Dietary Copper Restriction-Induced Changes in Myocardial Gene Expression and the Effect of Copper Repletion

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Dietary copper (Cu) restriction leads to cardiac hypertrophy and failure in mice, and Cu repletion (CuR) reverses the hypertrophy and prevents the transition to heart failure. The present study was undertaken to determine changes in myocardial gene expression involved in Cu deficient (CuD) cardiomyopathy and its reversal by CuR. Analysis was performed on three groups of mice: 4-week-old CuD mice that exhibited signs of cardiac failure, their age-matched copper-adequate (CuA) controls, and the CuD mice that were re-fed adequate Cu for 2 weeks. Total RNA was isolated from hearts and subjected to cDNA microarray and real-time reverse transcription–polymerase chain reaction analysis. Dietary CuD caused a decrease in cardiac mRNA of β-MHC, L-type Ca^{2+} channel, K-dependent NCX, MMP-2, -8, and -13, NF-κB, and VEGF. The mRNA levels of ET-1, TGF-β, TNF-α, and procollagen-I-α1 and III-α1 were increased in the CuD cardiac tissue. Copper repletion resulted in cardiac mRNA levels of most of the genes examined returning to control levels, although the K-dependent NCX and MMP-2 values did not reach those of the CuA control. In addition, CuR caused an increase in β-MHC, L-type Ca^{2+}channel, MMP-13 to levels surpassing those of CuA control, and a decrease in ET-1, and TNF-α mRNA levels. In summary, changes in gene expression of elements involved in contractility, Ca^{2+} cycling, and inflammation and fibrosis may account for the altered cardiac function found in CuD mice. The return to normal cardiac function by CuR may be a result of the favorable regression in gene expression of these critical components in myocardial tissue. Exp Biol Med 229:616–622, 2004

Key words: cardiomyopathy; copper deficiency; copper repletion; cDNA microarray; real-time RT-PCR

Introduction

Dietary copper deficiency (CuD) leads to cardiomyopathy in experimental animal models (1–5). Copper depletion–induced cardiomyopathy is characterized by concentric cardiac hypertrophy such as occurs in the pressure overloaded heart (4, 6). Although CuD heart hypertrophy may be initially an adaptive response to a stress stimulus, which normalizes wall tension, further progression leads to impaired cardiac muscle function and heart failure (7).

The transition from cardiac hypertrophy to failure in CuD mice has been investigated in our laboratory on the level of cardiac function under stress and nonstress conditions and morphology of cardiac tissue including histopathological changes examined by light microscopy and ultrastructural alterations by electron microscopy (7). We also have shown that the deterioration in cardiac function and morphology is reversible upon Cu repletion (CuR; Ref. 8). In particular, CuR improved CuD-induced myocardial structural lesions, normalized CuD-depressed cardiac systolic and diastolic functions, and restored the response of cardiac muscle to β-adrenergic stimulation (8).

Cardiac hypertrophy is often associated with poor prognosis and the onset of heart failure in patients (9). The transition from cardiac hypertrophy to failure is marked by a distinct alteration in gene expression profile, involving several major important cellular systems: loss of contractile proteins and the downregulation of Ca^{2+} handling proteins (10–16), both of which are implicated in the impaired cardiac function in heart failure patients, accumulation of extracellular matrix components that are involved in the increase in cardiac muscle stiffness (17), and changes in certain growth factors and cytokines such as VEGF and TNF-α (18–20), as well as the upregulation of the vasoconstrictor peptide ET-1 (21).

The changes in gene expression profile underlie the molecular mechanism of cardiac hypertrophy and its transition to heart failure. Depending on the initial hypertrophic stimuli such as hormones, cytokines, growth factors, vasoactive peptides, or catecholamines, the intracellular

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signaling pathways involved are different (22). However, the changes in gene expression with cardiac hypertrophy such as the upregulation of fetal genes including β-myosin heavy chain (β-MHC) and α-skeletal and α-smooth muscle actin, as well as atrial natriuretic peptide (ANF) and the downregulation of α-myosin heavy chain (α-MHC), are associated with several different etiologies including CuD-induced hypertrophy (23).

