Copper ions: The invisible killer of cardiovascular disease (Review)

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Abstract. Copper, a vital trace element, is indispensable for the maintenance of physiological functioning, particularly in the cardiac system. Unlike other forms of cell death such as iron death and apoptosis, copper-induced cell death has gained increasing recognition as a significant process influencing the development of cardiovascular diseases. The present review highlights the significance of maintaining copper homeostasis in addressing cardiovascular diseases. This review delves into the crucial roles of copper in physiology, including the metabolic pathways and its absorption, transport and excretion. It provides detailed insights into the mechanisms underlying cardiovascular diseases resulting from both excess and deficient copper levels. Additionally, it summarizes strategies for treating copper imbalances through approaches such as copper chelators and ion carriers while discussing their limitations and future prospects.

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1. Introduction

Cardiovascular diseases, including cardiac arrhythmias, atherosclerosis and heart failure (HF), are diseases of the circulatory system and are a leading cause of morbidity and mortality worldwide (1). According to the World Health Organization, cardiovascular disease accounts for 31% of the deaths worldwide (2). Despite the numerous therapeutic interventional methods, cardiovascular diseases account for approximately one-third of all deaths worldwide (3). Cardiovascular diseases aggravate healthcare costs and are a significant economic burden (4). Currently, the present understanding of the mechanisms and risk factors underlying the pathogenesis of cardiovascular diseases is limited, although there is increasing interest in the role of trace elements in cardiovascular diseases (5). Therefore, it is crucial to elucidate the composition of trace elements in cardiovascular diseases relative to physiological levels and provide valuable insights into treatment strategies for cardiovascular diseases.

Copper is primarily obtained from the consumption of vegetables, shellfish, meat, seeds and nuts (6), and plays a crucial role as a trace element in maintaining physiological functioning (7). There are two ionic forms of copper found in the body, Cu^{+} (cuprous ion, reduced form) and Cu^{2+} (copper ion, oxidized form) (8), both of which are involved in regulating enzymatic cellular functions (9). Generally, Cu^{2+} is converted to Cu^{+} by reductase enzymes such as duodenal cytochrome b (DCYTB) and six-layer epithelial antigen (Fig. 1) (10). Once taken up by copper transporter 1 (CTR1), Cu^{2+} is transported to different organelles in the cytoplasm and is metabolized by a copper chaperone for cytochrome c oxidase [CCO; COX (cytochrome c oxidase assembly homolog (COX)] 17 (11) and antioxidant-1 (ATOX1) (12).

Copper acts as a catalyst for numerous physiological processes, including energy metabolism, mitochondrial respiration and antioxidant activity (13). Copper is present in the body in its ionic form (14). When the homeostatic balance of copper ion levels is disrupted, such as through excess copper ions, this imbalance can trigger cellular toxicity and induce cell death via various pathways (15). Dysregulation of copper ions can disturb lipid metabolism, resulting in oxidative stress, mitochondrial damage and endothelial cell dysfunction (14), and induce atherosclerosis and other cardiovascular diseases such as arrhythmia and cardiomyopathy (15). While there is

a considerable body of literature describing the initial causes of copper dysregulation, there is a dearth of comprehensive exploration into the underlying pathological consequences (16). The primary treatment for dysregulated copper ion levels in cardiovascular diseases involves copper chelating agents; however, alternative methods such as copper ion carriers may also be used but are constrained by technical limitations that necessitate further research and improvement (17).

The present review aims to elucidate the mechanisms by which copper ions are involved in cardiovascular diseases, summarize the impact of copper ion abnormalities on cardiovascular diseases and potential therapeutic approaches, and investigate whether modulating copper ion levels can ameliorate cardiovascular diseases. This review offers innovative perspectives for managing cardiovascular diseases via the regulation of copper levels and paves the way for novel research directions. The inclusion criteria for the present study included: i) Research hypotheses and methods were similar to the research content of the present article to ensure the accuracy of the narrative; ii) the exact date that the research was conducted or published; iii) clear regulations on sample size; iv) clear criteria for patient selection, case diagnosis and staging; v) clear measures for intervention and control; vi) can provide OR (odds ratio) [relative risk (RR), rate difference and hazard ratio (HR)] and its 95% confidence interval, or can be converted into OR (RR, rate difference and HR) and its 95% confidence interval; and vii) if it is measurement data, the mean, standard deviation and sample size should be provided. The literature exclusion criteria were as follows: i) Duplicate reports; ii) research design flaws or poor quality; iii) incomplete data or unclear outcome effects; iv) the statistical method was incorrect and could not be corrected; v) OR was not provided or could not be converted into OR (RR, rate difference, HR) and its 95% confidence interval; vi) the measurement data could not provide mean and standard deviation; and vii) inaccurate animal experiments.

2. Roles and metabolic pathways of copper

Copper, a trace element essential for life, plays a crucial role in various physiological functions such as respiration, connective tissue formation, wound repair, nutrient energy metabolism and catecholamine synthesis (18). Additionally, copper serves as an important regulator of numerous enzymes involved in physiological processes including neuromodulation and angiogenesis. Dyla *et al* (19) demonstrated the key role of P-type Wilson ATPase in preventing copper deficiency or toxicity by facilitating the transfer of copper from the liver to the secretory pathway. Maintaining copper homeostasis relies on copper transport proteins; dysregulation and subsequent copper toxicity can occur if this process is disrupted (20). Recent studies have revealed that copper toxicity significantly impacts normal cardiac function (21) and contributes to pathologies including myocardial ischemia/reperfusion (I/R) injury (22), HF (23), atherosclerosis (24) and arrhythmias (25).

The physiological processes of copper metabolism are multifaceted but can be broadly categorized into three main stages: Absorption, transportation and excretion (Fig. 1). The most effective form of copper absorption occurs within the intestinal epithelial cells, where dietary copper is digested and absorbed as Cu^{2+} mediated by divalent metal transport protein 1 (26). While dietary copper typically exists in the form of Cu^{2+}, only Cu^{+} can be absorbed and utilized by the body (27). Therefore, in various cell types, Cu^{2+} often requires reduction to Cu^{+} through the action of reductases such as DCYTB, and uptake via a high-affinity mechanism involving CTR1 (28). COX17 facilitates the transport of copper ions to specific proteins such as cytochrome C oxidase (SCO) 1, SCO2 and COX11 to activate enzyme activity within the respiratory chain (29). Chaperone protein copper chaperone for superoxide dismutase (CCS) facilitates the transport of copper ions to superoxide dismutase 1 (SOD1) (28), and ATOX1 plays a crucial role in transporting copper ions to the nucleus for binding with transcription factors to regulate gene expression while also transferring them from the trans-Golgi network (TGN) to ATPase α-peptide (ATP7A) and ATPase β-peptide (ATP7B) (30). ATP7A facilitates the efflux of Cu^{+} from the intestinal epithelium into the circulation whereas ATP7B stores excess Cu^{+} in intracellular vesicles to maintain normal homeostasis (31). Copper bound with ceruloplasmin (CP) or albumin can be transported within specific organelles or secreted from cells and transferred to the liver via the bloodstream (32). In hepatocytes, ATP7B plays a crucial role in facilitating the p62-mediated release of copper from intracellular stores into the cytoplasm, thereby enabling the excretion of surplus copper through its incorporation into bile (33).

