MINIREVIEW

A Unified Perspective on Copper Deficiency and Cardiomyopathy (43599)

DENIS M. MEDEIROS,[1] JEANETTE DAVIDSON, AND JAMES E. JENKINS
Department of Human Nutrition and Food Management, The Ohio State University, Columbus, Ohio 43210-1295

Abstract. **Dietary copper restriction in rats results in cardiomyopathy. In rats fed copper-restricted diets from weaning for 5 to 8 weeks, a concentric hypertrophy is apparent, whereas postweaning copper restriction does produce cardiomyopathy without apparent hypertrophy. Both sets of circumstances appear to affect the integrity of the basal laminae of cardiac myocytes and capillaries. In rats fed copper-restricted diets from weaning, decreases in cytochrome c oxidase are related not only to copper's role as a coenzyme, but also to a marked decrease in the nuclear encoded subunits of the enzyme complex. Decreased levels of the δ-subunit of ATP synthase have been observed. However, such aberrations in mitochondrial enzymes, as well as morphologic alterations, apparently do not affect cardiac levels of ATP. This review suggests mechanisms of cardiac adaptation and initiation factors leading to cardiac hypertrophy. We present a hypothetical working model explaining the events leading to cardiac failure in the copper-deficient rat heart based on the present body of knowledge, and compare the pathology with other models of cardiomyopathies.** [P.S.E.B.M. 1993, Vol 203]

Copper is an essential trace element known to exert an influence upon cardiac pathology and metabolism. Experimental studies conducted by feeding primarily rodents purified diets lacking in copper results in cardiac lesions, hypertrophy, altered biochemical function, and pathology. Within a time period of only 5–8 weeks, feeding a rat a copper-deficient (Cu−) diet from weaning often results in death by hemothorax or aneurysms. Presumably, some of these observations may be mediated through cuproenzymes. Key copper-dependent enzymes include lysyl oxidase (cross-linking of elastin and collagen), cytochrome *c* oxidase (electron transport chain), ceruloplasmin (ferroxidase activity), superoxide dismutase (free radical detoxification), and dopamine β-hydroxylase (catechol production) (1–3).

Over the past 10 years, we have attempted to characterize the Cu− heart, using primarily rats, but also have conducted one study with pigs. A basic question that we have attempted to answer is what mechanism(s) leads to cardiac hypertrophy in copper deficiency. While the above cuproenzymes may affect cardiac biochemical metabolism, as may occur in other tissues, it is uncertain whether the changes in enzyme activities produce cardiac hypertrophy and/or dysfunction. Studying nutrient deficiency effects upon an animal or particular organ is problematic in the sense the end result may not be caused by the deficiency, but could be related to cellular injury leading to secondary alterations. In the case of the heart, cardiac hypertrophy often leads to cardiac failure. The characteristics of the failed heart may be much different from the initial set of circumstances or event that resulted in the initial onset of hypertrophy. Therefore, studying the end result of a disease condition often leads to more confounding questions than to real answers.

[1] To whom requests for reprints should be addressed at Department of Human Nutrition and Food Management, The Ohio State University, 265 Campbell Hall, 1787 Neil Avenue, Columbus, OH 43210-1295.

0037-9727/93/2033-0262$3.00/0

Initially, our basic objective was to first characterize the Cu– rat heart. This was done to allow us to determine how different or similar the results were to other models of hypertrophic cardiomyopathy. Once this was accomplished, we began to study the temporal sequence of events from the time of copper restriction to the end-stage disease state prior to death, and also the consequences of feeding a Cu– diet to mature postweanling rats.

Dietary Copper Deficiency in Humans

Information as to the dietary intake of Cu by humans is limited and estimated daily intakes equivocal. Pennington *et al.* (4, 5) reported average Cu intakes of women to be 0.93 mg/day and of men to be 1.24 mg/day, whereas Klevay *et al.* (6) estimated that the mean intake of many Americans is 1.55 mg/day.

There is no recommended dietary allowance (RDA) for Cu, because of the uncertainties surrounding quantitative dietary Cu needs (7), but 1.5–3 mg/day has been established as a safe and adequate dietary intake range by the National Research Council in 1989. This is a wider range than the 2–3 mg/day established in 1980, because it was believed that there was a discrepancy between the intake estimated from balance studies and reported intakes, without obvious clinical signs of Cu deficiency, specifically anemia and neutropenia (7).

Naturally occurring Cu deficiency in "healthy" humans has not been reported. Some incidence of Cu deficiency in severely malnourished young Peruvian and Chilean children, developing premature babies, adults on total parenteral nutrition, and people with Menkes' kinky-hair syndrome have been recorded (3, 7). However, some concern has been expressed as to the adequacy of current Cu intakes by healthy people generally (6, 8, 9) and athletes (10). In the study by Reiser *et al.* (9), four out of 23 humans developed heart-related problems when the subjects were fed a typical American diet with a high sucrose, but not starch, content (diet Cu level was 1 mg/day). The bioavailability of Cu and extent of dietary interaction are not known precisely (5, 11), but factors such as zinc, ascorbic acid, fructose or sucrose, iron-deficient anemia, fiber and phytates, and antacids (5, 11) may decrease absorption or increase needs of Cu.

Cardiomyopathy Aspects

Myocardial hypertrophy in humans is seen as an adaptive process that takes place to enable the heart to compensate for conditions of overload (12). However, the hypertrophic myocardium is not normal and eventually cardiac failure will result. Meerson (13) described three phases of the hypertrophic process as compensatory hypertrophy following acute and chronic overload, characterized by stable hyperfunction but decreased cardiac reserve capacity, and overcompensation or hyperadaptation leading to cardiac failure. Hypertrophic cardiomyopathies have also been induced experimentally in rats, by banding of the aorta, aortic stenosis, and renal arterial constriction (13).

Cardiac function may fail through inadequate venous emptying or reduced venous ejection fraction against arterial pressure (12). The types and pathophysiology of pathologic cardiac hypertrophy have been reviewed recently (12, 14, 15). Concentric hypertrophy is characterized by increased ventricular wall thickness, and is associated with pressure overload conditions, such as hypertension and aortic stenosis. Eccentric hypertrophy is characterized by increased left ventricular chamber volume and a relatively thin wall. Volume overload in aortic insufficiency, anemia, ischemia, and reduced volume load of mitral stenosis underlie eccentric hypertrophy. Congenital cardiomyopathies with hypertrophy may have increased left ventricular volume and symmetrical or asymmetrical increases in ventricular wall thickness (13–15). The conditions leading to the various types of hypertrophy are summarized in the diagram of Figure 1 and actual hearts representing normal, eccentric and concentric patterns in rats are presented in Figure 2.