The purpose of this study was, thus, to extend the investigation of dietary CuD-induced cardiomyopathy to the molecular level to determine the changes in gene expression during the transition from heart hypertrophy to failure. Furthermore, the changes in gene expression patterns associated with the recovery from CuD-induced cardiomyopathy by CuR were also within the scope of this investigation. To this end, we have compared three groups of animals: CuD mice that were fed CuD diet until 4 weeks old, the stage at which functional and morphological deteriorations were marked (the transition phase from cardiac hypertrophy to failure); age-matched CuA controls; and CuD mice that were re-fed adequate Cu for an additional 2 weeks. We used cDNA microarray to determine changes in the expression of genes related to extracellular matrix components, cytoskeletal function, apoptosis, DNA damage, and acute-phase response. Real-time reverse transcription–polymerase chain reaction (RT-PCR) was used for assessment of genes involved in contractile function, Ca^{2+} cycling, and others.

Materials and Methods

Animals and Treatment. Friend Virus B-type (FVB) mice were bred and maintained at the University of Louisville animal facilities and housed in plastic cages at 22°C on a 12:12-hr light:dark cycle. Dams of the pups were fed copper-deficient (CuD) AIN-93 diet containing 0.33 ppm Cu or copper-adequate (CuA) diet containing 6.0 ppm Cu starting on the third day after delivery. The diet was prepared according to a previously published report (24). The primary ingredients are cornstarch (53%), casein (20%), sucrose (10%), and soybean oil (7%) with essential vitamins and minerals. After the pups were weaned on the 21st day after birth, they were fed the same diet until they were 4 weeks old, at which point some of the CuD animals were sacrificed and others were switched to CuA diet for an additional 2 weeks. Animals had free access to double distilled water. Cages, feeding jars, and water bottles were rinsed regularly with water containing EDTA first and then with distilled water. Body weight was monitored weekly starting from the third week after birth. All procedures were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) certified University of Louisville Institutional Animal Care and Use Committee.

Microarray Analysis. The procedure for the cDNA microarray was described previously (25). Briefly, the whole heart obtained from three mice in each treatment group was used and total RNA was isolated from the heart tissue using RNeasy columns (Qiagen, Valencia, CA). Aliquots from the purified RNA sample from each mouse were pooled, and the pooled RNA samples were used for microarray analysis in order to eliminate individual variability; however, individual RNA samples were used for real-time RT-PCR analysis. Total RNA was converted to [α-^{32}P]-dATP-labeled cDNA probe by Moloney murine leukemia virus reverse transcriptase and the Atlas customer array-specific cDNA synthesis primer mix and purified with NucleoSpin columns (Clonetech). Membranes were prehybridized with Expresshyb from Clonetech for 60 mins at 68°C, followed by hybridization with probe overnight at 68°C. Membranes were washed 4 times in 2× standard saline citrate/1% sodium dodecyl sulfate, 30 mins each, and 2 times in 0.1× standard saline citrate/0.5% sodium dodecyl sulfate. Membranes were then wrapped and exposed to a Molecular Dynamics Phosphoimage Screen (Sunnyvale, CA). The hybridization procedure was repeated 3 times using the same pooled RNA sample for each treatment. The images were quantified densitometrically by using Atlas v2.01 software (Clonetech, Palo Alto, CA). Gene expression intensities were first corrected with background and then normalized with the sum of all nine housekeeping genes on the array. A total of 560 genes were analyzed. After acquiring imaging intensities of CuA, CuD and CuR groups, values that were less than 5000 or not significantly different from CuA control were eliminated ($P < 0.05$). The final data entry to Table 1 includes only the genes whose alterations in expression by either CuD or CuA in comparison with the CuA controls reached 2-fold or more, either increased (with the value equal to or more than 2) or decreased (with the value equal to or less than 0.5).

Real-Time Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Analysis. Method for analysis of selected genes using real-time RT-PCR followed the published procedure (25, 26). In brief, total RNA was isolated from each mouse heart using RNeasy columns (Qiagen, Valencia, CA). RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase and oligo-dT primers. Forward and reverse primers were designed using Primer Express software (Applied Biosystems, Foster City, CA; Table 2). The Sybr green DNA PCR kit (Applied Biosystems) was used for real-time RT-PCR analysis. Relative differences between groups were evaluated using cycle time values and expressed as relative increases or decreases setting the controls as 1.00. Assuming that the cycle time value is reflective of the initial starting copy and that there is 100% efficacy, a difference of one cycle is calculated from each gene's standard curve.

Data Analysis. A Student's t test was used for all experimental data analysis. Differences were considered significant at $P < 0.05$.

