Copper Metabolism in Heart Disease

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Copper is an essential trace metal element that significantly affects human physiology and pathology by regulating various important biological processes, including mitochondrial oxidative phosphorylation, iron mobilization, connective tissue crosslinking, antioxidant defense, melanin synthesis, blood clotting, and neuron peptide maturation. Increasing lines of evidence obtained from studies of cell culture, animals, and human genetics have demonstrated that dysregulation of copper metabolism causes heart disease, which is the leading cause of mortality in the US. Defects of copper homeostasis caused by perturbed regulation of copper chaperones or copper transporters or by copper deficiency resulted in various types of heart disease, including cardiac hypertrophy, heart failure, ischemic heart disease, and diabetes mellitus cardiomyopathy.

Keywords: copper deficiency ; heart disease

1. Copper Transporters and Heart Physiology and Pathology

The molecular mechanisms by which copper deficiency promotes cardiomyopathy were also revealed by studies defining the roles of copper transporters. The cellular copper level is precisely coordinated by its uptake and efflux via various copper transporters, including CTR1, CTR2, ATP7A, and ATP7B. CTR1 is the major high-affinity copper importer that localizes to the plasma membrane and endosomes, whereas CTR2 is a low-affinity copper importer that localizes to endosomes and lysosomes. Ablation of CTR2 reduces the generation of truncated CTR1 lacking a copper-binding echo domain and, thus, increases tissue copper contents [1]. Compared with controls, cardiomyocyte-specific *Ctr1*-knockout mice show low cardiac copper levels and severe cardiomyopathy with cardiac hypertrophy, endocardial fibrosis, and disordered sarcomere arrays. These mice also display defects in copper homeostasis in plasma and the liver, with a decrease in hepatic copper content and an increase in serum copper concentrations due to upregulation of ATP7A expression in the liver and small intestine [2]. Consistently, intestinal epithelial cell-specific *Ctr1*-knockout mice show diminished dietary copper absorption and, thus, reduced cardiac copper uptake. These mice exhibit cardiac hypertrophy, with an increased heart-to-body mass ratio and enlarged mitochondria harboring disordered cristae and excess large vacuoles. These abnormalities can be partially rescued by postnatal copper administration [3]. A copper-deficient diet reduces heart CTR2 protein expression by 46% compared with a copper-adequate diet in rats, demonstrating a potential association of a reduction in CTR2 in cardiac copper deficiency and heart disease [4]. However, further in vivo and in vitro studies are required to determine the role of CTR2 in heart disease.

ATP7A and ATP7B are copper exporters belonging to the P-type ATPase family and contain an ATP hydrolysis domain to provide energy for copper trafficking. ATP7A is ubiquitously expressed, with the exception of the liver in normal states, whereas ATP7B is predominantly expressed in the liver and some regions of the brain, placenta, kidney, and mammary tissue [5]. Under normal conditions, ATP7A and ATP7B localize to the trans-Golgi network (TGN), where they supply copper to copper-dependent enzymes in the secretory pathway. When cytosolic copper level rises, ATP7A or ATP7B interacts with the p62 subunit of dynactin (DNCT4) and traffics to endosome-like vesicles and then to the plasma membrane, pumping excess copper into the extracellular space, or into bile in the case of the liver, to reduce the intracellular copper level [6]. By contrast, when the intracellular copper level is low, ATP7A or ATP7B recycles to TGN and transports copper from the cytoplasm into the Golgi. In Menkes disease, the loss-of-function of ATP7A impairs apical absorption of copper in enterocytes; thus, this disease is characterized by accumulation of excess copper in the small intestine and copper deficiency elsewhere. A clinical study of 95 Menkes disease patients demonstrated a 4-fold increase in the frequency of congenital heart disease (4.2%) compared with a prevalence of 1% in the general population [7]. These results confirm that dysfunction of copper transporters causes cardiomyopathy due to an imbalance of cellular copper.

2. Copper Deficiency and Heart Disease

2.1. Cardiac Hypertrophy and HF

Progression from cardiac hypertrophy to HF was divided into three stages by Meerson [8]. In the first early developing stage, the metabolic requirement of the body exceeds cardiac output, and this stage is characterized by compensatory increased protein synthesis, mitochondrial biogenesis, and enlargement, followed by increased growth of myofibrils. In the second compensatory stage, cardiac output is induced to sustain the increase in cardiac mass and performance, and this stage is characterized by an increase in myofibril growth but impaired contractility. In the last decompensated stage, the mitochondrial-to-myofibrillar ratio decreases with ventricular dilation and a decline in cardiac output.