The primary stimulus for cardiac hypertrophy seems to be chronic pressure or volume overload, associated with changes in work load and energy and oxygen demands, leading to activation of nucleic acid and protein synthesis (13). The precise signal is not yet understood, but some evidence (16) suggests that the stimulus may be associated with the signal transduction pathways of myocyte proto-oncogenes. Experimental hypertrophy using pressure overloads such as aortic banding results in the expression, often within hours of the banding, of the proto-oncogenes c-*fos* and c-*jun*, followed by the expression of fetal forms of α-actin and V_3 isomyosin proteins (17). Zinc finger genes such as *Erg*-1 have also been identified as candidate genes that are expressed under conditions of pressure overload hypertrophy and are thought to respond to α-adrenergic stimulations of myocardial cells. These genes are expressed rapidly upon onset of external stimuli such as aortic banding and are expressed in *in vitro* and *in vivo* (cell culture) models of myocardial hypertrophy. Cardiac fibroblast proliferation normally occurs as well (18). While the initial signaling mechanism(s) has yet to be identified to show what activates these immediate sets of genes, a possible candidate has been suggested to be endothelin-1, which is thought to be produced and released from capillaries adjacent to myocytes of intact myocardium. Endothelin-1 binds to ventricular myocyte receptor sites, is a vasoconstrictor, and can simulate the expression of these proto-oncogenes, leading to expression of contractile protein genes such as *MLC-2*. It is possible that hemodynamic factors in the

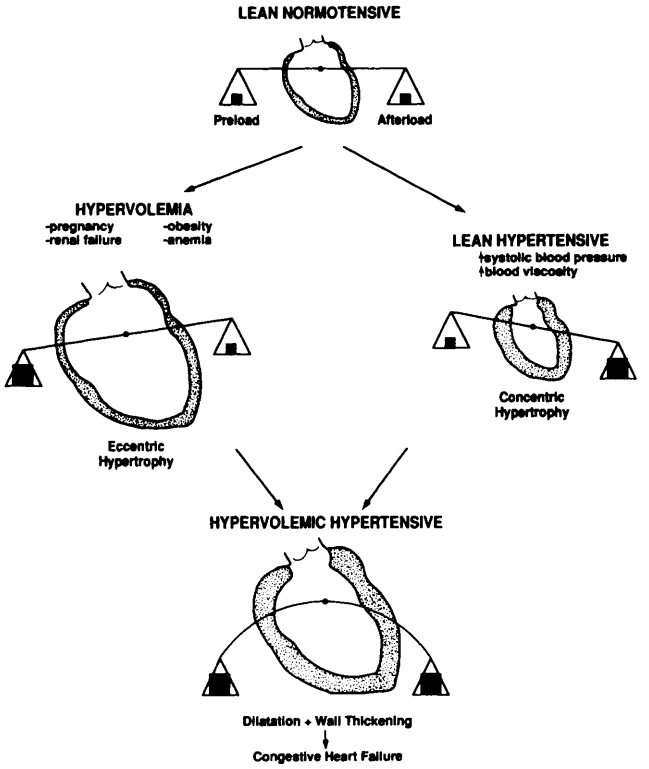


Figure 1. Schematic illustrating cardiac adaptations to either pressure overload or volume overload conditions—effects on the left ventricle. Increased preload, such as that observed in pregnancy, renal failure, obesity, and anemia, leads to eccentric hypertrophy, whereas an increased afterload, such as increased systolic blood pressure and increased blood viscosity, leads to concentric hypertrophy. A "mixed" hypertrophy under conditions with increased preload and afterload is illustrated at the bottom. Cu– rat hearts exhibit a predominantly concentric pattern of hypertrophy. (Illustration adapted from FH Messerli). Cardiovascular effects of obesity and hypertension. Lancet **1**:1165–1168, 1992.

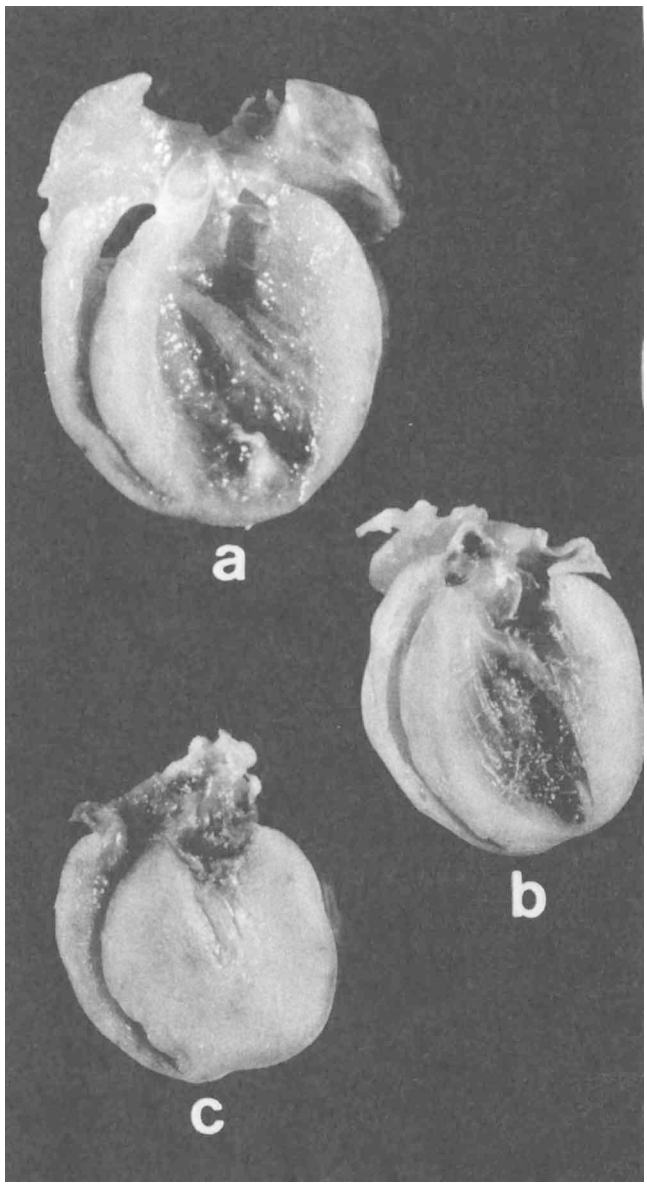


Figure 2. Rat hearts either (a) exhibit eccentric hypertrophy, (b) are normal, or (c) exhibit concentric hypertrophy. Note that in eccentric hypertrophy, the ventricles appear larger, but walls and intraventricular septum appear thinner. Increased weight of the heart is due to a greater perimeter. In the concentric pattern, note that the ventricle is hardly visible and the free walls and intraventricular septum are grossly thickened.

overloaded heart may trigger the expression of several proto-oncogenes, such as c-*fos*, c-*jun*, and c-*egr1* (12, 16), that seem to precede the hypertrophic process. The expression of "fetal" forms of the contractile proteins, creatine kinase, myosin light and heavy chain, α-actin and β-tropomyosin, and atrial natriuretic polypeptide has been confirmed in the hypertrophic process. Changes in the sarcolemma and calcium transport processes have been noted (13). Myosin isoenzyme form V_3 in myosin heavy chains is increased relative to the "normal" V_1 in hypertrophic rat ventricles (19). Since V_3 is associated with slow contractile myosin-ATPase, this would decrease myocardial ATP demand and subsequent oxygen consumption initially. However, increased V_3 may lead to decreased contractility and ultimately contribute to myocardial failure (13, 19). The locus for familial hypertrophic cardiomyopathy has been shown to map on chromosome 14, closely linked to cardiac myosin heavy chain genes (20).

Functional abnormalities in hypertrophic cardiomyopathies include decreases in contractility (power and velocity), relaxation, and decreased cardiac reserve (13). Sasson *et al.* (21) reported abnormal electrocardiogram patterns with atrial and ventricular arrhythmias. Abnormal ST segments were sometimes increased, Q waves were prominent, R waves demonstrated increased amplitude, and P-R intervals and QRS waves had decreased duration. Previously, it was thought that cardiac norepinephrine concentration was decreased in cardiac failure. More recently (22) it was shown that there is an increased cardiac catechol concentration, with selective downregulation of β_1-adrenergic receptors relative to β_2-receptors, that effectively reduces

norepinephrine sensitivity and sympathetic adrenergic influence.