3. Copper ions: The 'killer' of cardiovascular disease

Mechanisms of copper ion-induced apoptosis in cardiovascular disease. Apoptosis induced by copper ions is a crucial step in several cardiovascular diseases, involving oxidative stress, copper-mitochondrial crosstalk and vascular homeostasis (34). These mechanisms can contribute to the development of atherosclerosis, myocardial injury and coronary heart disease (35). Oxidative stress is associated with excess copper ions, while copper ion deficiency affects the association between copper and the mitochondria, as well as copper and vascular regulation (36).

Cells meticulously maintain a delicate balance between oxidation and antioxidant capacity (37). Disruption of oxidative homeostasis in the cardiovascular system can lead to oxidative stress, causing cellular damage and cardiovascular disease (38). Copper ions undergo cycles of oxidation and reduction, generating hydroxyl radicals which can cause DNA damage and lipid peroxidation (39). Excess copper promotes glutathione (GSH) oxidation, leading to catecholamine oxidation (39). Copper-mediated Fenton reactions induce oxidative stress, which disrupts lipid metabolism and induces DNA fragmentation (14). Direct binding of copper ions to fatty acylated components in the tricarboxylic acid (TCA) cycle results in protein aggregation and dysregulation (40), blocking the TCA cycle and inducing proteotoxic stress and cell death (Fig. 2) (41).

Mitochondria coordinate cellular metabolic processes and serve as a comprehensive source of metabolism and energy (42). Micronutrients are essential for proper mitochondrial function, particularly in the cardiac muscle cells (43). In the latter scenario, it is crucial for the activation of Cu^{+} enzyme function within the respiratory chain and for ensuring the

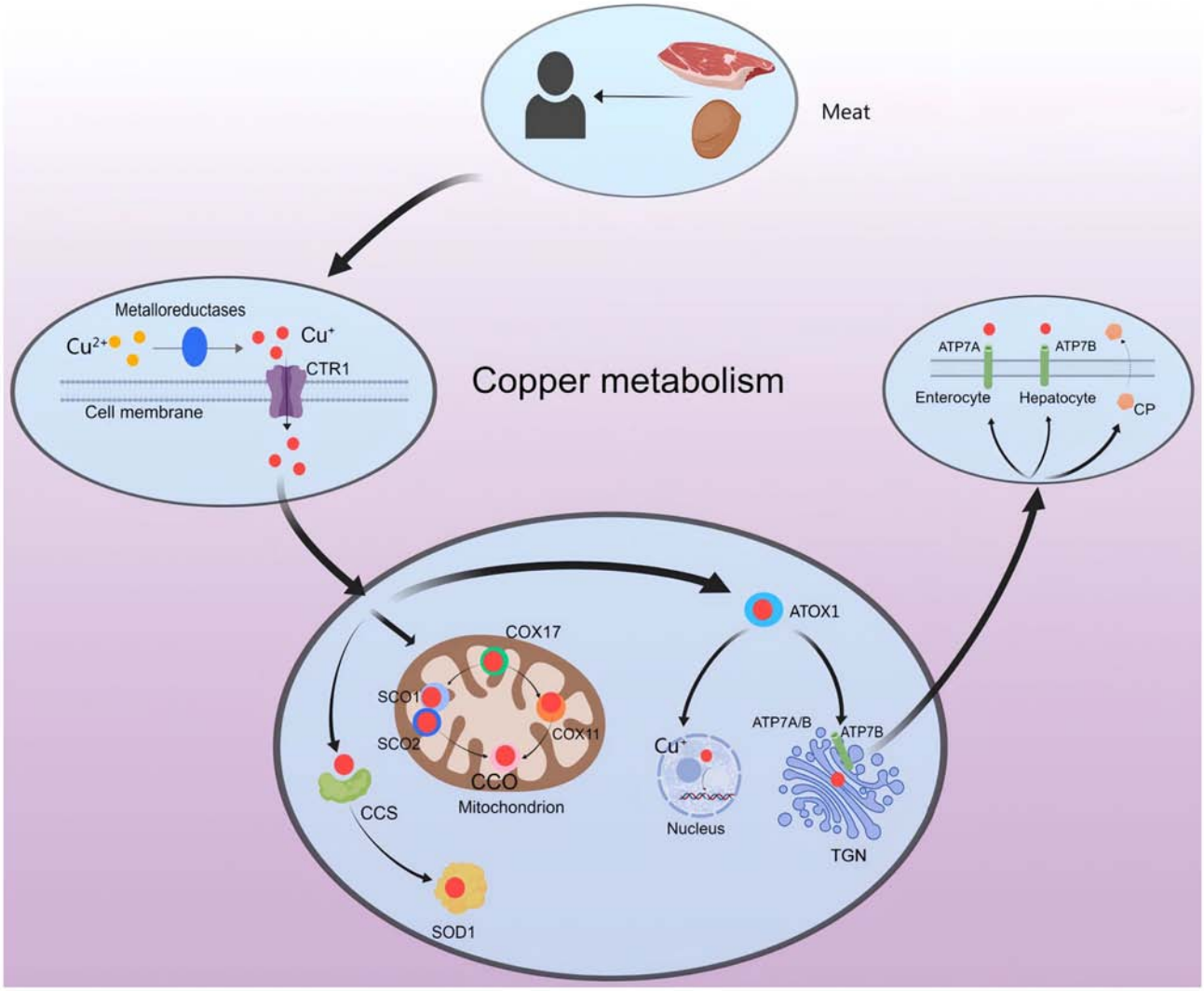


Figure 1. Intracellular copper metabolism. CTR1, copper transporter 1; CP, ceruloplasmin; TGN, trans-Golgi network; CCO, cytochrome c oxidase; CCS, copper chaperone for superoxide dismutase; ATP7A, ATPase α-peptide; ATP7B, ATPase β-peptide; COX, cytochrome c oxidase; SOD1, superoxide dismutase 1; SCO1,cytochrome C oxidase 1; SCO2, cytochrome C oxidase 2.

physiological function of CCO (44). Copper deficiency results in reduced transport via COX17 to SCO1/SCO2 and COX11, leading to diminished CCO synthesis (45). In addition, mitochondrial dysfunction occurs due to copper deficiency via an increase in the expression of other mitochondria-associated protein molecules (46). Specifically, increased expression of peroxisome proliferator-activated receptor-γ coactivator-1α protein, a key regulator of mitochondrial biosynthesis, disrupts the mitochondrial structure and leads to dysfunctional proliferation, which is involved in the development of certain cardiac diseases, such as heart failure and atherosclerosis (45). CCO activity and expression, leading to cardiomyocyte fibre stiffening, ultimately contribute to fatal cardiovascular diseases (44). In addition, exacerbation of the reduction in ATP and observed phosphocreatine levels, is accompanied by an increase in ADP and orthophosphate levels, in both cardiac tissue and other organs (30). Changes in the structure of the cristae and mitochondrial membranes are concomitant with these alterations, eventually leading to mitochondrial rupture, which impairs energy metabolism and induces myocardial injury (46) (Fig. 3).

