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The role and molecular mechanisms of copper in regulating animal lipid metabolism

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ABSTRACT. Copper serves as a crucial nutrient in animal organisms, acting as a cofactor in metabolic processes and facilitating enzyme formation. Research indicates that high copper levels can enhance animal production performance. However, excessive copper intake can lead to its accumulation, causing metabolic disruptions linked to various diseases. Moreover, copper supplementation can effectively reduce lipid synthesis, especially cholesterol and triglycerides, while enhancing fat oxidation and breakdown by regulating the activity of ATPase copper transporting beta (ATP7B), cyclic adenosine monophosphate (cAMP) levels and endoplasmic reticulum stress, thereby decreasing lipid accumulation *in vivo*. Additionally, copper deficiency in humans has been closely associated with diseases characterised by fat accumulation. Thus, this article comprehensively outlines the intricate interplay between copper and lipid metabolism, elucidating potential underlying mechanisms.

Introduction

The concentration of copper, the third most abundant essential trace element in animal bodies, typically ranges from 1 to 3 mg/kg, and this element is widely distributed in tissues and organs such as the liver, brain, kidneys, heart and hair (Wang et al., 2021). Copper plays a crucial role as an essential trace element in various physiological processes, including the elimination of free radicals, respiration, energy production, iron and oxygen metabolism, connective tissue development, neuropeptide formation, extracellular matrix maturation and neuroendocrine signalling (Ingle et al., 2018). Recommended dietary copper levels for piglets and fattening pigs range from 6 to 3 mg/kg, respectively

(NRC, 2012), while broilers require a dose of 8 mg/kg (NRC, 1994). Research has indicated that excessive copper supplementation beyond these requirements promotes growth and improves feed efficiency by inhibiting the development of intestinal pathogens (Pesti and Bakalli, 1996; Onifade and Abu, 1998; Villagómez-Estrada et al., 2020). Consequently, the amount of copper added to the diet often exceeds the livestock demand; however, the European Food Safety Authority has stipulated a reduction in the maximum allowable copper supplementation in animal feed (EFSA Panel on Additives and Products or Substances used in Animal Feed [FEEDAP], 2016). Studies have suggested that elevated levels of copper supplementation positively promote growth (Huang et al., 2013, 2015; Feng et al., 2020). Nonetheless,

to high copper administration can reduce zinc and iron absorption, leading to deficiencies of these nutrients, adversely affecting animal health (Wang et al., 2023; Helman et al., 2023). Moreover, over-supply of this nutrient can cause soil copper pollution, detrimental to physical, chemical and biological soil function and fertility (Liao et al., 2018). As copper has the ability to move through the food chain, it is speculated that it eventually accumulates in the human body, potentially posing a threat to our health (Chen et al., 2021).

The liver, the primary site of copper metabolism, reflects the body's copper status, with dietary copper supplementation elevating its levels (Wu et al., 2020; Zhang et al., 2020). Excessive and prolonged administration of copper supplements can result in the accumulation of hepatic copper, causing damage to liver cells and disrupting metabolic processes, including lipid metabolism (Gao et al., 2020; Zhang et al., 2020; Nguyen et al., 2022). Studies on broiler chickens have demonstrated that elevated copper levels significantly reduce plasma cholesterol and lipid concentrations (Wu et al., 2019). Conversely, insufficient copper content can induce dyslipidaemia, which may contribute to the pathogenesis of cardiovascular diseases and non-alcoholic steatohepatitis (Olivares et al., 2019; Liu and Miao, 2022). This review aims to elucidate the specific mechanisms underlying the regulation of lipid metabolism by copper.

Copper homeostasis and metabolism

Copper absorption

Dietary copper is primarily absorbed in the duodenum and proximal jejunum by binding to ligands to form absorbable chelates (Lawson et al., 2016; Collins, 2021). These chelates are transported into intestinal mucosal cells mainly through bivalent metal ion transporter (DMT1) and cell membrane receptor copper transporter (CTR; including CTR1 and CRT2), with CTR1 exhibiting the highest affinity for copper (Ren et al., 2019). Copper absorption typically ranges from 10 to 55% (Felix et al., 2012; Wang et al., 2018; Kim et al., 2022), with faecal excretion being the major route of its excretion (>90%), and a minor fraction excreted in urine (Czech et al., 2023). It is worth noting that copper is efficiently absorbed in the intestine. The absorption rate of copper is influenced by the chemical form of dietary copper, with copper bound to small organic compounds absorbed more efficiently than inorganic copper salts. Studies have demonstrated that organic forms, such as glycine

copper or proteinate-bound copper in finishing pigs (Wen et al., 2022) and copper methionine in broilers (Wen et al., 2019), show increased absorption efficiency compared to inorganic forms like copper sulphate. This enhancement is likely due to the fact that Cu is endocytosed into cells in the small intestine with organic matter. The compound is subsequently dissociated upstream of alanine-serine-cysteine transporter, type-2 (ASCT2) and peptide transporter 1 (PepT1), thereby improving the absorption and utilisation of copper (Wen et al., 2022).