2.1.1. Cardiac Hypertrophy

The most notable early response of the heart to copper deficiency is the initiation and progression of cardiac hypertrophy [9][10][11][12]. Cardiac hypertrophy is an independent risk factor for the development of heart diseases, including acute myocardial infarction, arrhythmia, valvular heart disease, and HF. The relationship between copper deficiency, mitochondrial defects, and cardiac hypertrophy was first described in 1970 by Goodman et al. They showed that enlargement of the mitochondrial compartment is a major contributor to cardiac hypertrophy in copper-deficient rats [13]. Copper deficiency-induced cardiac hypertrophy is concentric, resembling the effects of pressure overload, and is manifested by a thickening of the ventricular wall and interventricular septum with no change or a slight decrease in the size of the ventricular lumen. Anemia was also speculated to be a contributor to cardiac hypertrophy with copper deficiency [14]. However, other studies demonstrated that hypertrophy in copper deficiency can occur before and in the absence of anemia [15][16]. In fact, the degree of anemia appears to have no association with the degree of cardiac hypertrophy [13][17][18]. Rather, decreased CCO activity and ATP synthase function, with compensatory enlargement of mitochondria and mitochondrial biogenesis, contribute to cardiac hypertrophy [9].

In addition, Kang et al. demonstrated that in a mouse model of ascending aortic constriction-induced cardiac hypertrophy, copper supplementation attenuates cardiac hypertrophy partly by restoring expression of myocardial vascular endothelial growth factor (VEGF) and angiogenesis. Mechanistically, direct binding of CCS to hypoxia inducible factor 1α (HIF1α) mediates copper-dependent increases in HIF1α transcription activity and subsequently *Vegf* gene expression in cultured cardiomyocytes [19]. A subsequent study of rat cardiac H9C2 cells by the same group supported the critical role of VEGF in copper deficiency-induced cardiac hypertrophy [20]. Treatment with 5 μM copper sulfate attenuates hydrogen peroxide-induced cell hypertrophy, which is blunted by treatment with an anti-VEGF antibody in these cells. VEGF functions as a key regulator of induction of myocardial angiogenesis by promoting endothelial cell differentiation and migration and, thus, is crucial to sustain heart function. Numerous studies confirmed that VEGF is critical for heart disease and that inhibition of VEGF results in a transition from compensated cardiac hypertrophy to decompensated HF [21][22][23]. Indeed, although studies determining the role of VEGF in vivo using genetically modified mice are lacking, low VEGF expression is associated with HF in humans [24]. Aharinejad et al. showed that cardiac expression of VEGF was reduced in patients with dilated cardiomyopathy and HF. Therefore, activation of VEGF specifically in cardiomyocytes may be a promising approach to treat copper deficiency-induced cardiac hypertrophy. Subsequently, Kang et al. showed that hypertrophic cardiomyocytes re-enter the cell cycle and undergo mitosis and proliferation, evidenced by increased Ki-67 and phosphorylated histone H3 levels, which contribute to the development of copper deficiency-induced cardiac hypertrophy [25]. The contribution of copper deficiency to cardiac hypertrophy is clear; however, additional studies are warranted to elucidate the underlying molecular mechanisms, given that copper is involved in a variety of critical cellular processes.

In addition, the association between copper deficiency and hypertension has been reported in humans [26][27] and murine models [28][29][30]. Prolonged high blood pressure is a risk factor for cardiac hypertrophy and can result in cardiac hypertrophy by increasing the cardiac workload to meet the body's requirement. Several lines of evidence suggest that copper-regulatory proteins play a role in regulation of blood pressure. As a type of compensatory regulation, angiotensin II upregulates expression and activity of antioxidant 1 copper chaperon (ATOX1), which increases transcription and activity of extracellular SOD3 in aortas by acting as a copper-binding transcription factor and chaperone for SOD3. Therefore, *Atox1*-knockout in mice blunts the induction of SOD3 by angiotensin II and, thus, exacerbates increased vasoconstriction in mesenteric arterioles and hypertension induced by angiotensin II [31]. It was also reported that angiotensin II promotes APT7A-SOD3 interaction and delivery of copper to SOD3 in cultured vascular smooth muscle and mouse aortas. Consistently, *Atp7a*-knockout worsens angiotensin II-induced hypertension by blunting SOD3 activity in mice [32].