Ultrastructural changes in the myocyte in cardiac hypertrophy appear to be dependent upon the stage of hypertrophic development (13). In the early, acute stage, there is an increase in protein synthesis, with an early increase in mitochondria, followed by an increase in myofibrils. In the stable stage of hypertrophy, there do not seem to be significant changes in the myocyte ultrastructure. The stable phase of compensated hypertrophy is associated with increased myofibrillar growth and an essentially normal ratio of mitochondrial to myofibrillar volume densities. The third stage of decompensated hypertrophy and myocardial failure has a variable response. Pressure overload, but not volume overload or physiologic hypertrophy, is associated with early increased mitochondrial volume density (12, 13, 23, 24). The increased width of the hypertrophied myocyte with pressure overload, associated with the lateral addition of sarcomeres, stimulates mitochondrial synthesis to comply with increased sarcomere energy needs. However, when sarcomeres are added end-to-end at intercalated disk, as in volume overload eccentric hypertrophy, increased mitochondrial size and number are unnecessary. The needs of the increased cell length are met by endothelial hyperplasia and capillary lengthwise growth. Furthermore, mitochondrial volume density may be disproportionally increased in response to relative anoxia in hypertrophy associated with anemia, hypoxia, or ischemia (25, 26).

With progression of the cardiomyopathy to the last stage of overcompensation and ultimately failure, there seems to be a decreased mitochondrial to myofibrillar ratio in the hypertrophied myocyte, with a resultant imbalance between ATP production (mitochondria) and demand (myofibrils) (12, 13). Transverse capillary density is decreased and arterial lumen diameter diminished with progressive myocardial failure so that oxygen diffusion distance is increased (24–26), of special significance in the stressed heart. Cell mass has been reported to be increased relative to cell surface, thus suggesting reduced sarcolemmal surface and ion transport receptors (13). Fibrosis and foci of increased interstitial collagen deposition are increased (13–15, 21, 23), contributing to progressive myocardial stiffness and decreased contractility.

Copper-Deficient Cardiomyopathy

Copper deficiency seems to act as stimulus for the development of compensatory cardiac hypertrophy characterized by gross, functional, and ultrastructural changes, with a high morbidity and mortality (27, 28). Recently, we have reported that postweaning rats fed Cu− diets for 6 weeks have characteristics similar to those of the younger Cu− rats, except that the alterations appear to occur in the absence of overt hypertrophy (29). Specifically, mitochondria volume density and the mitochondria to myofibril ratio increase, and the mitochondria contain vacuoles and fragmented cristae as opposed to the usual parallel array of these fine structural components. Lipid droplets, glycogen granules, and separation of the myofilaments also appear greater in postweaning, copper-restricted rats. This is significant since the conventional wisdom was that copper deficiency only affected cardiac pathology and metabolism in weanlings and not in older animals as determined solely by the heart weight to body weight ratio. An unanswered question is whether suboptimal copper (i.e., marginal) in the diet can also lead to a similar fate if the experiments are of longer duration.

A pertinent characteristic of the Cu− hypertrophied rat heart is the predominantly concentric nature of the organ. As noted above, this occurs when the ventricular walls and septum are thickened, with either no change in ventricular lumen size or even a decrease in size. The occurrence of concentric hypertrophy in humans is often thought to be congenital. However, as reported above, aortic stenosis and hypertension in laboratory animals can produce this pattern. The pattern of hypertrophy is useful in that it readily allows us to determine whether some abnormalities of the Cu− rat heart could lead to this condition. As will be discussed later, the valves of the Cu− heart are compromised in terms of structure. However, one would expect an eccentric pattern of hypertrophy if a mitral valve prolapse or other valvular weakening factor were the primary stimulus in the hypertrophy. Likewise, the anemia associated with copper deficiency would lead to an eccentric or volume overload hypertrophy. Additionally, anemia often occurs after the hypertrophy in copper deficiency studies (30, 31) and some have reported hypertrophy due to copper deficiency in the absence of anemia (32). Earlier studies by Shields *et al.* (33) reported that cardiac hypertrophy in iron-deficient pigs was not as great as in Cu− pigs despite the presence of a greater level of anemia in the iron-deficient pigs.

Heart rate and electrical abnormalities have been reported in a number of studies. Prohaska and Heller (34) reported decreased heart rates in Cu−deficient rats, while Kopp *et al.* (35) found no significant change in heart rate, although it was somewhat lower in Cu−deficient rats. Electrocardiographic abnormalities included abnormal ST segments, increased P-R intervals, R wave duration and amplitude (27, 35, 36), and ventricular and supraventricular beats (27). Kopp *et al.* (35) reported increased H-V intervals in His bundle electrography, and suggested this was indicative of slow conductivity of the His-Purkinje system. Additionally, Medeiros *et al.* (36) and Davidson *et al.* (29) recorded increased QRS amplitude and QT duration in hearts of Cu− rats.

Altered catecholamine levels and sensitivity in the

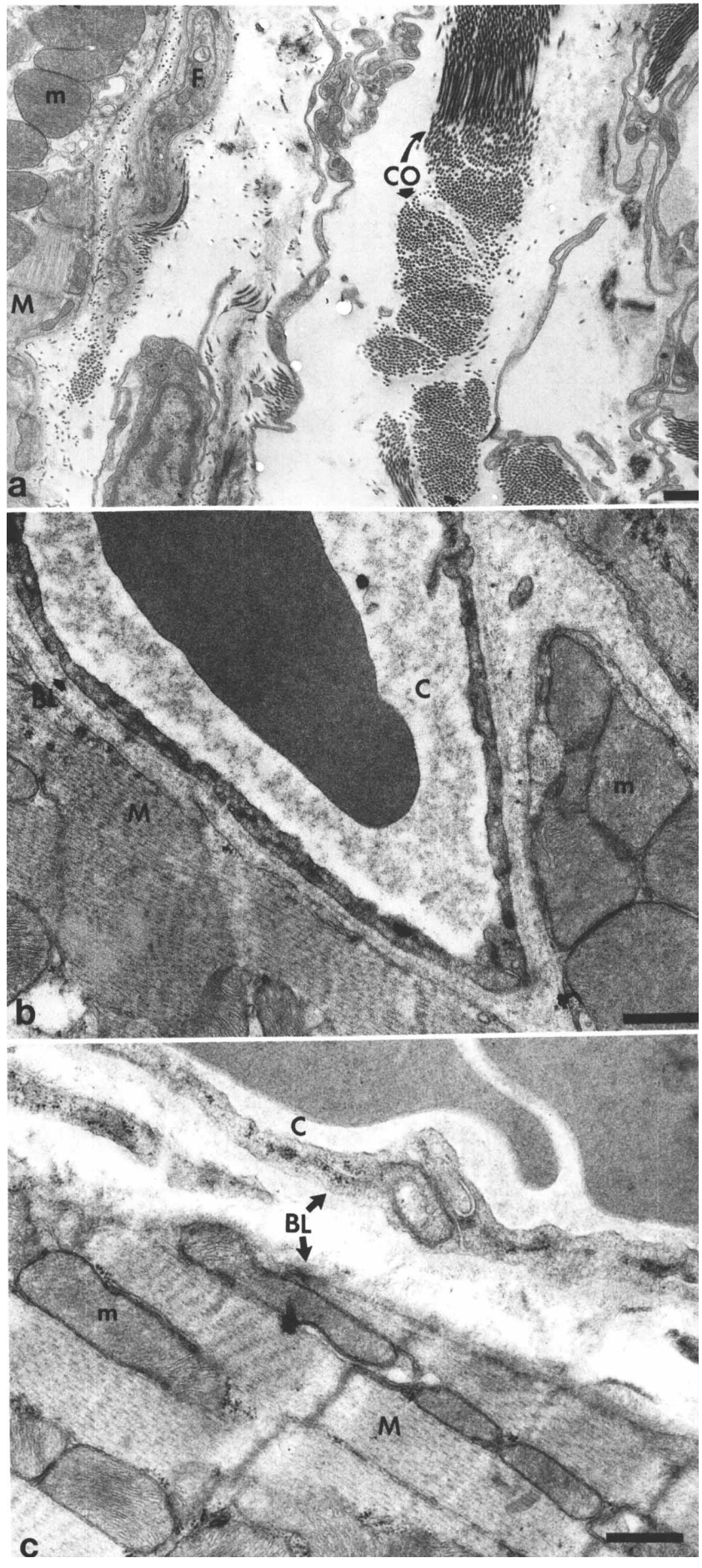
heart and nervous tissue may contribute to the cardiac hypertrophy and functional changes in Cu deficiency. There is some indication that norepinephrine levels are decreased with increased dopamine levels in Cu–deficient rats (34, 37), which could involve the cuproenzyme dopamine β-hydroxylase.