Hypoxia-inducible factor 1 (HIF-1) is the primary transcription factor regulating angiogenesis (47). Following ischemic injury, the copper concentration in the heart gradually decreases and is positively associated with HIF-1-mediated angiogenesis and the expression of angiogenesis and glycolysis-associated genes (48). HIF-1α, a crucial subunit of HIF-1, plays a major role in regulating HIF-1 activity during a myocardial infarction (49). Copper plays a role in multiple aspects of HIF-1 regulation, including the stabilization of HIF-1α, formation of transcriptional complexes and binding to hypoxia-responsive element (HRE) sequences of target genes (50). Copper transport into the nucleus is facilitated by CCS and subsequently mediated by the copper-binding protein (CuBP) (49). The 'GGAA' core motif is critical for binding to copper-dependent gene sites (49). Additionally, p300, also termed CREB-binding protein, and steroid receptor coactivator-1 act as cofactors to form the HIF-1 transcriptional complex 38 (49). Copper also plays a role in mediating the interaction between HIF-1 and HRE to initiate the expression of copper-dependent genes such as VEGF (49). For vascular maturation, lysyloxidase (LOX) is critical, and copper can modulate LOX production through

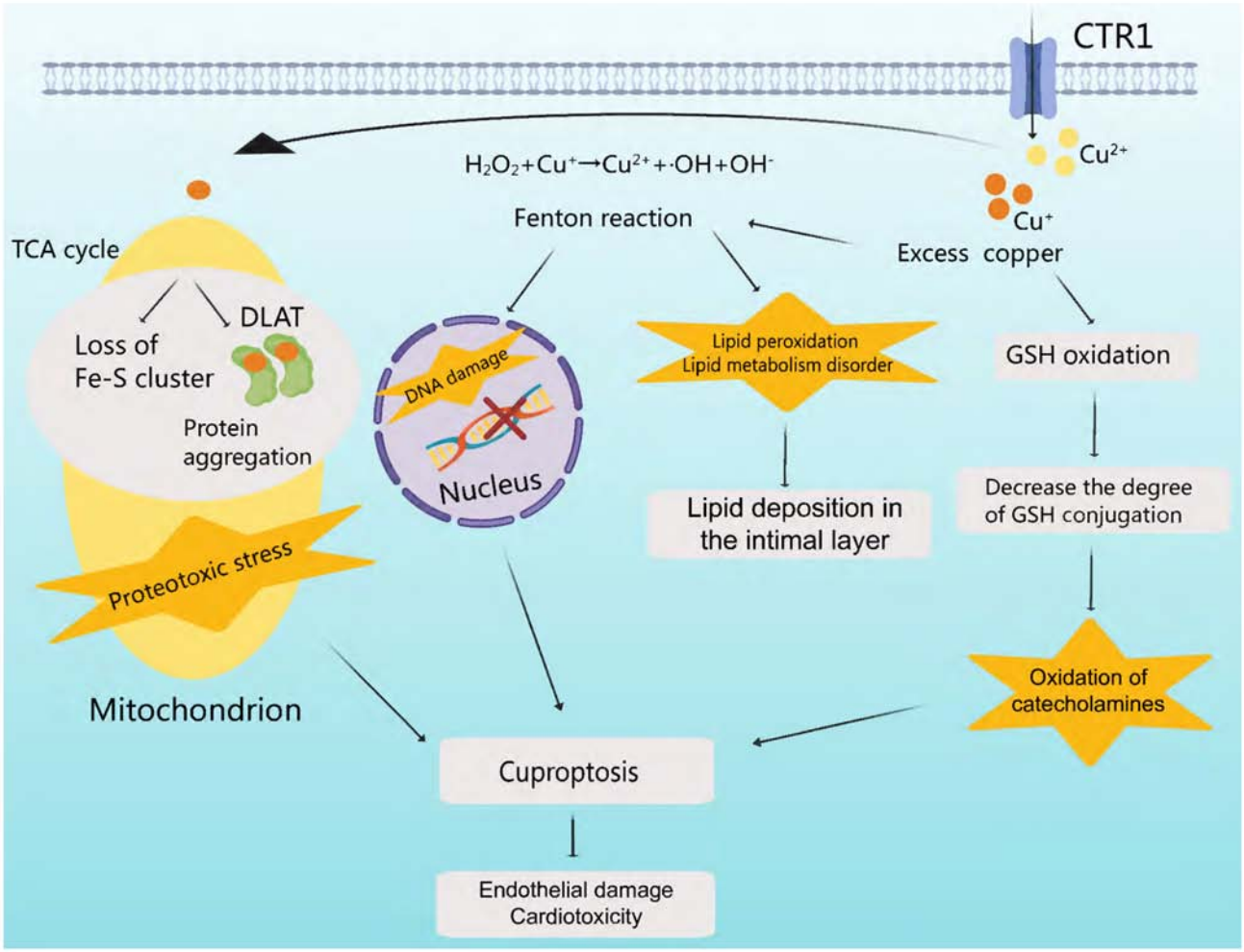


Figure 2. Mechanisms of oxidative stress. CTR1, copper transporter 1; GSH, glutathione; TCA, tricarboxylic acid; DLAT, dihydrolipoamide S-acetyltransferase.

ATOX1, ATP7A and Ras-related C3 botulinum toxin substrate 1 (RAC1) (51). Increased copper efflux is observed during ischemia and under hypoxic conditions (50). Inhibition of these mechanisms by copper efflux results in reduced vascular wall tone, increased myocardial fragility, reduced angiogenesis and ultimately myocardial injury (Fig. 4) (52).

Effects of copper excess on cardiovascular diseases. A summary of the effects of excess copper on cardiovascular diseases is provided in Table I. When excess copper is ingested, oxidative stress caused by excessive copper can lead to various problems, including lipid metabolism disorder, thus inducing (14) cardiovascular diseases such as arrhythmia, atherosclerosis and HF.

When a patient experiences an arrhythmia, it may indicate the presence of an abnormal heart rhythm, such as premature beats, atrial fibrillation, ventricular fibrillation or ventricular tachycardia (53). Elevated serum copper levels are often observed in these patients due to the release of copper-containing enzymes after myocardial injury or because sympathetic nerves promote the release of copper from liver stores into the bloodstream during stress (54). Hsiao *et al* (54) demonstrated that copper disrupts the cardiac rhythm of zebrafish larval embryos. A trial with Mediterranean mussels showed that high Cu^{2+} concentrations led to valve closure and a decreased heart rate in mussels (55). Bobbio *et al* (56) found a high prevalence of ventricular fibrillation and tachycardia in patients with arrhythmia, which was associated with myocardial copper accumulation.

Atherosclerosis, inflammation and the accumulation of lipids in the inner layer of blood vessel walls lead to vascular blockage and contributes to the development of coronary heart disease, cerebrovascular disease and peripheral arterial vascular disease, and is associated with significant clinical morbidity and mortality rates (57). Extensive research has demonstrated a close association between elevated serum copper levels and atherosclerosis (58). Copper serves as a cofactor for numerous enzymes and participates in normal physiological processes. However, excessive levels can exert toxic effects, causing cellular damage or even death (58). A previous study revealed a positive association between copper levels and the progression of atherosclerosis (59). Another study involving patients with acute myocardial infarction have supported these findings by showing significantly higher serum copper levels in those with acute myocardial infarction compared with non-acute cases (60). Copper plays a role in the oxidative modification of low density lipoprotein (LDL) and influences their susceptibility to oxidation; increased copper levels promote LDL oxidation and stimulate the production of oxidized lipoproteins, thus promoting the formation of

