16). Surgery was performed using ketamine hydrochloride (100 mg/ml, 0.75 μl/g BW) and Rompun (20 mg/ml, 38 μl/g BW) as anesthesia injected into the hindlimb of each rat. Animals were allowed to recover for 3 days, and weight gain was monitored. All rats were gaining weight at the time of measurements. Nonfasted rats were removed from their home cage and placed into another normal sized cage in the laboratory. The arterial catheter was connected to a calibrated Icomatic GM751 pressure transducer and polygraph (Harvard Apparatus, Edenbridge, KY) for recording the resting arterial pressure and HR. These recordings were made 15–30 min after the initial handling of the animal. Several measures were obtained and averaged for each rat. The criteria used were less than a 10% difference in measurements from the preceding 2-min period. In general, the animals kept the arterial pressure and HR to less than 5% variation for a 10–15-min interval.

Morphometric Analysis of Hearts. Formalin fixed hearts were cut sagittally to view the ventricle walls and chambers and corresponding atria from a ventral view. Using metric calipers and an Agfa magnifying loupe (Leverkusen, Germany) at 8× magnification, measurements were obtained to the nearest 0.2 mm. The following dimensions were determined for all hearts: right and left ventricular free wall thickness, right and left ventricular lesser diameter, interventricular septum thickness, median length,

Table I. Biochemical Parameters and Organ Weights of Rats Fed Control or Iron-Deficient Diets

Variable	Control ($n = 9$)	Iron-deficient ($n = 8$)	P
Hemoglobin (g/100 ml)	14.2 ± 5.64	5.8 ± 1.37	<.001
Hematocrit	45.5 ± 4.24	23.3 ± 6.02	<.0001
Transferrin saturation (%)	28.0 ± 2.0	5.0 ± 1.2	<0.01
Body weight (g)	346 ± 42.7	200 ± 54.1	<.0001
Heart weight (g)	1.00 ± .109	1.17 ± .116	<0.01
Heart:Body weight ($\times 10^{-3}$)	2.92 ± .335	6.23 ± .556	<.0001
Heart:Brain weight	0.52 ± 0.58	0.63 ± .046	<.001
Liver non-heme iron (μmol/g)	129.4 ± 18.0	33.6 ± 4.5	<.0001

Note. Means ± SD.

apical thickness, and heart length and width. Left ventricular circumferential volume (cm^3), left ventricular volume (cm^3), and left ventricular wall volume (cm^3) were calculated as described previously (17, 18). The left ventricular volume assumes symmetry with respect to the major and minor axes that describe an ellipse. The left ventricular major length and the left ventricular lesser diameter represent the major and minor ellipsoid axes, respectively. The left ventricular major length was defined as the distance perpendicular from a line drawn joining cranial aspects of mitral and tricuspid valves to the distal aspect of the left ventricular lumen. Left ventricular lesser diameter was defined as the width of the left ventricular lumen, measured perpendicular to the line describing the left ventricular major length, at a point 2/3 the distance from apex to left ventricular base. The left ventricular chamber volume was calculated as: $(4/3)\pi$ (left ventricular major length/2)(left ventricular lesser diameter/2)^2.

The apical dimension was determined as a caudal extension of the left ventricular major length axis, measured from the apical termination of the lumen to the epicardial apical surface. This made possible the calculation of the left ventricular circumferential volume as: $(4/3)\pi$ {(left ventricular major length + apical dimension/2}{(interventricular septum + left ventricular lesser diameter + left ventricular free wall)/2}^{2}.

The left ventricular wall volume was calculated as: $(4/3)\pi$\{(left ventricular major length + apical dimension)/2\}\{(interventricular septum + left ventricular lesser diameter + left ventricular free wall)/2\}^{2} – left ventricular volume.

After the hearts were photographed, the left ventricle free wall was dissected away and weighed.