Contractility studies on the Cu– rat heart have not produced consistent results. We have noted that when using the Long-Evans rat strain, copper deficiency did not appear to alter + or – dP/dT max's (36) when studied over a period of 2–6 weeks after weaning. In another study (38), we fed a copper deficient diet to the SHHF/Mcc-cp rat, a strain that develops both hypertension and hypertrophy as it ages and develops an eccentric pattern of hypertrophy. Rats of this strain yield three genotypes but only two phenotypes: homozygous corpulent (cp/cp), heterozygous lean (cp/±), and homozygous lean (±/±). The cp/cp strain is obese and develops diabetes as well as heart disease. The other two strains are lean but the cp/± strain develops hypertrophic cardiomyopathy and hypertension as it ages. Usually the cp/± strain develops hypertrophy around 6–10 months of age and cardiac failure develops around 16 months for males and 22 months for females. We noted more severe cardiac abnormalities as determined by electrocardiograms and ultrastructure in the males than females when fed a Cu– diet from weaning to about 6 weeks thereafter. Additionally, the male rats fed the Cu– diet had a significant decrease in both + and – dP/dT max's when compared with the male copper-adequate rats and also compared with female copper-adequate and -deficient rats. This is especially meaningful when considering that the myocardiums were grossly thickened. If the myocardiums were thickened for a healthy heart, then we would have expected an increased rate of rise and fall of pressure. That –dP/dT max also decreased is consistent with altered left ventricular function, probably a decreased rate of resequestration of Ca^{2+} from troponin to the sarcoplasmic reticulum. Changes in + and – dP/dT max's could be due to a reduction in available ATP as indicated by Kopp *et al.* (35). In our Long-Evans strain of rat, we did not detect any changes in ATP levels between copper-deficient and -adequate fed rats (39). This could explain partly any ability to determine changes in contractility using the Long-Evans strain. The SHHF cp/± strain used also developed a mixed pattern of hypertrophy, with gross thickening of the ventricular free walls and septum, but also an increase in the heart perimeter. Perhaps expression of the genetic material leading to hypertrophy was occurring and was superimposed upon the copper deficiency condition, in which a concentric pattern would be expected.

Blood pressures in different studies are more variable and may be species and age dependent. Blood pressure seems to be decreased in younger Cu–deficient rats (40–42), while blood pressure may be increased in older rats (43, 44) and also exacerbated by stress (i.e., physical restraint), as demonstrated by Klevay and Halas (45).

Interstitial inflammation with focal necrosis, subendocardial fibroplasia, and hemorrhage have been found in studies by Kelly *et al.* (32) and Allen and Klevay (28). Farquharson and Robins (46), using immunohistochemical methods in copper-deficient rat hearts, confirmed the presence of abnormal Type III and I collagen in focal areas, associated with necrosis and fibrosis. Type IV collagen was found exclusively in the basal laminae surrounding the capillaries and myocytes. Focal areas of distortion, fragmentation, and disorganization of a sometimes thickened or collapsed basal laminae supporting myocytes were indicated, whereas capillaries were less affected. Additionally, it also appeared that basement membrane was disrupted around both the myocytes and capillaries in both younger and older Cu– or restricted rat hearts (29). Results revealed both apparent increased distances between capillary lumen and sarcolemmal surface. Deposition of fibrillar collagen in the pericapillary-myocyte areas with phagocytic invasion has been demonstrated with our ultrastructural studies (Fig. 3). The basement membrane is composed of predominantly three proteins: fibronectin, laminin, and Type IV collagen. Integrity of basement membranes is of interest since it has been established that it has a role in regulating cell growth, differentiation, and polarity. A disruption of the basement membrane could exert some influence upon the initiation of the hypertrophic process. In agreement with Farquharson and Robins (46), it seemed as if the capillary wall was less affected than that of the sarcolemma. These changes suggest it would be possible for increased oxygen distances to exist, and raise the question of whether it may be involved in the development of myocardial pathology. Could the swelling of the mitochondria be an attempt to increase surface area to maximize uptake of both oxygen and copper to increase mitochondrial respiration? The abnormal connective tissue is associated with decreased activity of lysyl oxidase and inhibition of the crosslinking of lysyl residues of collagen and elastin (47, 48) and with an increased ratio of Type III to Type I collagen (49) in both rats and pigs (50). Decreased crosslinking in Cu– pigs has been observed (51) and tricuspid and bicuspid valves from both species are thickened with sparse connective tissue relative to control animals (30, 51). Aortal distortion and depletion of elastic tissue, rupture, and cardiac aneurysms, hemothorax, pleural effusion, rupture, and hemopericardium are present (27, 28, 47). Low cytochrome *c* oxidase activity may contribute to cardiac lesions (32).

Collagen metabolism is altered in the hypertrophied heart, particularly that exhibiting a concentric


m
F
CO
M
a
C
BL
M
m
b
C
BL
m
M
c

pattern (52). In normal hearts, the major fibrillar heart collagens, Types I and III, allow for transduction of the contractile force through the myocardium, providing a structural framework to withstand the contractile force generated (53). In the Cu− rat heart, not only is there an increase in Type III/I collagen (49), there is also a significant shift in the connective tissue weave that surrounds myocyte bundles (47). Cardiac collagen is stable, with a half-life of approximately 80–100 days (54, 55). Types I and III collagen are under the gene control of fibroblasts (56), and Type IV collagen, comprising the basement membrane, appears to be under genetic control and is produced in both cardiac fibroblasts and cardiac myocytes.

Types I and III collagen normally increase in response to pressure-overload-induced cardiac hypertrophy (57) resulting in concentric hypertrophy similar to observations in the Cu− rat heart. Deposition of collagen in such a model (53) resembles the pericapillary and intercellular accumulation of collagen we have observed in our studies with Cu− rats. Myocardial injury results when there is damage to the basal laminae (58) which leads to an infiltration of macrophages to clean up cell debris, perhaps by secretion of various proteases. Macrophage infiltration into the compromised basal laminae integrity is similar to our observations in Cu− rat hearts (Fig. 3). In our studies, we have noted the deposition of fibrillar collagen in the areas surrounding the capillaries and myocyte interfaces in postweaning copper-restricted rats (Fig. 3). Rats raised from weaning on a Cu− diet do not appear to deposit much collagen in this area, nor is the presence of macrophages as apparent as in the older rats fed copper-restricted diets. In younger and older copper-restricted rats, the commonality appears to be the disruption and thickening of the basal laminae. It may be that a younger rat is incapable of responding to injury to the basal laminae through cell-mediated mechanisms that result in inflammation. Macrophages secrete the cytokine interleukin 1, which in hearts stimulates fibroblasts. If a mature animal responds to the insult of the basal laminae by the presence of more macrophages to the injured site, such a response may lead to the production of more collagen via fibroblast stimulation as compared with a less mature animal. While this is speculation on our part, this is one potential idea to explain why an older animal fed a copper-restricted diet develops cardiac damage in the absence of overt hypertrophy while younger animals develop the hypertrophy. The presence of collagen fibers in the older animal may lead to maintenance of normal contractile force, whereas in the younger animal the myocardial contractile force must be met by myofibrillar enlargement. Alternatively, it can be argued that older mature animals have more collagen cross-linked prior to being fed a copper-restricted diet and have greater copper stores to mobilize, compared with the younger rat. The slow collagen turnover in mature rats, for instance, may mean that a longer period of copper depletion could lead to cardiac hypertrophy if cross-linking of collagen becomes impaired for newly synthesized proteins.