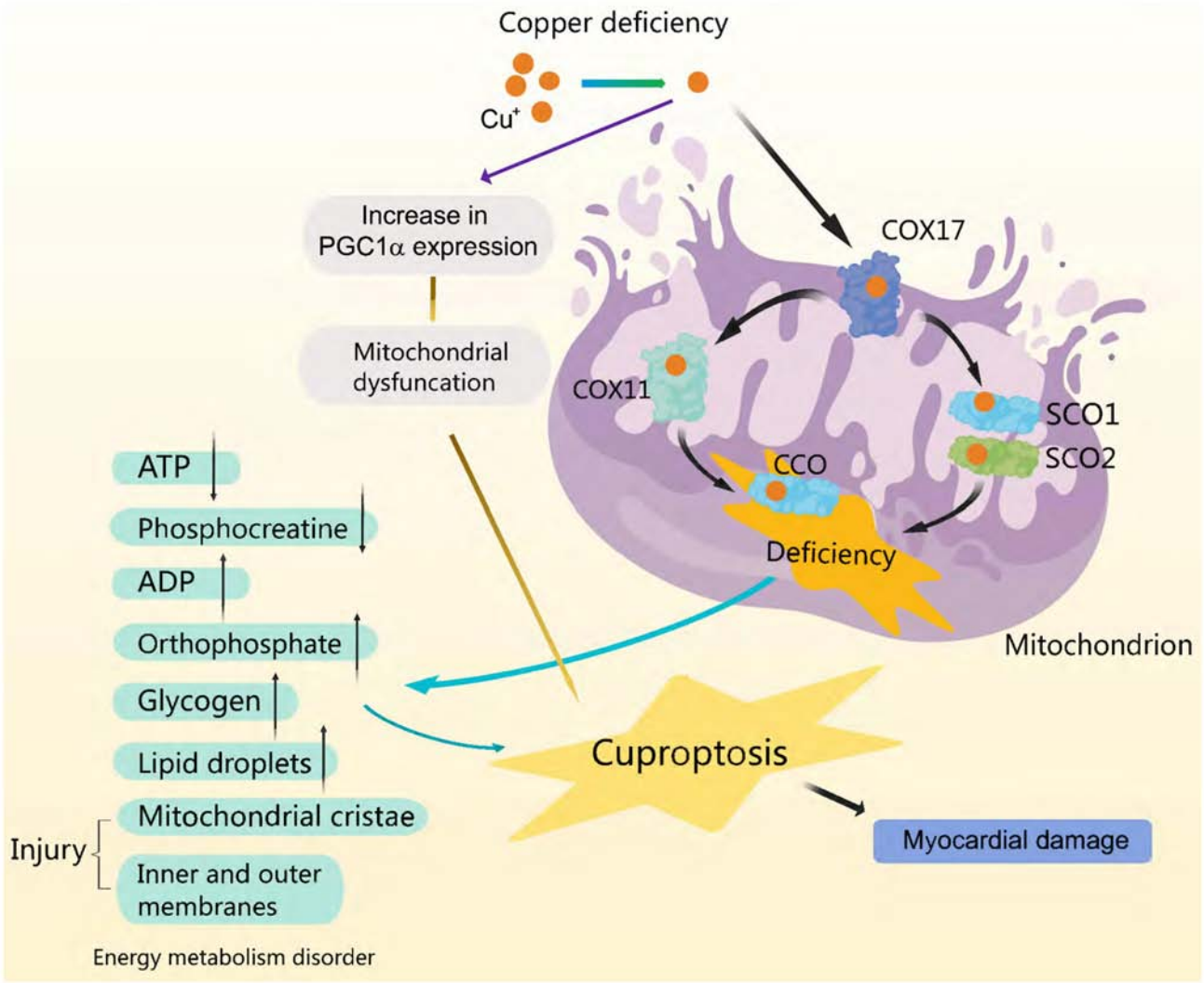


Figure 3. Copper and mitochondrial function. CCO, cytochrome c oxidase; PGC1α, peroxisome proliferator-activated receptor-gamma coactivator-1α protein; COX, cytochrome c oxidase copper chaperone; SCO, synthesis of cytochrome C oxidase.

atherosclerotic plaques (61). Furthermore, increased copper levels can increase reactive oxygen species (ROS) production and activate the NF-κB signalling pathway, exacerbating inflammatory changes within the vascular wall and promoting atherosclerosis (25). A study involving mice also found that the release of free copper ions induced neointimal thickening and contributed to the development of atherosclerotic lesions in damaged rat carotid arteries (62).

HF is characterized by impaired pumping function of the heart and inadequate cardiac output, which cannot meet the metabolic demands of bodily tissues due to multiple causes. It represents the terminal stage in the progression of various cardiovascular diseases (63). Mitochondrial energy supply, inflammation levels and intracellular oxidative stress are key mechanisms in the pathogenesis of HF (64). Of particular importance, copper plays a regulatory role in several biological processes associated with HF (65). A meta-analysis (66) incorporating 1,504 patients revealed a significant association between elevated serum copper levels and HF. Similarly, an animal model of diabetes-induced HF showed altered expression levels of copper transporter proteins and disturbed copper metabolism in rat cardiomyocytes (67). Fluctuations in copper homeostasis during HF may disrupt mitochondrial function and exacerbate oxidative stress (66). Elevated copper levels have been demonstrated to decrease the activity of antioxidant enzymes such as catalase and GSH peroxidase in rat tissues, leading to DNA damage via peroxygen-derived free radicals (68). In primary cardiac cells, Cu^{2+} has been shown to increase interleukin-6 release and activate MAP kinase (69), contributing to cardiac inflammation and hypertrophy (59). Copper-induced oxidative stress also promotes inflammation through ROS production, resulting in lipid, protein and DNA damage (70).

Mutations in sarcomeres in hypertrophic cardiomyopathy (HCM) lead to cardiac fibrosis, which affects contractility (71). Typical histopathological features of the disease include myocyte disorganization and changes in myocardial fibrosis (72). A study on patients with HCM revealed abnormal copper accumulation, and the use of the Cu^{2+} selective chelator tretinoin hydrochloride was found to slow or reverse disease progression in HCM (73). Accumulation of ROS plays a crucial role in the pathogenesis of cardiomyopathy, leading to myocardial fibrosis, ventricular remodelling and direct damage to cardiomyocytes (74). Excess copper ions can