Copper transportation

Once absorbed, copper enters portal vein blood, which is facilitated by copper-transporting P-type ATPase (ATP7A), an active ion transporter (Hartwig et al., 2019). Most copper ions entering the portal vein circulation are primarily taken up by liver cells, where CTR1 also plays an important role. Subsequently, copper initially binds to copper chaperone proteins, such as copper chaperone for superoxide dismutase (SOD), cytochrome oxidase 17 (COX17) and antioxidant protein 1 (ATOX1) (Prohaska, 2008), before being transported to various cellular compartments like the cytoplasm, mitochondria and *trans*-Golgi network (TGN), where it participates in enzymatic reactions (Polishchuk and Lutsenko, 2013). These reactions involve SOD1 in the cytoplasm, cytochrome c oxidase in the mitochondria and copper-transporting ATPase ATP7A/ATP7B in the TGN, thus maintaining physiological functions (Lutsenko et al., 2007). ATP7A protein functions as a copper-dependent enzyme, aiding in copper transportation to the mucosa of the Golgi apparatus via TGN (Lutsenko et al., 2007). Under high copper concentration, ATP7A relocates to the plasma membrane, enabling copper transportation to vesicles for exocytosis (Tümer and Møller, 2010). Copper absorbed into hepatic cells is subsequently transferred to ATP7B with the help of copper chaperone Atox1. ATP7B, located in the TGN, facilitates the binding of copper to apo-ceruloplasmin (Apo-Cp) within the endoplasmic reticulum, resulting in the formation of holo-ceruloplasmin (Holo-Cp), which is subsequently released into the bloodstream and distributed throughout the body (Gromadzka et al., 2023; Karpenko et al., 2023).

Copper utilisation and elimination

The utilisation of copper *in vivo* involves various biochemical processes and physiological activities. Under physiological conditions, copper participates in the formation of various redox

enzymes, including ceruloplasmin (Cp), COX, electron transport and enzymes associated with the oxidative phosphorylation system, SOD, antioxidant enzymes and lysyl oxidase. Additionally, copper is also involved in the synthesis of melanin, dopamine and pituitary hormones (Linder and Hazegh-Azam, 1996). Tissues or cells use these copper-containing compounds or enzymes for metal ion transport, energy supply, antioxidation and neurotransmitter conduction. Recent studies have revealed the involvement of copper in immune regulation, contributing to the anti-inflammatory properties of macrophages (Zangiabadi et al., 2023). These observations shed light on a novel aspect of copper's functionality. Collectively, copper have been shown to play a wide range of biological functions, including maintaining the integrity of cell structure and function, protecting the health of the digestive system and modulating the body's endocrine system and gene expression (Aigner et al., 2010; Li et al., 2014; Wang et al., 2015).

Excess copper is sequestered by metallothionein in intestinal mucosal cells (Felix et al., 1990) and removed through regular cell metabolism and ageing, thereby eliminating the surplus. The liver is the primary organ responsible for copper excretion (Wijmenga and Klomp, 2004), with ATP7B relocating to the hepatocyte canalicular membrane

to enable copper excretion into bile and subsequent elimination via the digestive tract (Lee et al., 2012).

Disturbance of copper homeostasis affects animal health

Maintaining copper homeostasis is crucial for animals, as both its deficiency and excess can lead to detrimental effects (Figure 1) (Dalto et al., 2023; Chen et al., 2023; Daniel et al., 2023). In particular, increased dietary copper intake correlates with significant liver deposition of this element (Hamdi et al., 2018; Zhang et al., 2020; Blavi et al., 2021; Hu et al., 2023). Excessive copper accumulation in the liver induces morphological alterations in the liver and hepatocytes, primarily affecting the mitochondria, leading to oxidative damage of their membranes, inactivation of tricarboxylic acid cycle enzymes and other related effects (Hu et al., 2023). Elevated copper concentrations have also been linked to increased aspartate aminotransferase and alanine aminotransferase activity in chicken serum and pathological tissue lesions, including hepatocyte swelling, degeneration of cytoplasmic granules and disruption of the core and sinusoidal structure of liver cells. In addition, pathological damage, including increased cytoplasmic vacuolisation, aberrant mitochondrial membranes, and electron