2.1.2. HF

Copper deficiency deleteriously affects all stages of progression from cardiac hypertrophy to HF. The hallmarks of copper deficiency-induced HF are diastolic dysfunction and a blunted response to β-adrenergic stimulation. Kang et al. showed that diet-induced copper deficiency in mice for 5 weeks starting from PND 3 results in systolic and diastolic dysfunction, including a significant 17% decrease in left ventricular peak systolic pressure (LVPSP), a ~50% decrease in the maximum rate of the rise of left ventricular pressure (+dP/dt) and in the maximum rate of the decline of left ventricular pressure (−dP/dt), as well as increases in left ventricular end diastolic pressure (LVEDP) and the duration of relaxation by 115% and 23%, respectively. Furthermore, hearts of copper-deficient mice have a blunted response to isoproterenol, a β-adrenergic agonist [33]. After 9 or 15 months of copper-restricted diet feeding, rats show cardiac diastolic and systolic dysfunction, evidenced by a blunted response of +dP/dt, −dP/dt, and LVEDP to isoproterenol [34]. Feeding a copper-adequate diet for 4 weeks to diet-induced copper-deficient mice completely restores cardiac diastolic and systolic function and the response to β-adrenergic stimulation [35], suggesting that the cardiac response to β-adrenergic stimulation requires copper.

The molecular mechanisms by which copper deficiency induces HF also include perturbation of cellular calcium homeostasis and elevated NO. Intracellular calcium homeostasis is regulated by sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA), sodium/calcium exchanger (NCX), and ryanodine receptors (RyRs) [36]. Kang et al. showed that dietary copper deficiency significantly changes expression of calcium cycling genes in mouse heart, including a decrease in the L-type calcium channel, which causes a release of calcium from the sarcoplasmic reticulum through RyRs and potassium-dependent NCX. Although cardiac function data were lacking, copper repletion via copper-adequate diet feeding compellingly normalized expression of these calcium regulatory genes in copper-deficient mice [37]. In addition, copper deficiency impairs cardiac contractile function and calcium homeostasis by elevating expression of phospholamban (PLB), which inhibits SERCA2a-dependent calcium uptake [38]. Calcium cycling is complex and requires multiple types of regulation, and additional calcium channels and regulatory proteins are reportedly involved in cardiac function and pathologies, including calcium release-activated calcium channel protein 1 (Orai1), stromal interaction molecule 1 (STIM1), and transient receptor potential cation channel, subfamily M, member 7 (TRPM7) [39][40][41]. It will be of great interest to determine whether copper regulates these proteins and their upstream regulators.

Copper deficiency reduces NO production by inhibiting SOD activity in endothelial cells and consequently impairs endothelial function [42]. However, the effect of copper deficiency on NO production in the heart differs from that in endothelial cells. Sarri et al. suggested that in the rat heart, copper deficiency enhances NO production by increasing the protein levels of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). Copper deficiency also upregulates cyclic guanosine monophosphate (cGMP) and, thus, contributes to impaired cardiac contraction in rats [43][44][45]. These studies highlight the importance of NO in copper deficiency-induced heart disease. The molecular mechanisms underlying the role of NO in regulation of heart pathology by copper deficiency warrant further investigation.

However, it is still debated how copper deficiency affects cardiac contractility. Saari et al. attributed the increased contractility of cardiomyocytes isolated from copper-deficient rats to the induction of a compensatory survival pathway through an enhancement of sensitivity to insulin-like growth factor-1 (IGF-1) [46][47]. Nonetheless, it is clear that increasing the amount of fat in the diet worsens the impaired cardiac contractile function induced by a marginal copper-deficient diet in rats [38][48][49], presumably due to dysfunctional calcium cycling in cardiomyocytes and a blunted response to β-adrenergic receptor activation [50][51][52].