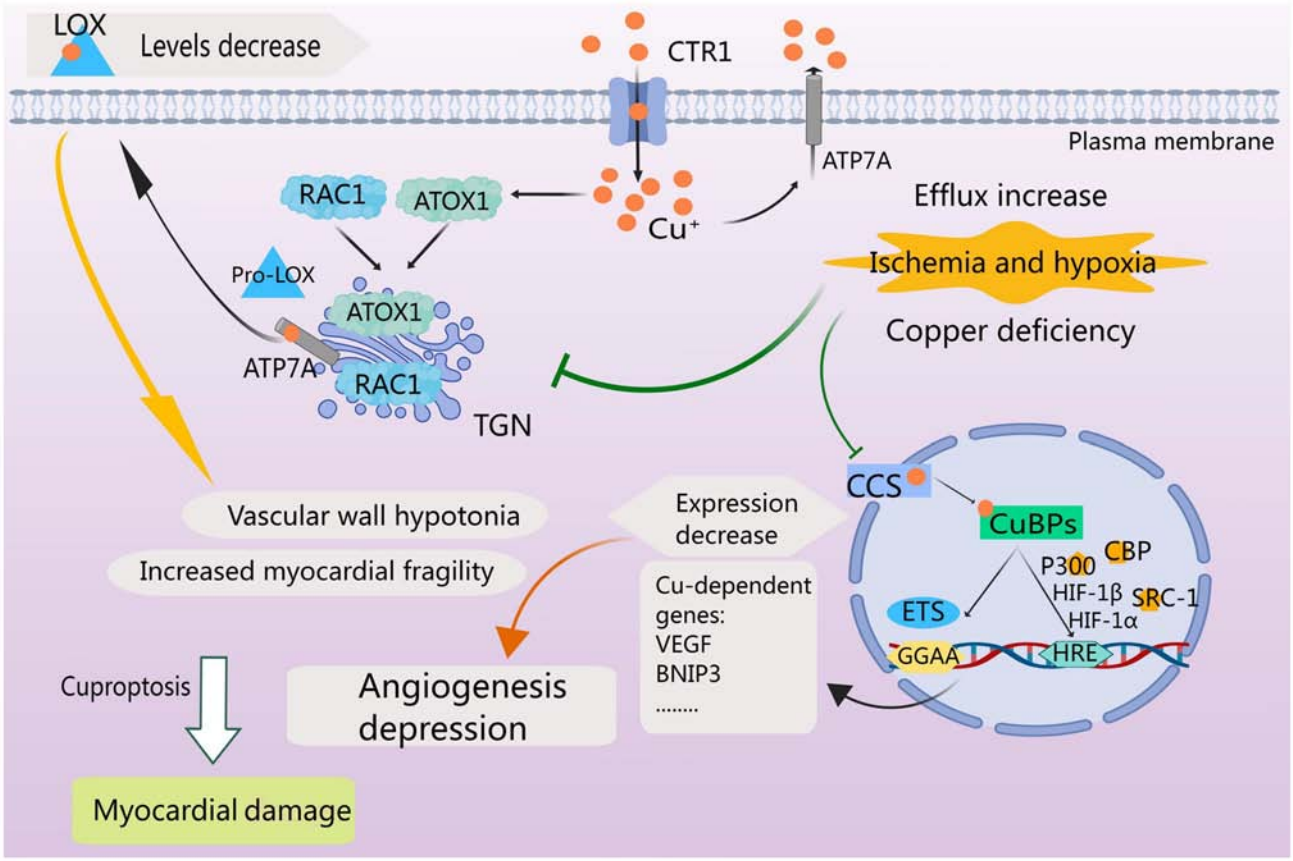


Figure 4. Copper and vascular regulation. CTR1, copper transporter 1; ATOX1, antioxidant-1; CCS, copper chaperone for superoxide dismutase; HRE, hypoxia-responsive element; HIF, hypoxia-inducible factor; SRC-1, steroid receptor coactivator-1; BNIP3, pro-apoptotic mitochondrial protein; LOX, lysyloxidase; ATP7A, ATPase α-peptide; RAC1, Ras-related C3 botulinum toxin substrate 1; CuBPs, copper-binding protein; CBP, colostrum basic protein; ETS, biomanipulation.

catalyse the formation of destructive hydroxyl radicals via the Fenton reaction, resulting in oxidative stress and inflammatory responses that cause structural and morphological changes in cardiac tissue (75). In addition to oxidative stress, excessive copper ion accumulation alters energy metabolism patterns within cardiomyocytes by inducing abnormal aggregation of thioctylated proteins and loss of iron-sulfur cluster proteins in the respiratory chain complex through direct binding to thioctylated proteins in the mitochondrial TCA cycle (76).

Excess copper can lead to the development of aortic aneurysms, which are characterized by abnormal dilation of the aortic wall and compression of the surrounding organs (77). The aetiology of aortic aneurysms is complex, with a previous a study suggesting possible links to inflammation, copper toxicity and endothelial cell damage (78). In cases of pathological inflammation in aortic aneurysms, tissue copper levels are significantly elevated (79). Excess copper disrupts the balance between NO production and degradation, which plays a crucial role in regulating vascular tone and endothelial function (79). Elevated copper levels upregulate inducible NO synthase expression, leading to excessive NO production; peroxynitrite is then formed as a potent oxidant that can cause further oxidative damage (80). Furthermore, copper interacts with atherosclerotic risk factors such as homocysteine, resulting in increased hydrogen peroxidation and oxidative stress (81). Please refer to Table I for details (82-92).

Effects of copper deficiency on cardiovascular disease. Not only does an excess of copper lead to cardiovascular disease, but copper deficiency may also result in alterations to cardiac morphology, swelling of the mitochondria, and fragmentation and enlargement of myocytes (93). During a copper-deficient state, the copper deficiency leads to mitochondrial dysfunction and ROS accumulation, which in-turn lead to cardiovascular diseases including myocardial I/R injury (94). Nutritional surveys conducted in the West as far back as the 1990s revealed a significant decline in dietary copper content, with half of adults consuming less than the amount recommended by the European Community and the United Kingdom (95). Additionally, at least a quarter of adults were found to consume less than the average estimated requirement published by the United States and Canada, which suggests that diseases such as Alzheimer's disease, ischemic heart disease and osteoporosis are linked to a low intake of copper (96). Data from one study summarized information from >60 medical publications incorporating >2,500 patients suffering from cardiovascular, musculoskeletal and neurological disorders due to copper malnutrition. Of these patients, >1,000 benefited from copper supplementation (97). Saari (98) demonstrated that dietary copper deficiency resulted in various cardiovascular defects with systemic effects including hypertension, increased inflammation, anaemia decreased blood clotting and possibly atherosclerosis, which also has effects on specific organs or

Table I. Effects of excessive high levels of copper on cardiovascular disease.

First author/s, year	Type of disease (animal/human)	Treatment	Duration	Pathological manifestations	(Refs.)
Hsiao *et al*, 2020	Arrhythmia (zebrafish)	Copper sulfate pentahydrate	48 h	The action of copper makes the heart rate of zebrafish embryos irregular.	(54)
Bagheri *et al*, 2015	Patients with angina pectoris (human)	Coronary angiography	12 years	The content of copper in serum is higher than that in normal individuals.	(57)
Chen *et al*, 2023	Atherosclerosis (human)	N	16 years	The level of serum copper in patients who died of coronary heart disease was higher than that in other patients.	(59)
Yuan *et al*, 2022	Heart failure (human)	N	5 years	Copper content is proportional to the risk of heart failure.	(65) (82)
Kunutsor *et al*, 2021	Atherosclerosis (human)	N	27 years	The high copper content in serum increases the incidence of atherosclerosis.	
Zhu *et al*, 2020	Atherosclerosis (human)	N	10 years	The incidence of atherosclerosis is proportional to the content of urinary copper.	(83)
Alexanian *et al*, 2014	Heart failure (human)	N	5 months	The content of serum copper is proportional to the risk of heart failure and related to left ventricular systolic and diastolic function.	(84)
Malek *et al*, 2006	Heart failure (human)	N	1 years	The content of serum copper is inversely proportional to the prognosis of heart failure.	(85)
Nyström-Rosander *et al*, 2009	Aortic aneurysms (human)	Operation of thoracic aortic aneurysm	2 years	The increase of serum copper content can increase the incidence of thoracic aortic aneurysm in patients.	(86)
Koksal *et al*, 2007	Aortic aneurysms (human)	Surgery for abdominal aortic aneurysm or aortic occlusive disease	5 years	The high levels of iron and copper in the patient's body lead to an increase in oxidative pressure, which may be one of the factors leading to the formation of aneurysms.	(87)
Qin *et al*, 2010	Atherosclerosis (mouse)	High fat/ cholesterol diet	12 weeks	The downregulation of ATPase copper transport α can attenuate the oxidation of low density lipoprotein through hunman monocytic leukemia-derived macrophages.	(88)
Ploplis *et al*, 2001	Atherosclerosis (mouse)	1-1.5 mm copper/ silicone cuff	3 weeks	Copper induces intimal regeneration and increases adventitia collagen deposition.	(89)
Bini *et al*, 2015	Arrhythmia (Lobster)	Cu	3 h	Copper lowers the heart rate of the lobster.	(90)
Li *et al*, 2021	Myocardial injury (pig)	Cu	80 days	High density of copper can cause cardiotoxicity.	(91)
Li *et al*, 2017	Myocardial injury (chicken)	Copper sulfate	12 weeks	Copper can increase myocardial enzyme activity and induce cardiac injury and autophagy.	(92)

N, no information found.

tissues such as diminished structural integrity of the heart and blood vessels, impaired energy use by the heart, decreased contractility of the heart, altered ability for blood vessels to control their diameter growth and structural-functional changes in circulating blood cells.