Statistical Analysis. Mean differences in variables by dietary iron treatment were determined by Student's t test. The level of statistical significance was set at a P of 0.05 or less *a priori.*

Results

Descriptive indicators of growth and viability indicated differences by dietary treatments. Iron-deficient rats were significantly ($P \leq 0.0001$) lighter in body weight (Table I) and were anemic as determined by both hemoglobin and hematocrit levels ($P \leq 0.001$ and 0.0001, respectively). However, heart ($P \leq 0.01$) and heart:body weight ($P \leq 0.0001$) were greater in iron-deficient rats as compared to controls. Another measure of cardiac hypertrophy, heart:brain weight, revealed greater ratios in the iron-deficient rats compared to controls ($P \leq 0.001$). This suggests that a true heart hypertrophy independent of body weight decreases in the iron deficient group. Liver nonheme iron was significantly less ($P \leq 0.0001$) in iron-deficient versus control rats. Plasma transferrin saturation was lower in rats fed iron-deficient diets compared to controls ($P \leq 0.01$). Thus these data suggest that rats fed the iron deficient diets did develop iron-deficiency with anemia.

Heart rate was greater in iron-deficient rats ($P \leq 0.01$) compared to controls (Table II). Systolic, diastolic and mean arterial pressures did not differ by treatment ($P > 0.05$). Heart NE levels were greater in control rats than in iron deficient rats (≤ 0.01).

Morphometric data (Table III) revealed that hearts from iron-deficient rats had smaller left ventricular lesser diameters compared to controls ($P \leq 0.05$). However, the apical thickness and left ventricular volumes were significantly greater ($P \leq 0.01$ and 0.05, respectively) in hearts from iron-deficient rats compared to controls. Left ventricular wall volume and wall mass tended to be greater in the hearts of iron-deficient rats compared to controls, but there was also much greater variability among this group for these variables, resulting in nonsignificant F values. Overall, Figure 1 clearly demonstrates an eccentric hypertrophy present in the iron-deficient rats. For comparison, Figure 2

Table II. Heart Rate, Heart Norepinephrine and Blood Pressures of Rats Fed Control or Iron-Deficient Diets

Variable	Control ($n = 9$)	Iron-deficient ($n = 8$)	P
Heart rate (beats/min)	373 ± 16	438 ± 13	<0.01
Systolic pressure (mmHg)	153 ± 6	162 ± 7	
Diastolic pressure (mmHg)	117 ± 5	118 ± 6	
Mean arterial pressure (mmHg)	134 ± 5	133 ± 5	
Heart norepinephrine (ng/mg)	700 ± 34	405 ± 32	<0.01

Note. Means ± SD.

Table III. Cardiac Morphometry in Rats Fed Control or Iron-Deficient Diets

Variable	Control (n = 9)	Iron-deficient (n = 8)	P
Right ventricular free wall (cm)	.15 ± .131	.13 ± .020	
Right ventricular lesser diameter (cm)	.12 ± .171	.16 ± .093	
Interventricular septum (cm)	.36 ± .082	.31 ± .056	
Left ventricular free wall (cm)	.36 ± .145	.38 ± .060	
Left ventricular lesser diameter (cm)	.21 ± .129	.36 ± .086	<.05
Median length (cm)	1.66 ± .885	1.46 ± .090	
Apical thickness (cm)	.048 ± .0311	.091 ± .0181	<.01
Heart length (cm)	1.69 ± .252	1.55 ± .093	
Heart width (cm)	1.29 ± .212	1.28 ± .090	
Left ventricular circumferential volume (cm^3)	.69 ± .097	.84 ± .246	
Left ventricular volume (cm^3)	.041 ± .0452	.102 ± .055	<.05
Left ventricular wall volume (cm^3)	.648 ± .090	.724 ± .219	
Left ventricular wall mass (g)	.680 ± .095	.760 ± .229	

Note. Means ± SD.

demonstrates hearts from rats fed control and copper-deficient diets from weanling for a 6-week period. The ventricular walls and septum are thicker but the ventricular chamber much smaller for the copper-deficient hearts (Figure 2) as compared to the iron-deficient hearts (Figure 1).