2.2. Ischemic Heart Disease

Copper deficiency results in ischemic heart disease (IHD), commonly called coronary heart disease [53][54][55], via dyslipidemia through multiple mechanisms involved in dysregulation of cholesterol, fatty acid, triglyceride, and lipoprotein metabolism [56][57][58]. Copper deficiency results in the accumulation of free fatty acids in the heart and liver [58][59][60]. One possible explanation is that copper deficiency increases fatty acid synthesis by increasing nuclear localization of mature sterol regulatory element-binding transcription factor 1 (SREBP1) and thereby increasing expression of de novo lipogenic genes, including fatty acid synthase (FASN), in rat livers [59]. Copper deficiency also suppresses fatty acid oxidation and utilization. Transcriptomic analysis of the small intestine of copper-deficient rats showed that copper deficiency suppresses the expression of mitochondrial and peroxisomal fatty acid β-oxidation genes, including carnitine palmitoyltransferase 1 (*Cpt1*); L-3-hydroxyacyl CoA dehydrogenase (*Hadhb*); acyl-CoA synthetase long-chain family member 1 and 3 (*Acsl1* and *Acsl3*); δ-2-enoyl-CoA isomerase (*Peci*); and carnitine-octanoyl transferase (*Crot*) [61]. Mao et al. reported that, in hearts of copper-deficient rats, the medium-chain acyl-CoA dehydrogenase (MCAD) transcript level is low, and this contributes to cardiac lipid accumulation [62]. Dietary copper supplementation (45 mg/kg) increased fatty acid uptake and oxidation by upregulating gene expression of fatty acid transport protein (*Fatp*), fatty acid-binding protein (*Fabp*), *Cpt1*, and *Cpt2* in the liver, skeletal muscle, and adipose tissue of rabbits [63]. In addition, copper deficiency alters

the fatty acid composition toward a profile that is positively associated with IHD incidence, with an increase in medium- and long-chain saturated fatty acids in various tissues and in the circulation [64][65], while copper supplementation decreases the proportions of unsaturated fatty acids [66][67].

Moreover, copper deficiency causes hypercholesterolemia. Dietary copper restriction results in low plasma copper levels and low CP and SOD activities but increased plasma free cholesterol in humans [68] and rodents [69][70]. Conversely, supplementation of hypercholesterolemic patients with 5 mg/day copper for 45 days decreased total plasma cholesterol and slightly increased high-density lipoprotein (HDL) cholesterol [71]. It has not been thoroughly defined how copper controls cholesterol homeostasis, but animal studies provide some insights. Diet-induced copper deficiency increases hepatic glutathione (GSH) levels and subsequently increases the activity of cardiac hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, which controls the rate-limiting step of cholesterol biosynthesis [69]. Furthermore, copper deficiency significantly increases the total plasma pool of cholesterol due to enlargement of plasma volume. Mice fed a marginal copper-deficient diet (1.6 mg/kg) first show a larger pool of plasma cholesterol at 3 weeks, followed by an increase in plasma cholesterol concentrations at 5 weeks. However, in rats fed a copper-deficient diet (0.6 mg/kg), both elevations of the plasma pool size and cholesterol concentration are detected at 3 weeks [70].

Changes in the composition of lipoprotein components by copper deficiency are another contributor to IHD. In copper-deficient rats, the plasma levels of triglyceride-containing low- and very low-density lipoproteins (LDLs and VLDLs) increased by 1.6- and 2.7-fold, respectively. In addition, copper deficiency increases the susceptibility of LDLs and VLDLs to oxidation [72]. Increased lipoprotein oxidation during copper deficiency is due to increases in the triglyceride content of these lipoproteins [56] and decreases in SOD1 activity [73]. Although copper deficiency increases the expression of the lipoprotein ApoE [61] and HDL cholesterol concentrations [74][75], it does not alter the lipid composition of HDLs. In addition, the activity, but not mRNA expression, of plasma lecithin: cholesterol acyltransferase (LCAT), which catalyzes esterification of free cholesterol in HDLs, is reportedly reduced in copper-deficient rats [76]. However, it is unclear whether these HDLs in the copper-deficient state improve or worsen cholesterol efflux activity. Nonetheless, copper deficiency increases the ratio of atherogenic VLDL and LDL cholesterol to protective HDL cholesterol [74][75]. A better understanding of the molecular mechanism resulting in dysregulation of lipid metabolism caused by copper deficiency will provide new insights to reduce the incidence of IHD.