Given our observations that the copper-deficient rat heart has significant structural impairment in the basement membranes of both myocytes and capillaries (29, 46), we believe that further investigation of the basal laminae in the etiology of copper-deficiency-induced hypertrophy would yield new information on a mechanism that could trigger the disease state.

The increase in fibrillar collagen in cardiac hypertrophy is usually interpreted as a mechanism for the heart to maintain mechanical strength and physiologic pumping. Increased blood pressure would lead to induction of collagen in response to the pressure overload. In the Cu− rat, the collagen cross-linking is decreased. This would increase the work load upon the heart to maintain adequate blood flow. In essence, one possibility is that normal blood pressure level in the Cu− rat may be hypertensive in the relative sense if the connective tissue is weakened. Such an explanation is consistent with the concentric pattern of hypertrophy in Cu− rats.

Fibronectin is of special interest since those models in which mechanical work load is induced experimentally, or in which hypertension is induced, fibronectin synthesis and expression are upregulated (59, 60). Fibronectin in humans dying from atherosclerotic complications revealed greater levels compared with fibronectin in humans dying from other causes (61). Studies of basement membranes of the heart are virtually nonexistent. In searching the literature, we did find that experimental infarct among rats led to an increased level of fibronectin, particularly between interstitial spaces between myocytes and beneath arterial, venous, and capillary endothelium. The thickening of our basement membranes in Cu− rat hearts may be due to increased fibronectin. It has been suggested by some that this increase allows Types I and III collagen a scaffold to accumulate when synthesized (62).

Figure 3. Electron micrographs illustrating the myocyte-capillary juncture of myocardium from rats fed copper-restricted diets. Rats fed a Cu− diet from weaning rarely demonstrate any accumulation of collagen in the area of the capillary-myocyte juncture. However, those rats raised from weaning on a copper-adequate diet and then switched to a Cu− diet for 6 weeks clearly demonstrate (a) the presence of collagen deposition, fibroblast and macrophage infiltration, and disruption of the basal laminae, as opposed to (b) the normal appearance of myocardium from Cu−adequate rats. The (c) thickening and disruption of the basal laminae in rats fed Cu− diets from weaning or postweaning is a common feature. M, myocyte; m, mitochondria; C, capillary; BL, basal laminae; CO, collagen fibrils; F, fibroblast. Bar = 1 μM.

Ultrastructural changes in the myocardium of young Cu− rats are well established. Hypertrophy of myocytes, due to increased size and possible number, and mitochondria (30, 35, 63) have been documented. Abnormal mitochondrial DNA molecules, in association with megamitochondria, have been reported in rats treated with cuprizone, an established Cu chelator (64). Recently, Medeiros *et al.* (30) reported that increased myofibril content contributed to myocyte hypertrophy. Nonspecific, subcellular, degenerative changes in myocytes have been reported (30), characterized by fragmented mitochondrial cristae and vacuolization of matrix (30) and distorted sparse myofibrils with poorly aligned Z bands. Sarcomeres appeared separated at the Z bands and unbanded myofibrils were observed (47).

The different response of mitochondria, observed in the increased mitochondrial volume density and mitochondrial to myofibril ratio in our laboratory and other studies on Cu deficiency (as compared with other models of cardiomyopathy), may be coupled to the role of Cu as a cofactor in the synthesis of cytochrome *c* oxidase (CCO). CCO, on the inner mitochondrial membrane, is essential to mitochondrial respiration and oxidative phosphorylation, catalyzing the transfer of electrons from ferricytochrome *c* to oxygen (65). CCO is composed of several subunits whereby the largest first three subunits are mitochondrially encoded and the remaining subunits are nuclear encoded (65). It appears that the mitochondrial and nuclear transcripts are coordinately expressed (66). Our laboratory reported several years ago that in the mitochondrial fraction, a subunit of approximately 23 kDa was diminished or absent in the Cu− heart (63). Using a polyclonal antibody to cytochrome *c* oxidase, we have tentatively identified this peptide as subunit IV (67). Further studies suggested that all nuclear encoded peptides were decreased but mitochondrial encoded peptides were not altered among Cu− rat hearts. CCO is turned over cooperatively, with a $t_{1/2}$ of CCO of 4–5 days (68). When even one subunit is absent, the enzyme cannot be assembled, causing decreased respiration and breakdown. This includes degeneration of the intermitochondrial membrane, loss of cristae and vacuolization of the matrix, loss of DNA, RNA and proteins, and disintegration of the outer mitochondrial membrane with swelling of the organelle (65, 68). Once integrity of the mitochondria is breached, and respiratory function is compromised, mitochondria are rapidly degraded by lysosomal and cytoplasmic proteolytic enzymes. Disruption of these membranes may lead to increased phospholipid peroxidation and free radical damage. It is thought possible that the mitochondrial inner membrane disruption may be a signal for early degeneration (65, 68). Decreased CCO activity in the heart and skeletal muscle of rats (69) and mice (70) with Cu deficiency has been reported. This decrease in CCO need not be linked to changes in ATP concentration, since ATP may be augmented by the creatinine-phosphate kinase system, and anaerobic glycolysis of glucose and glycogen. The finding of increased glycogen granules in Cu−deficient myocytes lends credence to this theory (30, 36). Also, ATP levels may not change if there is a shift toward the slower velocity myosin V_3 type, as described earlier.

Since the nuclear encoded subunits appear to be dramatically diminished in copper-deficient rat hearts, we conducted some Northern hybridization studies where we used oligonucleotide probes for subunits II and IV of cytochrome *c* oxidase. Our data revealed that subunit II expression of mRNA did not appear to be altered as a function of copper status, but that for subunit IV, the mRNA transcript was elevated in the Cu− rat heart despite lower levels of this peptide as determined by Western blot analysis. The upregulation of cytochrome *c* oxidase subunit IV (and perhaps even some of the other nuclear encoded subunits) may be a stimulus for increased volume density of the mitochondria to myofibril ratio observed in Cu− rat hearts.

Recently, we have succeeded in identifying another decreased peptide, with an apparent 16 kDa molecular mass, in the Cu− rat heart through peptide sequencing. The peptide demonstrated 80% homology with bovine cardiac δ-subunit of ATP synthase when the amino acid sequence was computer matched (67). The cytochrome *c* oxidase and ATP synthase complexes are adjacent to one another on the inner mitochondrial membrane. If the mitochondria are compromised in the Cu− rat heart, then it is not surprising that both of these peptides could be affected to a similar degree. One approach we are currently using to determine whether membrane integrity could be responsible for these peptide alterations is to administer a powerful antioxidant to Cu− fed rats. Since the enzyme superoxide dismutase activity is known to decrease with copper deficiency, a lack of membrane protection from the free radicals generated could be partly responsible. Treatment of Cu− rats with concurrent administration of other antioxidant compounds has resulted in reduced cardiac hypertrophy and mitochondrial damage (71). Examination of some of the enzymes in the copper-deficient rat hearts administered antioxidants could provide useful information to further elucidate the mechanism(s) responsible. However, with respect to copper and cytochrome *c* oxidase, the work of Keyhani and Keyhani (72) on Cu− yeast cells demonstrated that copper is needed to assemble the nuclear encoded subunits to the final mitochondrial complex. We intend to determine whether this is the case in the Cu− rat heart by examining intracellular localization of subunit IV by use of immunogold and transmission electron microscopy.