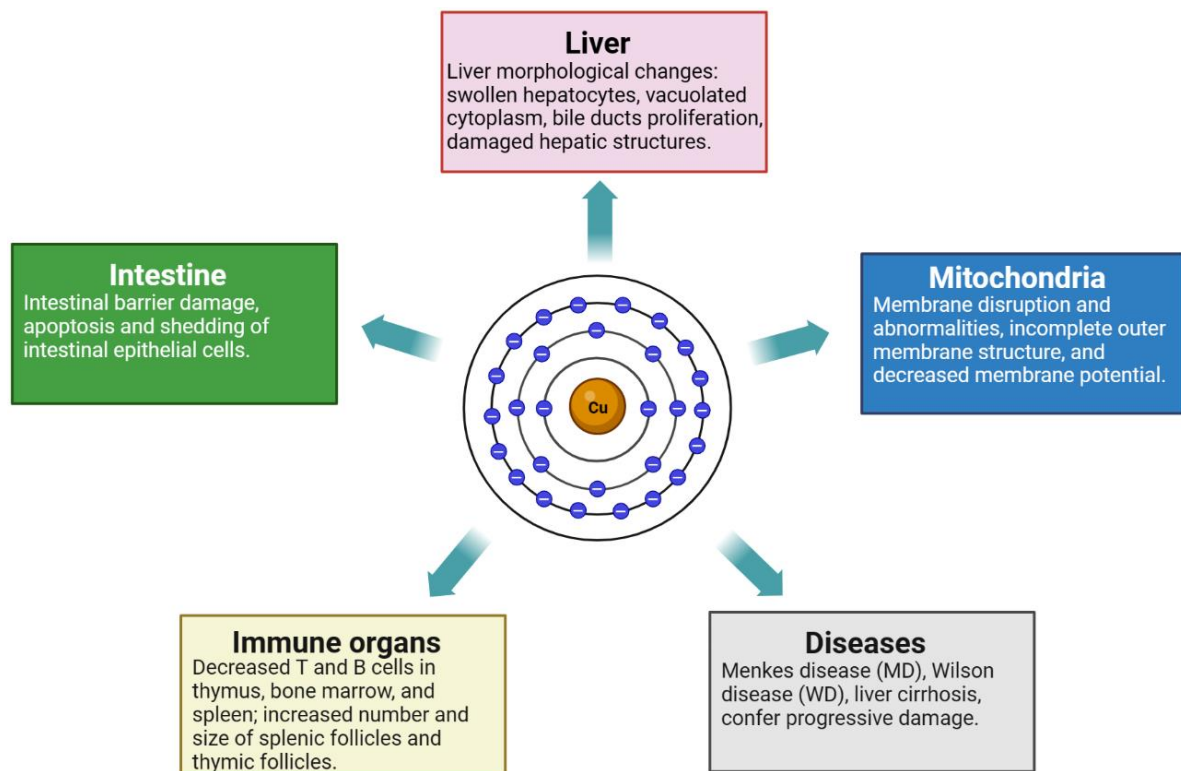


Figure 1. Adverse effects of excess copper

dense deposits in the cytoplasm and nucleus, can be observed using electron microscopy (Cao et al., 2010). Comparable observations were made by Hosseini et al. (2014) in rats, wherein the analysis of isolated mitochondria demonstrated a significant increase in the copper-induced generation of free radicals in the liver, leading to lipid peroxidation and a significant decline in mitochondrial membrane potential, ultimately causing mitochondrial swelling. The incorporation of copper ions exerts a significant influence on the functioning of electron transport chain complexes I, II and IV, thereby affecting mitochondrial ATP production and causing impairment to the structural integrity of the outer mitochondrial membrane, which results in the release of cytochrome C. Increased copper accumulation in the liver leads to the emergence of liver nodules, along with a range of pathological alterations, including inflammation, hepatocyte hypertrophy and bile duct hyperplasia (Huster et al., 2006; Ralle et al., 2010). Absorbed copper binds to metallothionein for storage in the liver. However, in ruminants, the transcription of metallothionein in lysosomes cannot effectively respond to rapidly increased copper levels. Therefore, ruminants are particularly susceptible to hepatic copper accumulation compared to non-ruminant species due to their limited capacity to adapt to excess dietary copper (Clarkson et al., 2020). Initial stages of liver disease resulting from copper deposition manifest as liver steatosis and slight nuclear enlargement (Huster, 2014). As copper deposition intensifies, hepatic pathological changes become more pronounced. Importantly, investigations have demonstrated a key effect of the Golgi membrane protein GP73 in triggering inflammation, fibrosis and developmental abnormalities in ATP7B knockout mice aged 2046 weeks (Wright et al., 2009). Notably, cardiolipin, a phospholipid abundantly present in the inner mitochondrial membrane, plays a crucial role in preserving mitochondrial membrane integrity and functionality. Yurkova et al. (2011) discovered that the absence of ATP7B in mice resulted in copper accumulation within liver mitochondria, leading to oxidative stress, and subsequent cardiolipin degradation, resulting in phosphatidic acid and phosphatidylhydroxyacetone production. These alterations in lipid composition are induced as a consequence of morphological changes in liver mitochondria. Increasing copper deposition results in mitochondrial cristae expansion, intermembrane space enlargement and higher permeability (Zischka et al., 2011; Su et al., 2011)

Disruption of cellular copper homeostasis can lead to various diseases, such as Menkes disease