During numerous attempts to identify the causes of IHD, copper supplementation and adequate dietary copper have been demonstrated to reduce the risk of IHD. In a study of adult men with moderately high cholesterol, 2 mg/day copper supplementation for 4 weeks increased both erythrocyte SOD1 and lipoprotein oxidation lag time, the latter of which is a risk factor for IHD [77]. In another study of adult women with moderate hypercholesterolemia, 2 mg/day copper supplementation for 8 weeks elevated erythrocyte SOD1 and plasma CP levels. However, no significant changes in plasma cholesterol concentrations by copper supplementation were observed, which may be due to the small number of patients recruited and the different diets used. In addition, copper reduces the mean plasma oxidized LDL level (21 of 35 subjects showed a decrease), which helps to lower IHD risk [78]. In healthy young women, copper supplementation (6 mg/day for three 4-week periods with 3-week washouts between periods increased erythrocyte SOD1 activity and decreased fibrinolytic factor plasminogen activator inhibitor type 1 concentrations, suggesting that copper supplementation reduces IHD risks [79]. Antioxidants, particularly carotenoids, are tightly linked to IHD [80]. In humans, low levels of carotenoids are associated with a higher risk of myocardial infarction and the development of atherosclerosis and hypertension, as well as higher levels of circulating inflammatory cytokines [80]. Low circulating carotenoid levels are also associated with high oxidative stress evidenced by decreased circulating SOD levels [81]. Interestingly, in healthy adults, while copper supplementation increased red blood cell hemolysis time, which was positively and significantly correlated with circulating carotenoid levels, the administration of copper chelators reduced levels of circulating carotenoids, including lycopene and carotenes [82]. However, the molecular mechanisms by which copper deficiency promotes IHD and copper supplementation benefits IHD have not been extensively investigated and must be further elucidated.

2.3. DM cardiomyopathy

Copper-deficient diet-induced cardiomyopathy is characterized by global decreases in circulating and cardiac copper concentrations. Cu^{+} comprises 95% of total copper and localizes intracellularly, whereas Cu^{2+} comprises 5% of total copper and localizes extracellularly [83]. In contrast to copper deficiency-induced cardiomyopathy, increases in circulating copper concentrations and 2–3-fold increases in extracellular myocardial Cu^{2+} levels, but decreases in intracellular myocardial Cu^{+} levels, were reported in humans and rodents with DM cardiomyopathy. The reduced myocardial copper content and elevated systemic and total cardiac copper content in DM cardiomyopathy reflect defective uptake of copper by myocardiocytes [84][85][86][87]. Glycosylation of proteins to form advanced glycation end-products (AGEs) is a deleterious consequence of hyperglycemia in diabetes and metabolic syndrome [88]. In hearts of rats with DM, increased extracellular

Cu^{2+} increases gene expression of *Tgfβ*, *Smad4*, and collagens, which results in collagen deposition and increases the formation of AGEs of collagens. These events cause vascular injury and increase susceptibility to IHD [89]. Elevated extracellular Cu^{2+} in DM cardiomyopathy is likely loosely bound to extracellular matrix components, such as collagens [84][85]. Interestingly, in rodents and humans with DM cardiomyopathy, Cu^{2+}-selective chelators, including trientine and triethylenetetramine (TETA) dihydrochloride, prevent excessive cardiac collagen deposition, improve cardiac structure and function, and restore antioxidant defense by promoting copper excretion [84][90][91][92][93][94]. Zhang et al. reported that the expression of the *Ctr1* gene was downregulated in hearts of rats with DM, which is consistent with impaired cardiac copper uptake in DM [92]. Although TETA decreases the expression of cardiac *Ctr1* in rats, it increases CTR2 localization to the plasma membrane and, thus, concomitantly normalizes the reduced cardiac Cu^{+} levels in DM. In addition, TETA increases localization of ATP7A to the TGN and peri-nuclear region and corrects the defects in copper delivery to the secretory pathway and, thus, improves the utilization of copper by cuproenzymes, including ATOX1 and SOD1 [92]. These data suggest that TETA normalizes cardiac copper homeostasis and restores cardiac function in DM by restoring expression and localizations of copper transporters and copper-binding proteins. In addition, TETA restores mRNA and protein expression of copper chaperones, including COX11, COX17, CCS, and SOD1 and, thus, restores copper availability and trafficking, and improves cardiac functions in hearts of rats with DM [91]. Although the highly selective Cu^{2+} chelator trientine efficiently treats DM cardiomyopathy, long-term clinical studies are necessary to determine whether the improvement of cardiac function by trientine is associated with long-term benefits for mortality. Similarly, additional studies investigating the effects of trientine for treatment of other cardiomyopathies, such as IHD, are also warranted.

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