Table 1. Altered Myocardial Gene Expression in CuD and CuR Mice in Comparison to CuA Mice by Microarray Analysis[a]

Gene/Protein	CuD/CuA	*P*	CuR/CuA	*P*	CuR/CuD	*P*
Procollagen, type I, α1	2.42	<0.05	1.19	<0.05	0.50	<0.05
Procollagen, type III, α1	2.52	<0.05	1.31	<0.05	0.52	<0.05
Thrombospondin 1	0.42	<0.05	0.84	<0.05	2.00	<0.05
Cyclin D1	1.78	<0.05	0.89	<0.05	0.50	<0.05

[a] Data are mean ± SEM of three hybridizations from each pooled sample of three hearts in each group. Only genes shown altered expression in the value of any ratio equal to or more than 2 or equal to or less than 0.5 were considered and included in this table.

Results

CuD-induced myocardial pathological and functional changes and systemic alterations and the effect of CuR on these manifestations in the mouse model under the same condition have been reported previously (7, 8). The cDNA microarray analysis has revealed that the expression of many genes was altered by CuD and these alterations were corrected by CuA. The results presented in Table 1, however, only showed the genes whose expression changes induced by CuD or CuA in comparison to CuA reached 2-fold or more. By this 2-fold change criterion, only four genes were included in the table. In general, procollagen genes (I-α1 and III-α1) were upregulated by CuD. This upregulation was suppressed by CuR. A significant decrease in the mRNA level of the matricelluar protein thrombosponsin 1 was found in CuD (greater than 50% decrease), and CuR caused elevation of the mRNA level. Cyclin D1 mRNA levels were increased by CuD and decreased by CuR. Real-time RT-PCR analysis (Table 3) complemented the results obtained from the cDNA microarray with several cardiac specific genes. Specific changes in the myocardial gene expression in the CuD mice and their recovery in the CuR mice are described below.

β-MHC mRNA was decreased in CuD heart compared to CuA controls by 35% ($P < 0.05$). Interestingly, CuR caused an increase of more than 3-fold of β-MHC mRNA levels ($P < 0.05$). The difference in mRNA level of β-MHC between CuD and CuR hearts is 5-fold ($P < 0.05$). Copper depletion led to a decrease to 85% of control in cardiac mRNA level of L-type Ca^{2+} channel and CuR not only reversed this alteration but also increased the mRNA levels for L-type Ca^{2+} channel more than 2-fold compared to CuA controls ($P < 0.05$). This elevation represents more than 3-fold increase compared to CuD cardiac tissue. Copper depletion caused a dramatic decrease in cardiac mRNA of K-dependent Na/Ca^{2+} exchanger and CuR caused an increase in the mRNA level.

CuD decreased matrix metalloproteins-2, -8, and -13 (MMPs) mRNA levels by 57%, 86%, and 73% compared to controls, respectively. Copper repletion normalized levels of both MMP-8 and MMP-13, however, did not recover the CuD-depressed expression of MMP-2. Cardiac TNF-α mRNA levels were increased in the CuD (by 30%, $P < 0.05$) and decreased significantly in the CuR cardiac tissue. ET-1 mRNA was increased more than 3-fold in CuD tissue and decreased to below 50% of control by CuR. A dramatic decrease in cardiac mRNA of VEGF was observed in CuD tissue (85% reduction compared to CuA control, $P < 0.05$); CuR corrected this decrease. The increase in VEGF mRNA by CuR represents greater than a 6-fold difference if compared to CuD mRNA levels. The levels of TGF-β mRNA were approximately 2-fold higher in CuD cardiac tissue ($P < 0.05$) and reversed by CuR. Copper depletion significantly decreased the expression of NF-κB and CuR corrected this depression.

Discussion

We have shown previously that CuD-induced cardiomyopathy leads to severe depression in cardiac contractility under stress and nonstress conditions (7). The effects on cardiac function and hemodynamics were all reversed upon reintroducing Cu into the diet of CuD animals, with a concomitant reversal of cardiac hypertrophy (8). In this study we present the results demonstrating the changes in myocardial gene expression that may account for the decreased cardiac function in CuD and the reversibility by CuR. We studied changes in several components essential for optimal cardiac function at the cardiomyocyte level (contractile apparatus and Ca^{2+} cycling proteins), as well as at the level of cardiac muscle as a whole (extracellular matrix components).