Discussion

Cardiac morphometric analysis demonstrated an eccentric pattern of hypertrophy in rats made anemic by feeding on an iron-deficient diet. This is supported by the finding that the left ventricular volumes, left ventricular lesser diameters and apical thickness were greater for hearts from

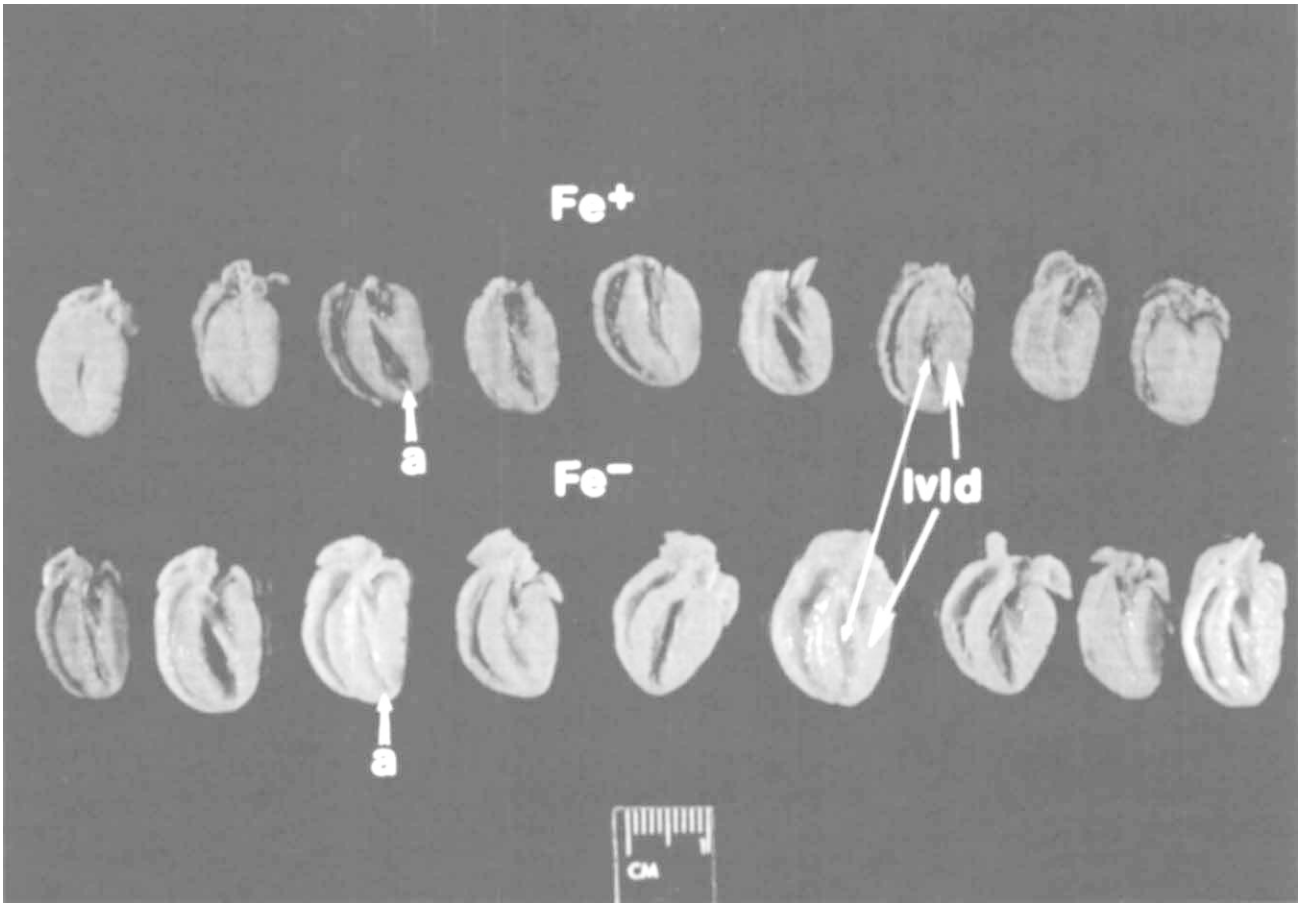

Figure 1. Hearts of rats fed diets either adequate (Fe+, top row) or deficient in iron (Fe–, bottom row). Note the greatly increase chamber volumes of the iron-deficient rats and increased circumference. The arrow with *lvld* indicates the lesser ventricular lesser dimension. The lvld was greater in the iron deficient rats. The apical thickness is indicated by the letter *a* with arrows, which is greater in the iron-deficient rats. The double-headed arrow indicates the left ventricular volume, which was greater in the iron-deficient hearts compared to the controls. See Table III for actual dimensions.

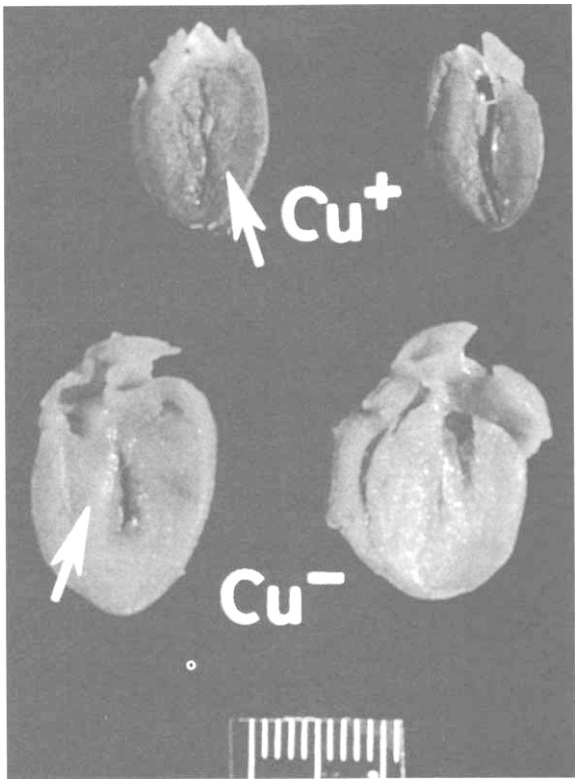

Figure 2. Hearts of rats fed copper-adequate (Cu+) and copper-deficient (Cu−) diets. The hearts from copper-deficient rats demonstrate a concentric hypertrophy as indicated by the grossly thickened left ventricular free walls (arrows).

iron-deficient rats. An eccentric pattern of hypertrophy is known to occur in volume overload conditions, such as floppy valves, where there is a back-flow of blood from the ventricles to the atria during systole. Other conditions leading to eccentric hypertrophy include pregnancy, renal failure, and obesity (19). All of these conditions result in an increased preload. The data from Table III and Figure 1 clearly demonstrate this. An increased afterload, or pressure overload, such as that which occurs in hypertension or valvular stenosis, leads to a concentric hypertrophy where the volume of ventricular lumens either decreases or remains unchanged, but the ventricular walls and interventricular septum are grossly thickened. A mixed hypertrophy is possible where there are both increases in preload and afterload (19).