Based on the current literature and findings from

our laboratory, we propose a tentative working model to explain the events leading to cardiac failure in copper deficiency (Fig. 4).

There have been numerous studies documenting mitochondrial defects in various muscle and neurologic diseases. DiMauro's group (73) has published extensively on this topic, including myopathies associated with deficits in cytochrome *c* oxidase. Fibroblasts isolated from a child afflicted with Leigh's syndrome revealed a disorder due to a nuclear mutation in cytochrome *c* oxidase, but all subunits were present to lesser degrees (74). Mita *et al.* (75) reported that a quadricep muscle biopsy from a young patient afflicted with Kearns-Sayre syndrome demonstrated a mitochondrial deletion to all of subunit III, parts of NADH-coenzyme Q reductase (subunits III and IV), all of ATP synthase subunit VI, and part of ATP synthase subunit VIII. DNA that encoded subunit IV was present but not mitochondrial encoded subunit II. They suggested that this may be due to lack of translation of all three mitochondrial encoded subunits due to lack of coordinate expression resulting from subunit III deletion. In another disorder, termed myoclonus epilepsy with ragged red fibers, which affects both brain and muscle, Western blot analysis from sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a decrease in subunit II relative to the other subunits, but Northern blot analysis failed to show any change in subunits I, II, and III (73). Finally, with respect to human heart disease, Schwartzkopff *et al.* (76) reported a case of a 30-year-old female exhibiting tachycardia in which there were no overt signs of cardiac failure. Subsequent biopsy of the right septal endomyocardium revealed enlarged and vacuolated mitochondria, increased mitochondria to myofibril ratio values similar to the Cu– rat heart, the appearance of glycogen granules and lipid droplets, and a marked decrease in cytochrome *c* oxidase activity. The similarity to our Cu– rat and pig hearts from the micrographs and cytochrome *c* oxidase activity reported are strikingly similar. Muller-Hocker *et al.* (77) and Zeviani *et al.* (78) reported similar observations with patients suffering from cardiomyopathy, particularly lower cardiac cytochrome *c* oxidase activity.

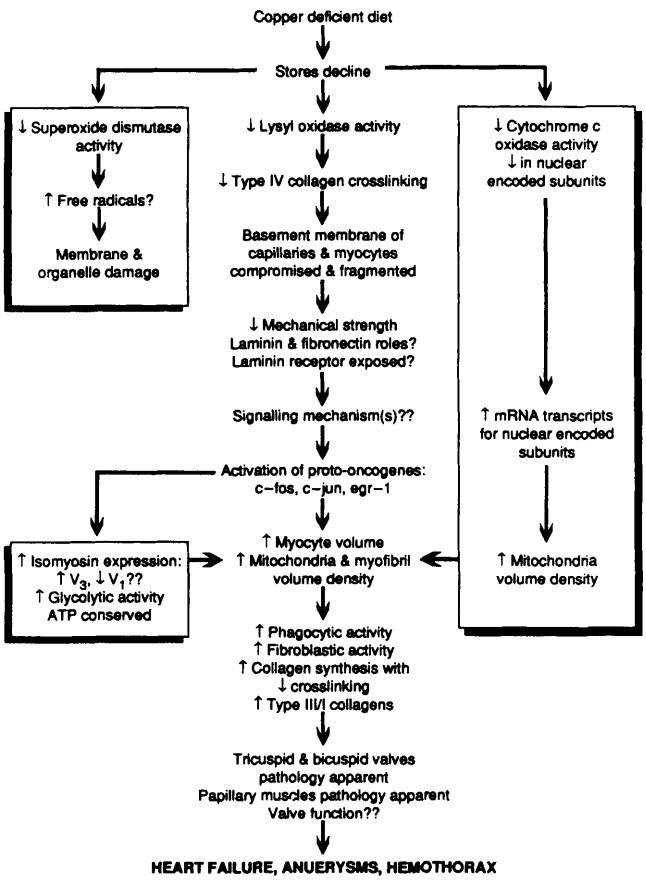


Figure 4. Hypothetical model describing a potential scenario of events leading to heart failure in young rats fed a Cu– diet. Differences in how weaning and postweaning rats respond to a Cu– diet are not fully understood. In the older rat, an increased expression of collagen mRNA may occur, whereas in the younger weaning rat, such an expression may not occur, leading to hypertrophy.

There are only three human studies reporting cardiovascular abnormalities with experimental Cu deficiency. Klevay *et al.* (6), reported increased blood total cholesterol and low density lipoprotein cholesterol and electrocardiogram abnormalities in a man after 15 weeks on a diet supplying 0.8 mg Cu/day. Reiser *et al.* (9) reported increased low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol, with no change in total cholesterol in 24 men with Cu intakes of 1 mg/day. At 4 weeks one man developed myocardial infarction and at 7 weeks one developed tachycardia; the study was terminated when one developed Type II heart block at 11 weeks. However, Turnland (11) could find no difference in blood cholesterol or cardiac function in 11 men after 6 weeks on diets containing 0.8 mg Cu/day.

Future Directions

The characterization of the hypertrophied Cu– rat heart as predominantly a concentric pattern allows the testing of several hypotheses as to molecular aspects of the dysfunction. Foremost is the induction of collagen synthesis. As with other concentric models reviewed, increased collagen deposition and a shift toward greater Type III:I suggest that the genes coding for these connective tissues are induced or altered. Due to the fact that the basement membrane architecture appears to be an early lesion in the copper-restricted rat (29, 46), the regulatory mechanisms responsible for these changes need to be addressed. Much of our information on basement membranes comes from findings of tumor biology. It is not unreasonable that disruption of the basement membrane may influence signaling mecha-

nisms pertaining to cardiac cell growth, metabolism, and differentiation characteristics.

Many of the characteristics of the failing hypertrophied heart suggest a shift toward a more fetal character, such as a shift from the V_1 to V_3 isomyosin. Such a shift may allow the heart to conserve more ATP since the V_3 is slower in contracting, although the increased glycogen deposition observed may also suggest increased glycolytic activity. One or both aspects may explain why ATP levels in the Cu− rat heart appear normal, despite lower cytochrome *c* oxidase activity, as demonstrated by several laboratories (35, 39, 79).

The possible role of the proto-oncogenes c-*fos*, c-*jun*, and *egr*-1, which have been known to be activated in hypertrophy, should be investigated in this model. To demonstrate any potential cause and effect between the basement membrane viability and hypertrophy, a time course where the basement proteins and ultrastructure are assessed concomitant with the expression of c-*fos* and c-*jun* may yield new information. We anticipate that in this model of hypertrophy, such lesions would be likely to precede expression of c-*fos*, c-*jun*, V_3, and collagen isoforms.

Valvular influences upon the pathogenesis should not be totally discounted in this model, although evidence suggests that they are not a primary factor. Since there is some eccentric pattern to the enlarged heart, valvular dysfunction could occur and may be developing later in the pathogenesis. It is also not inconceivable that damage to the myocardium initially could alter the function of the heart valves and papillary muscles independent of any direct influence caused by copper deficiency upon these structures. Echocardiograms may prove useful, using a longitudinal study, to assess the course of events with respect to valvular involvement.

The utility of the model is unique in that concentric hypertrophy, which does occur in humans, can be produced noninvasively (i.e., without aortic banding) and in the absence of hypertension. Others interested in studying cardiovascular aspects of this type of hypertrophy may find this model useful in their studies.

Finally, some studies on humans afflicted with cardiomyopathies of unknown etiology should focus on aspects of either copper nutriture or copper utilization. Apparent mitochondrial defects associated with some forms of human cardiomyopathies could implicate a link with copper metabolism.