Given that contractility is governed by two cardiac cellular components, contractile proteins and those involved in Ca^{2+} cycling, alterations in the expression of these proteins lead to manifest changes in cardiomyocyte function as well as cardiac function as a whole. A differential expression of MHC occurs in pathological conditions depending on the type of stimulus. For example, an increase in thyroid hormone or exercise causes an elevation of α-MHC, whereas aging and pressure overload cause β-MHC elevation (27). An increase in β-MHC expression relative to α-MHC allows for more economy in force generation given its slow contractile velocity; an increase in β-MHC is associated with cardiac hypertrophy in dietary CuD (23). Although β-MHC mRNA is increased in the hypertrophied CuD heart, the mRNA level of β-MHC decreased significantly when these hearts underwent transition to failure as shown in this study. In humans, the transition from hypertrophy to failure is marked by a decrease in

Table 2. Gene Name, Primer Sequence and Accession Number of Analysis Using Real-Time RT-PCR

Gene	Primer	Accession No.
Myosin light chain 2 (MLC2)	F TGACGTTACCGGCAATCTTG R TGGTTCAGGGCTCAGTCCTT	M91502
Myosin light chain 2a (MLC2a)	F GCCGGCAACATTGACTACAA R CTCAGCCTGTCTACTCCTCTTTCTC	S70785
α-myosin heavy chain (α-MHC)	F CCACTTCTCCTTGGTCCACTATG R ACAAACCCACCACCGTCTCA	M74752
β-myosin heavy chain (β-MHC)	F CCTCCTCACATCTTCTCCATCTCT R CTCCGGATTCTCCGGTGAT	AY056464
Myosin light polypeptide 1a (Myl1a)	F TGCCCATGATGCAAGCTATC R ACACGCAGACCCTCAACGA	NM021285
Cardiac actin	F CTTCAATGTGCCTGCCATGT R CAATGCCTGTGGTTCTTCCA	M15501
Troponin-C	F CAGGAGATGATCGACGAAGTAGAC R GCACCGAACCATCATGACAA	NM009393
Na^+/Ca^{2+} exchanger K-dependent (KNCX)	F TCTTCCTCCTGTGTGTCACCAT R AGAGTGGCCGTGATGAAGGT	AY156046
Na^+/Ca^{2+} exchanger (NCX)	F GATTCCGTGACTGCCGTTGT R CCTGGGTAGCTGCTACTTTGCT	AF004666
L-type Ca^{2+} channel	F CAGATGAGAGCGCCCGTATC R CCCGGTTCAGCAGCTTGA	NM014193
Sarcoplasmic reticulum Ca^{2+} ATPase (SRCA-2)	F GAGGGTCTGCCTGCTGTCA R CAGAAGGCAGACTTCGAACGA	AF039893
Phospholamban (PBL)	F CAGACCTGCAACATGCCAACT R GCAGCGGTGCGTTGCT	NM023129
Tumor necrosis factor-α (TNF-α)	F GACCCTCACACTCAGATCATCTTCT R CCTCCACTTGGTGGTTTGCT	XM-110221
Matrix metalloproteinase-13 (MMP-13)	F TGCATTCAGCTATCCTGGCC R GGCAGGGACCAACAGGG	NM-008607
Matrix metalloproteinase-2 (MMP-2)	F CACCTGGTTTCACCCTTTCTG R AACGAGCGAAGGCATACAA	M84324
Matrix metalloproteinase-8 (MMP-8)	F GGTAACTAACTCTGCAGCCCTCTT R CGAACCAGGGACGGAATATG	U96696
Matrix metalloproteinase-3 (MMP-3)	F CCCACCAAGTCTAACTCTCTGGAA R GGGTGCTGACTGCATCAAAGA	BC006725
NF-κB	F GGCGGCGTTTTACTCTTT R CCGTCTCCAGGAGGTTAATGC	AF069542
Fas-ligand (Fas-L)	F GGCTGGGTGCCATGCA R GGCACTGCTGTCTACCCAGAA	V06948
Bcl-2	F ACTTCGCAGATGTCCAGTCA R CACAATCCTCCCCCAGTTCA	M16506
Cdc6	F CCACTCCGTGTGTGGACGTA R GGAGTGTTGCACAGGTTGTCA	NM011799
TGF-β	F GACCCTGCCCCTATATTTGGA R GCCCGGGTTGTGTTGGT	M13177
Atrial natriuretic factor (ANF)	F AGGAGAAGATGCCGGTAGAAGA R GCTTCCTCAGTCTGCTCACTCA	K02781
HFL	F AGTGTGGTGGCCTATGAAGGA R GCGCTTGTTGGCCAGATT	AF114380
Endothelin-1 (ET-1)	F CTGGGAGCGAGTGCCTTTC R GGCATCGTGTCTCTGCTCTCT	NM010104
Vascular endothelial growth factor (VEGF)	F ACCCTGGTGGACATCTTCCA R TCATCGTTACAGCAGCCTGC	M95200