Results from this study support that one of the primary effects of chronic iron deficiency on the circulatory system is hypertrophy of the heart. There is an increase in heart size (20), ventricular wall thickness as presented here, cell size (21–23), and impaired mitochondrial function (24). Since both cellular growth and degeneration are evident in these studies, it is not clear if the adaptation is positive or negative (21–23). Iron deficiency is associated with increases in urinary and plasma NE concentrations (25, 26) and depressions in tissue NE concentrations and turnover (20, 27, 28). There is typically a 50% or more decrease in heart NE pool sizes that is directly inversely correlated with the severity of the iron deficiency anemia. Ischemia secondary to severe anemia is likely a direct stimulus of the heart hypertrophy (29), but volume overload or perhaps NE content are also highly likely as direct agents in this model of hypertrophy (30). The dramatically lower heart NE observed in the current study is similar to what has been seen in other studies (20, 27, 28) and may be attributed to alterations in recycling of NE as opposed to decrements in synthesis (20). Unpublished studies in isolated perfused rat hearts show lower ventricle NE pool sizes and release rates from iron-deficient rat heart in as little as 3–7 days of feeding a low-iron diet (PhD dissertation, B. Chew, The Pennsylvania State University, 1993). Since abnormalities in NE metabolism are frequently observed in the failing heart, it is worth noting that some of the effects of NE on the heart contractility in the failing heart are dependent on the presence of thyroid hormone (31). Iron deficiency has repeatedly been shown to lead to lower T3 concentrations and T3 turnover rates (27, 32, 33).

With respect to other nutrient deficiencies that lead to cardiac hypertrophy, copper-deficient rats have hearts that exhibit a concentric hypertrophy. Often copper-deficient rats become anemic or simply have lower hematocrit and hemoglobin levels compared to adequate copper rats (7, 8, 34). If the anemia in copper deficiency were responsible for the cardiac hypertrophy, then an eccentric pattern—not a concentric pattern—would occur. Figure 2 clearly demonstrates the concentric pattern in copper-deficient rats. The eccentric hypertrophy observed in iron-deficient rats with anemia (Figure 1) suggests that the anemia associated with copper deficiency is not the primary cause of hypertrophy. In both models, there are increases in the volume density of mitochondria and mitochondria to myofibrils, except that this increase is much more dramatic in the case of the copper-deficient rat hearts compared to hearts from iron-deficient rats (5). For both minerals, the increase in mitochondrial volume density could be related to the issue of both metals being needed in electron transport, with copper perhaps serving as the most critical need with respect to its role in the terminal end of oxidative phosphorylation *via* the cupro-enzyme cytochrome *c* oxidase. We have compared copper-deficient with iron-deficient rats and have noted the changes in hypertrophy already reviewed and the greater mitochondria volume density in copper-deficient rats as compared to iron-deficient rats (35). In that study, whereas the hematocrit was significantly lower in the iron-restricted group (39.1 ± 6.1) compared to the controls (45.9 ± 4.6), hemoglobin levels did not differ among the two treatment groups. Morphometric analysis suggested that both the copper-restricted and iron-restricted groups had a concentric hypertrophy. The presence of a concentric hypertrophy in the iron-restricted group occurred without physiological anemia, or at least to the extent observed in the present study, and the rats were only marginally iron-deficient when evaluating the iron stores com-

pared to the controls in the Wildman *et al.* study (35) and the present findings. Thus, although it appears that an increased mitochondria to myofibril ratio exists in iron deficiency, the presence of outright anemia appears to predominate and results in an increased preload and ventricular dilatation giving the eccentric hypertrophy reported here. Our study is supported by the findings of Petering *et al.* (36), in which iron-deficient rats with severe anemia had cardiac lesions characteristic of dilatation cardiomyopathy reported. Tanne *et al.* (37) reported similar lesions using both electron and light microscopy. Using morphometry analysis, we quantitatively have verified that the anemia produced by iron deficiency is eccentric and characteristic of a volume overload.

In summary, these data demonstrate that rats made anemic by feeding on an iron-deficient diet develop an eccentric hypertrophy. The anemia associated with iron deficiency most likely results in a volume overload that leads to the eccentric hypertrophy. Copper deficiency leads to concentric hypertrophy by some unknown factor; however, anemia is not likely to be the primary agent leading to this form of hypertrophy. In future studies the morphology of the heart by the approach used in this study can facilitate the search for the underlying mechanism responsible for the cardiac hypertrophy.

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