Copper deficiency hinders the function of mitochondria and energy production, which leads to impaired mitochondrial respiration and ECG abnormalities in copper-deficient hearts (99). For example, myocardial dysfunction has been linked to mitochondrial dysfunction caused by copper deficiency-induced expression of molecules related to mitochondria (100). Oxidative stress (101), inflammation, endothelial dysfunction and impaired lipid metabolism (102) may be associated with the mechanism behind copper deficiency-induced atherosclerosis. Enzyme function is also compromised by copper deficiency, including that of copper-zinc SOD (Cu-Zn SOD), resulting in a weakened antioxidant defence system and increased vulnerability to oxidative stress, contributing to the development of atherosclerosis (103). Furthermore, copper deficiency impairs the activity of certain Cu/Zn-oxides, which may lead to increased accumulation of ROS and oxidative stress, which further promotes inflammation and atherosclerosis (104). Other research has indicated that copper deficiency inhibits the expression of adhesion molecules, while activating endothelial cells through inducing leukocyte adhesion (105). Cholesterol levels are a significant risk factor for atherosclerosis, as demonstrated by a study by Habas and Shang (106), which found that copper deficiency elevated cholesterol levels and impacted lipid metabolism thereby influencing the development of atherosclerosis (107). Additionally, Jeney *et al* (108) suggested that reduced NO levels due to SOD1, itself induced by copper deficiency, may contribute to atherosclerosis by hindering vasodilation.

The mechanism of myocardial I/R injury caused by copper deficiency may be attributed to the upregulation of inflammatory factors such as interleukins and free radicals due to the excessive accumulation of ROS (109). By contrast, relatively low levels of copper may exacerbate the inflammatory response during I/R injury (22). Copper supplementation may mitigate tissue damage during this process (109). An early study indicated that copper deficiency decreases CCO activity in the heart (110). Thus, copper deficiency in cardiomyocytes significantly reduces the expression of copper chaperones and the enzymatic activity of CCO, leading to a decrease in left ventricular (LV) copper content and impaired LV contractile function in dilated cardiomyopathy (111). Copper deficiency also induces cardiac hypertrophy (112). However, a direct reduction in the size of certain hypertrophic cardiomyocytes and replication of other size-reduced hypertrophic cardiomyocytes contribute significantly to the regression of copper-deficient cardiac hypertrophy, resulting in the normalization of the size and number of cardiomyocytes in the heart (113). It has also been demonstrated that copper deficiency decreases vascular elasticity and increases platelet aggregation, thereby increasing the risk of ischemic vascular disease (114). Furthermore, a nutritional study has shown that prolonged periods of dietary copper deficiency can lead to elevated cholesterol, blood pressure, homocysteine, isoprostane and uric acid levels; adversely affect arteries and the cardiac rhythm; decrease dehydroepiandrosterone levels; impair glucose tolerance and paraoxonase activity; and promote thrombosis and oxidative damage (115).

In mouse experiments, Klevay and Viestenz (116) observed that copper-deficient rats exhibited a range of electrocardiographic abnormalities, including ST-segment depression spanning one-third to one-half of the RR interval, bundle-branch block with R-wave heights and widths three times the normal values, Q-waves, and second- and third-degree heart block. Copper deficiency reduced the life span of rats by 73%, and Viestenz and Klevay (117) also reported that dietary copper deficiency led to ECG abnormalities in rats.

4. Therapeutic approaches to address copper ion metabolism abnormalities and the associated cardiovascular diseases

Despite high levels of research into the understanding of the role of copper physiologically and pathophysiologically, there remain uncertainties regarding the accurate measurement of copper levels and the long-term effects of copper exposure on cardiovascular health (118). The World Health Organization has recommended a daily intake of 0.9 mg/day for adults weighing 70 kg and has specified a safe upper limit (119). The U.S. Environmental Protection Agency (EPA) has not formally established an oral reference dose for copper or a maximum acceptable dose for inclusion in its Integrated Risk Information System database (120). Toscano *et al* (121) demonstrated that Wistar rats exposed to copper for 30 days showed a significant increase in blood pressure and myocardial contractility. Another study emphasized the potential adverse effects of copper exposure on myocardial contractility at recommended daily doses, tolerable maximum intake doses, and twice the tolerable maximum intake level, but the validity of these doses requires verification through extensive experimentation (122). Serum copper concentration is influenced not only by food and environmental intake, but also by absorption, excretion and storage, due to human variability. Therefore, it is challenging to estimate the individual benefits or losses from serum copper concentration or determine an optimal level, and researching these issues remains a challenge (123).

Currently, treatments based on copper metabolism in cardiovascular disease can encompass a wide range of options, including copper chelating agents [such as triethylenetetramine (TETA), tetrathiomolybdate (TTM) and disodium ethylene diamine tetraacetic acid (EDTA)], small-molecule inhibitors of copper chaperone proteins (such as DCAL50), copper ion carriers and natural antidote agents (Table II).

Copper chelators are compounds that bind to copper ions to remove toxic copper from cells and prevent its accumulation (124). However, excessive chelation of copper by these compounds can lead to cellular copper deficiency and cell death (9) (Fig. 5). TETA is a chelator that specifically binds to Cu^{2+} ions and has been widely used for the treatment of Wilson's disease (125). It may improve myocardial function in diabetic patients by restoring mitochondrial CCO, CCS and SOD1 activity (67). Additionally, it inhibits the elevation of serum copper levels and effectively mitigates the increase in CP activity following myocardial ischemia (126). TTM is a small hydrophilic compound with high specificity as a copper chelator. It is commonly used to treat Wilson's disease and exhibits a favourable safety profile in this regard (127).

Table II. Treatment strategies for copper dysregulation.