myofibrillar and contractile protein content (28). It has been proposed that this loss of myofibrillar and contractile proteins constitutes a major reason for the systolic dysfunction observed in heart failure patients. Previous reports concerning myofibrillar protein content in CuD rat hearts also have shown a slight but significant shift in isomyosin types from V_1 (composed of two α heavy chains) to V_3 (composed of two β heavy chains) and no changes in other myofibrillar proteins (29, 30). However, possible changes in the myofibrillar proteins during the transition to heart failure have not been reported in rats. Interestingly, CuA caused a significant shift from CuD-depressed β-MHC mRNA levels to overexpression in the mouse model. This finding raises an important question: does the overexpres-

Table 3. Alterations in Gene Expression Analyzed by Real-Time RT-PCR[a]

	CuD/CuA	*P*	CuR/CuA	*P*	CuR/CuD	*P*
Contractile protein						
β-MHC	0.65	0.05	3.28	<0.05	5.04	<0.05
Ca^{2+} cycling proteins						
L-type Ca^{2+} channel	0.85	<0.05	2.70	<0.05	3.19	<0.05
K-dependent NCX	0.33	<0.05	0.48		1.44	<0.05
Fibrosis-related genes						
MMP-2	0.43	<0.05	0.62	<0.05	1.45	<0.05
MMP-8	0.14	<0.05	1.20		8.52	<0.05
MMP-13	0.27	<0.05	1.40	<0.05	5.25	<0.05
Other genes						
TNF-α	1.31	<0.05	0.46	<0.05	0.35	<0.05
ET-1	3.14	<0.05	0.45	<0.05	0.14	<0.05
VEGF	0.15	<0.05	0.99		6.48	<0.05
TGF-β	1.99	<0.05	0.99		0.50	<0.05
NF-κB	0.46	<0.05	0.74		1.62	<0.05

[a] The data were obtained from the RNA samples isolated from three separate hearts of each group. Only genes shown altered expression in the value of any ratio equal to or more than 2 or equal to or less than 0.5 were considered and included in this table.

sion of a low velocity contractile protein benefit a dysfunctional heart, and what is the mechanism behind such a phenomenon?

Alterations in myocardial contractility in end-stage heart failure are linked to major changes in Ca^{2+} cycling (10, 11, 14). A major player in the regulation of Ca^{2+} homeostasis in cardiac excitation-contraction coupling is the sarcolemmal L-type Ca^{2+} channel. The L-type Ca^{2+} channel is a voltage gated channel that plays a role in the initiation of contraction by allowing the influx of Ca^{2+} through the sarcolemmal membrane into cardiac myocytes, which causes a release of Ca^{2+} from the sarcoplasmic reticulum (SR) through the SR ryanodine receptor (RyR). A decrease in L-type Ca^{2+} channel density in animal models of cardiac failure occurs, although reports are not consistent, and correlates with moderate to severe congestive heart failure (31). Results from failing human myocardium are controversial, with some showing a decrease in the abundance of specific L-type Ca^{2+} channel subunits and others showing no change in tissue from patients with end-stage failure (11). The present study showed a drastic decrease in L-type Ca^{2+} channel mRNA level in dietary CuD. This is consistent with some of the reports mentioned above and may account for altered channel function and disturbed excitation-contraction coupling. Conversely, the CuR-caused recovery of the expression of the mRNA for the L-type Ca^{2+} channel may contribute to the improved contractility observed in CuR animals.

The failing heart has a decreased frequency-dependent upregulation of force due to altered SR Ca^{2+} load (11). One of the major factors involved in SR Ca^{2+} load is the activity of the SRCA, Ca^{2+} availability to SRCA, and Ca^{2+} storage in and leak from the SR (11). Another contributor to SR Ca^{2+} loading is the NCX that exchanges Ca^{2+} for Na^{+} and constitutes the major Ca^{2+} efflux mechanism in cardiomyocytes (11). In the CuD-induced heart failure model, a drastic decrease in K-dependent NCX mRNA was observed, which might be involved in the myocardial contractile defects.