1. Davis GK, Mertz W. Copper. In: Mertz W, Ed. Trace Elements in Human and Animal Nutrition. Orlando, FL: Academic Press, Vol **1**: pp301–364, 1987.
2. Prohaska JR. Biochemical changes in copper deficiency. J Nutr Biochem **1**:452–461, 1990.
3. Danks MM. Copper deficiency in humans. Annu Rev Nutr **8**:235–257, 1988.
4. Pennington JAT, Young BE, Wilson DB, Johnson RD, Vanderveen JE. Mineral content of foods and total diets: The selected minerals in food survey, 1982–1984. J Am Diet Assoc **86**:876–881, 1986.
5. Pennington JAT, Young BE, Wilson DB. Nutritional elements in U.S. diets: Results from the Total Diet Study. J Am Diet Assoc **89**:659–664, 1989.
6. Klevay LM, Inman L, Johnson LK, Lawler M, Mahalko JR, Milne DB, Lukaski HC, Bolonchuck W, Sandstead HH. Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism **33**:1112–1118, 1984.
7. Food and Nutrition Board. Recommended dietary allowances. Washington, DC: National Academy Press, pp224–230, 1989.
8. Milne DB, Johnson PE, Klevay LM, Sandstead HH. Effects of copper intake on balance, absorption, and status indices of copper in men. Nutr Res **10**:975–986, 1980.
9. Reiser S, Smith JC, Mertz W, Holbrook JT, Schofield DJ, Powell AS, Canfield WK, Canary JJ. Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am J Clin Nutr **42**:242–251, 1985.
10. Resina A, Fedi S, Gatteschi L, Rubenni MG, Giamberardino MA, Trabassi E, Imreh F. Comparison of some serum copper parameters in trained runners and control subjects. Int J Sports Med **11**:58–60, 1990.
11. Turnland JR. Copper nutriture, bioavailability and the influence of dietary factors. J Am Diet Assoc **88**:303–308, 1988.
12. Katz AM. Cardiomyopathy of overload. N Engl J Med **322**:100–110, 1990.
13. Meerson FZ. The failing heart: Adaptation and deadaptation. New York: Raven Press, 1983.
14. Frances GS, Cohn JN. Heart failure: Mechanisms of cardiac and vascular dysfunction and the rationale for pharmacologic intervention. FASEB J **4**:3068–3075, 1990.
15. Beyar R, Sideman S. Mechanical pathophysiology of some heart diseases: A theoretical study. Med Biol Eng Comput **28**:237–248, 1990.
16. Mulvagh SL, Roberts R, Schneider MD. Cellular oncogenes in cardiovascular disease. J Mol Cell Cardiol **20**:657–662, 1988.
17. Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc Natl Acad Sci USA **85**:339–343, 1988.
18. Morkin E, Ashford TP. Myocardial DNA synthesis in experimental cardiac hypertrophy. Am J Physiol **215**:1409–1413, 1968.
19. Mercadier JT, Lompre AM, Wisnewsky C, Samuel JL, Bercovici J, Swynghedauw B, Schwartz K. Myosin isoenzyme changes in several models of rat cardiac hypertrophy. Circ Res **49**:525–532, 1981.
20. Solomon SD, Geisterfer LA, Vosberg H, Hiller G, Jarcho JA, Moton CC, McBride WO, Mitchell AL, Bale AE, McKenna WJ, Seideman JG, Seideman CE. A locus to familial hypertrophic cardiomyopathy is closely linked to the cardiac myosin heavy chain genes, CRI-L436, and CRI-L329 on chromosome 14 at qiq12. Am J Hum Genet **47**:389–394, 1990.
21. Sasson Z, Rakowski H, Wigle ED. Hypertrophic cardiomyopathy. Cardiol Clin **6**:233–288, 1988.
22. Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB. β_1- and β_2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium. Circ Res **59**:297–309, 1986.
23. Ferrans VJ. Cardiac hypertrophy: Morphological aspects. In: Zak R, Ed. Growth of the Heart in Health and Disease. New York: Raven Press, pp187–239, 1984.
24. Zak R, (Ed). Factors controlling cardiac growth. In: Growth of the Heart in Health and Disease. New York: Raven Press, pp165–187, 1984.
25. Banchero N. Capillarity and the distribution of capillaries and