First author/s, year	Type of disease (animal/human)	Drugs	Pathological effects	(Refs.)
Toscano *et al*, 2022	Complications of diabetes (human)	EDTA	Reduce the recurrence of cardiovascular events in patients with diabetes mellitus.	(121)
Kannan *et al*, 2024	Atherosclerosis (mice)	TTM	TTM inhibits atherosclerosis in apolipoprotein E mice by reducing bioavailable copper and vascular inflammation.	(125)
Gromadzka *et al*, 2023	Hypertrophic cardiomyopathy (human)	Trientine	Improvement of mitochondrial function in patients with hypertrophic cardiomyopathy.	(128)
Zou *et al*, 2024	Myocardial injury (human)	Trientine	Restore myocardial expression and enzyme activity.	(127)
Ferrero *et al*, 2022	Vascular disease (rats)	DCAL50	Inhibit tumour growth and angiogenesis and prevent the formation of the endothelial cell network.	(130)
Ramli *et al*, 2022	Myocardial infarction (human)	Curcumin	Significantly protect cardiac function and reduce myocardial infarction size.	(133)
Banfi *et al*, 2007	Cardiac dysfunction (rat)	TTAT	Divalent copper chelate triethylenetetramine can improve cardiac pump function.	(143)
Alvarez *et al*, 2010	Myocardial ischemia (rat)	TTAT	Inhibit the increase of copper content in serum, effectively eliminate the increased ceruloplasmin activity after ligation and improve myocardial ischemia.	(144)
Zhang *et al*, 2013	Cardiomyopathy (rat)	TTAT	Restore left ventricular function and improve cardiomyopathy in rats.	(145)
Zhang *et al*, 2013	Myocardial injury (rat)	TTAT	Restore cardiac contractility, maintain cardiac structural integrity and muscle fibre calcium sensitivity.	(146)
Lu *et al*, 2010	Diabetic complications (mice)	TTAT	Strengthen the role of the antioxidant defence mechanism to limit the damage of diabetes to heart and blood vessels.	(147)
Gong *et al*, 2006	Left ventricular dysfunction (human)	TTAT	Improve left ventricular disease.	(148)
Pan *et al*, 2003	Vascular diseases (human)	TTM	Inhibition of NF-κB against angiogenesis and metastasis.	(149)
Ouyang *et al*, 2015	Complications of diabetes (human)	EDTA	Reduce cardiovascular risk in patients with diabetes mellitus.	(150)
Lamas *et al*, 2013	Myocardial infarction (human)	EDTA	Reduce the incidence of cardiovascular events in patients with myocardial infarction.	(151)
Ujueta *et al*, 2019	Complications of diabetes (human)	EDTA	There is a significant reduction in combined cardiovascular events in patients with diabetes.	(152)

EDTA, disodium ethylene diamine tetraacetic acid; TTM, tetrathiomolybdate; TTAT, triethylenetetramine.

Wilson's disease is typically characterized by excessive accumulation of copper in the liver (25). TTM chelates bioavailable copper by forming a TTM-Cu-protein triple complex (128). A study has found that TTM specifically forms a TTM-Cu-ATX1 complex with the intracellular chaperone ATX1 to inhibit copper transport to the TGN and its downstream incorporation

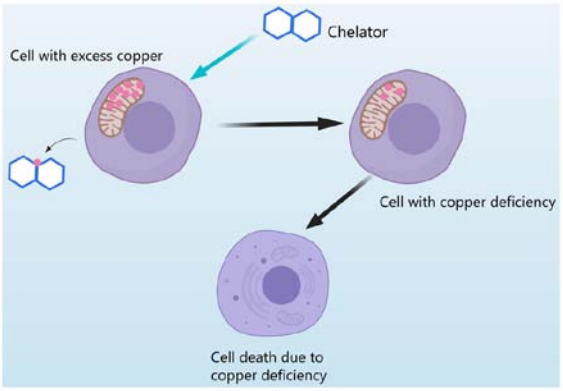


Figure 5. Copper chelation mechanisms.

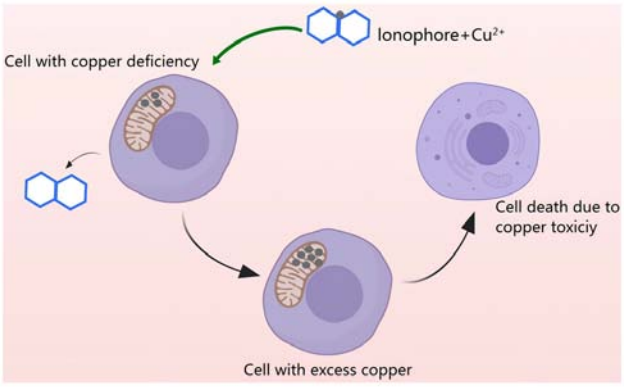


Figure 6. Copper ion carriers.

into copper proteins (127). Another study revealed that TTM inhibits atherosclerosis in ApoE-deficient mice by reducing bioavailable copper and vascular inflammation (129). In addition to TETA and TTM, EDTA also acts as a metal chelator for various metals including copper (130). A clinical trial demonstrated that EDTA disodium-based infusion reduced recurrent cardiovascular events in type 1 and type 2 diabetic patients with a prior myocardial infarction (131). Trientine diHClide is another common copper chelator used for treating Wilson's disease (132), which can selectively bind Cu^{2+} ions to improve mitochondrial function in patients with hypertrophic cardiomyopathy (73), as well as restore mitochondrial function and normalize myocardial expression and enzymatic activity of proteins involved in energy metabolism among diabetic patients (133).

The disadvantages of the use of metal chelators need to be considered. They have been shown to redistribute heavy metals from other tissues to the brain, increasing brain neurotoxicity and leading to the loss of essential metals, such as zinc, or even hepatotoxicity due to serious side effects due to hepatotoxicity (134). Therefore, when using metal chelators for diseases characterized by excess Cu intake, one must consider carefully their potential risk of neurotoxicity or loss of essential metals.

In addition to broad-spectrum metal ion chelators, drugs that specifically regulate the concentration and distribution of intracellular copper ions have greater potential. DCAL50, an inhibitor of copper chaperones, blocks intracellular copper ion transport and can bind to the copper chaperone proteins ATOX1 and CCS, thereby specifically inhibiting the proliferation of cancer cells without affecting normal cellular function (135). This mechanism may be attributed to the interference with copper ion transport leading to increased ROS levels, mitochondrial dysfunction and reduced ATP production, ultimately inhibiting Cu/Zn SOD1 activity (136). Inhibitors of copper chaperones address the concern of other copper chelators in that they non-selectively bind other metal cations and produce toxic side effects (11). Further research into these inhibitors may offer valuable insights for drug development for the management of cardiovascular diseases.

At present, the methodology for addressing copper deficiency involves the use of copper ion carriers, which are small molecules that form complexes with copper and facilitate its transport across the cell membrane and into the mitochondria (137). The entry of copper ion carriers into the cell leads to copper accumulation, which in-turn, triggers oxidative stress and induces cell death (Fig. 6) (138). Common copper ion carriers include elesclomol and disulfiram. Elesclomol is widely recognized to induce copper overdose and cell death (139), by acting as a copper ion carrier with a hydrophilic pore in its centre, to which Cu^{2+} ions can bind easily, facilitating copper entry into cells, thus increasing the intracellular copper concentration (140), and this, in-turn increases ROS levels, triggering oxidative stress (141) and ultimately cell death. Disulfiram is another known copper ion carrier that helps transport copper into cells; it has shown value in the treatment of alcoholism (138). Both these copper ion carriers have shown value in cancer treatment, but their ability to treat cardiovascular disease requires further study (11). It is important to note that copper ion carriers do not have a well-defined understanding of its specificity for copper ions and exhibit a range of functions, several of which remain incompletely understood (137). Additionally, they are not easy to manipulate during transport and inappropriate copper transport may lead to tissue-specific issues (25), thus, further study is required to improve the function of these carriers. There are natural antidotes, derived from herbs and their derivatives, that target multiple proteins and exhibit fewer side effects and toxicity, while showing higher stability than synthetic chelating agents. For example, turmeric, by itself and its derivatives are highly effective in the treatment of cardiovascular diseases (142). Please refer to Table II for details (143-152).