The accumulation of extracellular matrix (ECM) proteins in cardiac interstitium is one of the hallmarks of the hypertrophied (especially that associated with pressure overload) and failing heart and constitutes a major component in cardiac muscle dysfunction in addition to the adverse changes that occur in cardiac myocytes *per se* (17, 32). The increase in fibrillar collagen in pathological conditions is thought to be a result of fibroblast and/or myofibroblast increased synthesis and deposition of collagen (33). The accumulation of fibrillar collagen in the ECM accounts for the increased stiffness in cardiac muscle and impaired function (33). Previously we have shown an alteration in the time constant of relaxation Tau, as well as a prolonged relaxation time, both of which are indicative of increased myocardial stiffness in the CuD heart (7). These parameters identified increased muscle stiffness through direct measurement of heart muscle function. Previous reports have shown no change in the concentration of collagen in CuD but an increased proportion of collagen III protein in CuD hearts in rats and pigs (34, 35). Others have reported an increase in both collagen I and III, especially in areas where scar tissue and increased fibrosis occurred (37), and an increase in collagen deposition in the pericapillary areas in the CuD rat heart (37). We show here that mRNA levels of collagen I and III were indeed significantly increased in the CuD mouse hearts, agreeing well with our previous observations (7) and reports in the literature (34–37). Furthermore, TGF-β expression was increased in CuD, suggesting activation of fibroblast and/or myofibroblast proliferation, leading to increases in collagen synthesis and deposition in myocardial tissue. The expression of TNF-α was also increased by CuD, and, interestingly, TNF-α expression was repressed by CuR. The decrease in TNF-α mRNA expression constitutes an interesting finding given

the involvement of this cytokine not only in immune activation and coagulation but also in nitric oxide metabolism in vascular endothelial cells.

The accumulation of ECM collagen is governed by the rate of its synthesis by fibroblasts and myofibroblasts and its degradation by matrix metalloproteinases (MMPs; Ref. 34). MMP activity is under tight regulatory control at (i) the transcriptional level, (ii) the post-translation regulation level, and (iii) inhibition by endogenous (TIMP-1, -2, -3, and -4; Ref. 34). Under pathological conditions such as myocardial infarction and atherosclerosis, MMP activity is one of the major factors in myocardial matrix remodeling. The activation of MMP-1, -2, -3, and -9 during myocardial infarction occurs early in the pathological process and is thought to be involved in the tissue repair (38); however, a downregulation of MMP expression occurs in the prolonged pressure overload condition (39). In CuD-induced cardiomyopathy, a decrease in MMPs mRNA was observed, suggesting a similar mechanism to that observed in the prolonged pressure overload condition. The net result of increased ECM collagen and decreased MMP abundance favors the accumulation of ECM components in both CuD and prolonged pressure overload. Upon CuR, an increase in MMP-2, -8 and -13 were observed. In the case of MMP-13, mRNA levels were above those found in the control. The increase in MMP expression following CuR may contribute to the resolution of accumulated ECM collagens, leading to reduced fibrosis and improved cardiac function.

A drastic increase in the vasoconstrictor peptide ET-1 mRNA levels were found in CuD hearts. Cardiac ET-1 is an important endogenous vasoconstrictor that exerts its actions through specific receptors that are widely distributed in the cardiovascular system (21). The expression of ET-1 is found in various disease conditions such as hypertension, myocardial infarction, and heart failure (40). Furthermore functional deterioration of the left ventricle during the transition from cardiac hypertrophy to failure correlates well with ET-1 levels in the myocardium, suggesting an important role of ET-1 during the transition from compensatory hypertrophy to congestive heart failure in humans as well as experimental animal models (21). Interestingly, the reintroduction of Cu into the diet of CuD animals caused a drastic decrease in ET-1 mRNA levels to below those found in CuA controls. The mechanism by which this occurs warrants further investigation, especially given the beneficial effects of lowering ET-1 levels in heart failure.

Another important finding in this study is the decrease in VEGF mRNA level in the CuD myocardium. VEGF is a potent mitogen for endothelial cells, and if introduced to an ischemic limb induces angiogenesis and improves tissue perfusion (41). It was recently found that patients with congestive heart failure have reduced circulating VEGF concentrations (17). The mechanisms by which a decrease in circulating VEGF concentrations occurs are unknown; however, the decrease in myocardial VEGF mRNA in CuD suggests a change at least at the expression level.

In summary, CuD-induced cardiac failure is associated with gene expression changes in contractile proteins, Ca^{2+} cycling proteins, extracellular matrix collagens, and MMPs, as well as ET-1, VEGF, and TNF-α. Although these changes only reflect what would happen at the transcription level and do not necessarily demonstrate the changes in protein content and function, these findings provide important information for further studies regarding the molecular mechanism of CuD-induced cardiomyopathy and its recovery by Cu repletion.

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