mitochondria in cardiac growth. In: Legato M, Ed. The Stressed Heart. Boston: Martinus Nijhoff Publishers, pp67–86, 1987.
26. Tomanek RJ, Canby CA. Subcellular growth of cardiocytes during hypertrophy. In: Legato M, Ed. The Stressed Heart. Boston: Martinus Nijhoff Publishers, pp49–65, 1987.
27. Viestenz KE, Klevay LM. A randomized trial of copper therapy in rats with electrocardiographic abnormalities due to copper deficiency. Am J Clin Nutr **35**:258–266, 1982.
28. Allen KGD, Klevay LM. Cholesterolemia and cardiovascular abnormalities in rats caused by copper deficiency. Atherosclerosis **29**:81–93, 1978.
29. Davidson JA, Medeiros DM, Hamlin RL. Cardiac ultrastructure and electrophysiological abnormalities in postweanling copper-restricted and copper-repleted rats in the absence of hypertrophy. J Nutr **122**:1566–1575, 1992.
30. Medeiros DM, Bagby D, Ovecka G, McCormick R. Myofibrillar, mitochondrial and valvular morphological alterations in cardiac hypertrophy among copper deficient rats. J Nutr **121**:815–824, 1991.
31. Goodman JR, Warshaw JB, Dallman PR. Cardiac hypertrophy in rats with iron and copper deficiency: Quantitative contribution of mitochondrial enlargement. Pediatr Res **4**:244–256, 1970.
32. Kelly WA, Kesterson JW, Carlton WW. Myocardial lesions in the offspring of female rats fed a copper deficient diet. Exp Mol Pathol **20**:40–57, 1974.
33. Shields GS, Coulson WF, Kimball DA, Carnes WH, Cartwright GE, Wintrobe MM. Studies on copper metabolism. XXXII. Cardiovascular lesions in copper-deficient swine. Am J Pathol **41**:603–621, 1962.
34. Prohaska JR, Heller LJ. Mechanical properties of the copper-deficient rat heart. J Nutr **112**:2142–2150, 1982.
35. Kopp SJ, Klevay LM, Feliksik JM. Physiological and metabolic characterization of a cardiomyopathy induced by chronic copper deficiency. Am J Physiol **245**:H855–H866, 1983.
36. Medeiros DM, Liao Z, Hamlin R. Ultrastructure and longitudinal measures of electrocardiographic activity and cardiac function in copper-deficient rats. J Nutr **121**:1026–1034, 1991.
37. Schoenemann HM, Failla ML, Rosenbrough RW. Cardiac and splenic levels of norepinephrine and dopamine in copper deficient pigs and rats. Comp Biochem Physiol **970**:387–391, 1990.
38. Medeiros DM, Liao Z, Hamlin R. Copper deficiency in a genetically hypertensive cardiomyopathic rat: Electrocardiogram, functional and ultrastructural aspects. J Nutr **121**:1026–1034, 1991.
39. Chao JC, Medeiros DM, Altschuld RA, Hohl CM. Cardiac nucleotide levels and mitochondrial respiration in copper-deficient rats. Comp Biochem Physiol **104A**:163–168, 1993.
40. Medeiros DM, Lin KN, Liu CF, Thorne BM. Pregestational dietary copper restriction and blood pressure in the Long-Evans rat. Nutr Rep Int **30**:559–564, 1984.
41. Wu BN, Medeiros DM, Liu CF, Thorne BM. Long-term effects of dietary copper and sodium upon blood pressure in the Long-Evans rat. Nutr Res **4**:305–314, 1984.
42. Klevay LM, Moore RJ, Leslie M. Effect of copper deficiency on blood pressure and plasma and lung angiotensin-converting enzyme activity in rats. Nutr Res **8**:489–497, 1988.
43. Medeiros DM. Hypertension in the Wistar-Kyoto rats as a result of post-weaning copper restriction. Nutr Res **7**:231–235, 1987.
44. Klevay LM. Hypertension in rats due to copper deficiency. Nutr Rept Int **35**:999–1005, 1987.
45. Klevay LM, Halas ES. The effects of dietary copper deficiency and psychological stress on blood pressure in rats. Physiol Behav **49**:309–314, 1991.
46. Farquharson C, Robins SP. Immunolocalization of collagen types I, III, and IV, elastin and fibronectin within the heart of normal and copper-deficient rats. J Comp Pathol **104**:245–255, 1991.
47. Borg TK, Klevay LM, Gay RE, Siegel R, Bergin ME. Alteration of the connective tissue network of striated muscle in copper deficient rats. J Mol Cell Cardiol **17**:1173–1183, 1985.
48. Farquharson C, Duncan A, Robins SP. The effects of copper deficiency on the pyridinium crosslinks of mature collagen in the rat skeleton and cardiovascular system. Proc Soc Exp Biol Med **192**:166–172, 1989.
49. Dawson R, Milne G, Williams RB. Changes in the collagen of rat heart in copper-deficient-induced cardiac hypertrophy. Cardiovasc Res **16**:359–365, 1982.
50. Medeiros DM, Failla ML, Schoenemann HM, Ovecka GD. Morphometric analysis of myocardium from copper-deficient pigs. Nutr Res **11**:1439–1450, 1991.
51. Vadlamudi RK, McCormick RJ, Medeiros DM, Vossoughi J, Failla ML. Copper deficiency alters collagen types and covalent crosslinking in swine myocardium and cardiac valves. Am J Physiol (in press).
52. Weber KT. Cardiac insterstitium in health and disease: The fibrillar collagen network. J Am Coll Cardiol **13**:1637–1652, 1989.
53. Eghbali M, Weber KT. Collagen and myocardium: Fibrillar structure, biosynthesis and degradation in relation to hypertrophy and its regression. Mol Cell Biochem **96**:1–14, 1990.
54. Bonnin CM, Sparrow MP, Taylor RR. Collagen synthesis and content in right ventricular hypertrophy in the dog. Am J Physiol **10**:H703–H713, 1981.
55. Laurent GJ, Sparrow MP, Bates PC, Millward DJ. Collagen content and turnover in cardiac and skeletal muscles of the adult fowl and the changes during stretch-induced growth. Biochem J **176**:419–427, 1978.
56. Eghbali M, Czaja MJ, Zeyel M, Weiner FR, Zern MA, Seifter S, Blumenfeld OO. Collagen mRNAs in isolated adult heart cells. J Mol Cardiol **20**:267–276, 1988.
57. Chapman D, Weber KT, Eghbali M. Regulation of collagen types I and III and basement membrane type IV collagen gene expression in pressure overloaded rat myocardium. Circ Res **67**:787–794, 1990.
58. Vracko R, Cunningham D, Frederickson RG, Thorning D. Basal lamina: The scaffold for orderly cell replacement. Lab Invest **58**:77–87, 1988.
59. Saouaf R, Takasaki I, Eastman E, Chobanian AV, Brecher P. Fibronectin biosynthesis in the rat aorta in vitro: Changes due to experimental hypertension. J Clin Invest **88**:1182–1189, 1991.
60. Takasaki I, Chobanian AV, Sarzani R, Brecher P. Effect of hypertension on fibronectin expression in the rat aorta. J Biol Chem **265**:21935–21939, 1990.
61. Orekhov AN, Andreeva ER, Shekhonin BV, Tertou VV, Smirnov VN. Content and localization of fibronectin in normal intima, atherosclerotic plaque, and underlying media of human aorta. Atherosclerosis **53**:213–219, 1984.
62. Casscells W, Kimura H, Sanchez JA, Yu ZX, Ferrans UJ. Immunohistochemical study of fibronectin in experimental myocardial infarction. Am J Pathol **137**:801–810, 1990.
63. McCormick RJ, Ovecka GD, Medeiros DM. Myofibrillar and nonmyofibrillar myocardial proteins of copper-deficient rats. J Nutr **119**:1683–1690, 1989.
64. Guerineau M, Guerineau S, Gosse C. Abnormal mitochondria DNA molecules in megamitochondria from cuprizone treated rats. Eur J Biochem **47**:313–319, 1974.
65. Luzikov NV. Mitochondrial biogenesis and breakdown. New York: Consultant Bureau, 1985.
66. Hood DA. Co-ordinate expression of cytochrome c oxidase subunit III and IV_c mRNAs in rat tissues. Biochem J **269**:503–506, 1990.
67. Medeiros DM, Shiry L, Lincoln AJ, Prochaska L. Cardiac nonmyofibrillar proteins in copper-deficient rats: Amino acid sequencing and Western blotting of altered proteins. Biol Trace Elem Res **36**:271–282, 1993.

68. Desautels M. Mitochondrial proteolysis. In: Fiskum B, Ed. Mitochondrial Physiology and Pathology. New York: Van Nostrand Reinhold, pp40–65, 1986.
69. Paynter DI, Moir RJ, Underwood EJ. Changes in activity of the Cu–Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. J Nutr **109**:1570–1576, 1979.
70. Prohaska J. Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. J Nutr **113**:2048–2058, 1983.
71. Saari JT, Medeiros DM. Effect of dimethyl sulfoxide on enlarged hearts of copper-deficient rats. Biol Trace Elem Res **31**:249–263, 1991.
72. Keyhani E, Keyhani J. Cytochrome c oxidase biosynthesis and assembly in *Candida utilis* yeast cells: Function of copper in the assembly of active cytochrome c oxidase. Arch Biochem Biophys **167**:596–602, 1975.
73. DiMauro S, Zeviani M, Rizzuto R, Lombes A, Nakase H, Bonilla E, Miranda A, Schon E. Molecular defects in cytochrome oxidase in mitochondrial diseases. J Bioenerg Biomembr **20**:353–364, 1988.
74. Mirand AF, Ishii S, DiMauro S, Shay JW. Cytochrome c oxidase deficiency in Leigh's syndrome: Genetic evidence for a nuclear DNA-encoded mutation. Neurology **39**:697–702, 1989.
75. Mita S, Schmidt B, Schon E, DiMauro S, Bonilla E. Detection of "deleted" mitochondrial genomes in cytochrome c oxidase-deficient muscle fibers of a patient with Kearns-Sayre syndrome. Proc Natl Acad Sci USA **86**:9509–9513, 1989.
76. Schwartzkopff B, Zierz S, Frenzel H, Block M, Neuen-Jacob E, Reiners K, Strauer BE. Ultrastructural abnormalities of mitochondria and deficiency of myocardial cytochrome c oxidase in a patient with ventricular tachycardia. Virchows Arch [A] **49**:63–68, 1991.
77. Mullen-Hocker J, Johannes A, Droste M, Kadenbach B, Hubner G. Fatal mitochondrial cardiomyopathy in Kearns-Sayre syndrome with deficiency of cytochrome c oxidase in the cardiac tissue and skeletal muscle. An enzyme-histochemical ultraimmunocytochemical structural study in the long-term frozen autopsy tissue. Virchows Arch [B] **52**:353–367, 1986.
78. Zeviani M, Van Dyke DH, Servidei S, Bausermann SC, Bonilla E, Beaumont ET, Sharda J, Van der Laan K, DiMauro S. Myopathy and fatal cardiopathy due to cytochrome c oxidase deficiency. Arch Neurol **43**:1198–1202, 1986.
79. Rusinko N, Prohaska JR. Adenine nucleotide and lactate levels in organs from copper-deficient mice and brindled mice. J Nutr **115**:936–943, 1985.