5. Effects of Cu/Zn and Fe on cardiovascular diseases

The effects of Cu/Zn and Fe on cardiovascular diseases are summarized in Table III. Numerous factors can affect cardiovascular disease, including zinc and iron ions. Zinc ions play a critical role in several metabolic reactions and, when combined with copper, form part of the functional group of key enzymes, such as Cu-Zn SOD and endothelial NOS, which functions to prevent atherosclerosis (153). Copper ions have been found to accelerate the oxidation of LDL *in vitro*, leading to the formation of oxidized LDL and other pro-atherosclerotic by-products; meanwhile, zinc may affect copper bioavailability and metabolism (154) as well as cellular oxidation of LDL (155). Therefore, the ratio of copper to zinc in the diet may be an important determinant of coronary risk (156). Salehifar *et al* (157) demonstrated in a study that

Table III. Effects of other elements on cardiovascular disease.

First author/s, year	Type of disease (animal/human)	Treatment	Duration	Pathological effects	(Refs.)
Kunutsor *et al*, 2022	Heart failure (human)	N	3 years	The ratio of serum Zn/Cu is proportional to the incidence of heart failure, which can improve the risk assessment of heart failure.	(167)
Wang *et al*, 2023	Aortic aneurysm (human)	Operation of abdominal aortic aneurysm	1 year	The serum Zn/Cu of the patients is relatively high.	(168)
Majewski *et al*, 2019	Coronary heart disease (Wistar rats)	Copper nanoparticles (40 nm)	8 weeks	Decrease the plasma cubic zinc ratio and prostaglandin F2-α to enhance vasoconstriction.	(169)
Tousson and El-Gharbawy, 2022	Cardiac injury (rat)	Copper nanoparticles	2-4 weeks	Cause toxicity, injury and oxidative stress in rat hearts.	(170)
Huo *et al*, 2023	Diabetic complications (mice)	Cucl-2 and copper ions	48 h	Diabetic cardiomyopathy is associated with cuproptosis.	(171)
Piavchenko *et al*, 2020	Heart damage (Wistar rats)	Zn succinate	1 month	Toxic and atrophic changes in the heart were detected.	(172)
Karagulova *et al*, 2007	Myocardial injury (rat)	Zn pyrithione	1 week	Increase myocardial healing and reduce arrhythmias during reperfusion.	(173)
Yang *et al*, 2022	Arrhythmia (rat)	Ginseng pine heart capsules	N	Reduced AF sensitivity and inhibited electrical and structural remodelling by upregulating Fpn, reducing intracellular iron overload and decreasing reactive oxygen species production.	(174)
Fang *et al*, 2021	Arrhythmia (rat)	N	N	Fpn-mediated iron death is associated with new-onset AF due to LPS-induced endotoxemia by exacerbating calcium-handling protein dysregulation.	(175)

N, no information found; AF, atrial fibrillation; Fpn, ferroportin.

age was negatively correlated with zinc levels and positively correlated with copper levels in healthy volunteers. Patients with idiopathic dilated cardiomyopathy had lower levels of zinc compared with healthy volunteers, and there was a significant difference in the Zn/Cu ratios according to the New York Hospital Authority classification, suggesting that the balance between zinc and copper plays a key role in the development of idiopathic dilated cardiomyopathy (158). Patients with advanced HF, whether in atrial fibrillation or sinus rhythm, exhibit profound hypozincaemia and reduced Zn/Cu ratios due to activation of the adrenergic-angiotensin-aldosterone system and HF drugs (159). In recent years, there has been increasing research on copper and zinc nanoparticles as well as different metals and nanoparticle forms such as metal oxides, all having potential effects on the cardiovascular system (160). Iron death, characterized by oxidative stress due to intracellular Fe^{2+} accumulation, leading to large amounts of ROS production, ultimately results in cell death (161) and contributes to various cardiovascular diseases including atherosclerosis, I/R injury, HF, myocardial infarction and adriamycin cardiomyopathy (162). The underlying mechanism may be related to GSH metabolism (163), abnormal iron metabolism (164), lipid peroxidation (165) and mitochondrial dysfunction (166). Please refer to Table III for details (167-175).

6. Conclusions and future perspectives

Copper ion levels in cells must be maintained in a state of relative equilibrium, as both excessive and deficient amounts can significantly impact health (176). The present review provides an overview of the fundamental role of copper and its metabolic pathways in physiology. It discusses the

mechanisms underlying cardiovascular diseases resulting from copper excess and deficiency, particularly focusing on the pathogenic mechanisms related to oxidative stress and mitochondrial metabolism disorders. Furthermore, it explores potential therapeutic approaches for managing copper-related cardiovascular diseases, such as using copper chelating agents and ion carriers, while also highlighting the limitations of these methods. The balance between normal cellular oxidation and antioxidants must be maintained, as excess copper levels can induce oxidative stress, leading to cell death and cardiovascular disease (14). Therefore, special attention should be paid to this during treatment. For instance, Jomova *et al* (177) demonstrated that an excess of copper ions led to the aggregation of fatty proteins and abnormal expression of Fe-S cluster proteins, resulting in protein toxic stress and cell death. In addition to oxidative stress and mitochondrial metabolic disorders causing cell death, abnormal copper levels can induce cell death through ROS, ER and inflammatory responses (178). An association has been established between oxidative stress and inflammation, which inevitably plays a crucial role in the pathogenesis of cardiovascular diseases such as atherosclerosis, stroke and HF (41). The presence of copper ions in cells serves a dual function; clinical studies have yielded conflicting results regarding the relationship between copper ion levels and the development of cardiovascular disease (76). Thus, further in-depth research is necessary for future validation. It is worth delving into its pathogenic mechanism and the regulatory mechanisms both physiologically and pathophysiologically (179). While copper chelating agents and carriers may treat diseases effectively, their disadvantages are evident. For example, chelating agents may induce toxicity by binding with other metal ions while off-target effects make copper regulation using copper ion carriers difficult, thus additional studies on how to regulate copper levels are required (180). Furthermore, different organs have distinct copper ion requirements, further complicating the issue of tight copper regulation (181). Investigating the optimal concentration of copper ions in different organs can offer valuable guidance for the optimal treatment of copper ions. Future development of ion regulators should focus on organ/cell specificity and targeting. Recently, Liu *et al* (182) developed a multifunctional nanocomposite material capable of precisely delivering drugs for treating atherosclerosis. While this method addresses the issue of copper deficiency, further research is required to determine if it can also address excessive copper ion levels. Although various studies on copper-induced cell death have shown promising results, there still remain several challenges, such as how the aggregation of fatty acylated proteins triggers cell death, optimizing the performance of copper regulators and reducing their side effects, determining the mode of death induced by copper overload and deficiency, gaining a comprehensive understanding of the roles of copper in the mitochondria, and whether copper ion carriers can selectively deliver drugs to hypertrophic myocardial tissues through drug delivery systems (183). Further investigation into these questions will improve the understanding of the relationship between copper-induced cell death and heart disease, leading to the development of innovative therapeutic strategies for heart disease.

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Authors' contributions

YW wrote the manuscript, conceived the topic of review and collected the data; LF conceived the study and collected the data; AX and MZ collected and analyzed the data needed in the article; XM assisted in the collection of data and edited the manuscript; and JZ participated in drafting the initial draft. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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