(MD) and Wilson disease (WD; also known as hepatolenticular degeneration) (Gromadzka et al., 2020). MD is characterised by impaired copper absorption due to ATP7A dysfunction in intestinal mucosal cells and subsequent transport into the bloodstream, resulting in copper absorption disorders and severe copper deficiency (De Feyter et al., 2023). On the other hand, WD involves mutations in ATP7B that modify its functionality, hindering copper binding to Cp in the liver and its excretion with bile (De Feyter et al., 2023). Consequently, copper accumulates in the liver, kidneys, brain and cornea. Excessive copper accumulation in various organelles exerts cytotoxic effects on cells, manifesting as lipid peroxidation, glutathione (GSH) depletion, mitochondrial function impairment and lysosomal rupture (Liu and Miao., 2022; Chen et al., 2023). These deleterious consequences ultimately culminate in the development of liver cirrhosis or cause progressive damage to the nervous system (Horn et al., 2019). The harmful outcomes triggered by diets high in coppers, which lead to hepatic copper accumulation, share similarities with the aforementioned pathological conditions.

Disruptions in copper homeostasis also affect the immune system and intestinal integrity. Elevated levels of free copper in the body suppress T and B cells in the thymus, bone marrow and spleen (Cui et al., 2010; Li et al., 2014), resulting in inhibited lymphocyte proliferation and differentiation, as well as impaired macrophage phagocytic capacity, ultimately affecting both cellular and humoral immunity (Elgerwi et al., 1999; Cui et al., 2010). Yang et al. (2020) indicated that excess copper caused pathologic changes (increased number and area of splenic and thymic corpuscles), increased levels of inflammatory cytokines and MDA, and decreased antioxidant enzyme activity in the immune tissues of chickens. Moreover, long-term supplementation of large amounts of copper to animal feed was shown to lead to the high copper transport in the intestinal epithelium, producing super-negative ions, resulting in the synthesis of toxic hydroxyl radicals (Linder, 1991; Arredondo et al., 2000). This in turn caused apoptosis and shedding of intestinal epithelial cells, and subsequent damage to tight junction proteins and regenerative capacity of the intestinal barrier in pigs (Fry et al., 2012; Wang et al., 2015). Moreover, researchers have reported that too high copper intake may result in the oxidation of ferrous ions, generating excessive superoxide anion radicals in multiple tissues, reducing antioxidant enzyme activity and damaging lipid membrane structure (Gaetke and Chow, 2003). Therefore, free radical-mediated

damage rather than copper accumulation could be the direct cause of tissue damage (Gao et al., 2020).

Copper regulates lipid metabolism

Numerous studies have highlighted the role of copper in lipid metabolism (Klevay and Hyg, 1973; Zhong et al., 2022, 2023). Dietary copper supplementation has been shown to decrease serum triglyceride (TG), total cholesterol and LDL-C levels in rats compared to the basal diet group. Additionally, copper administration significantly reduces concentrations of oxidative stress markers such as lipid hydroperoxides and malondialdehyde (Galhardi et al., 2005), suggesting an inhibition of triglyceride and cholesterol synthesis in the body. Moreover, studies in farm animals have revealed an association between copper supplementation and lipid concentrations. For instance, copper has been found to exert a dual effect on cholesterol and fat levels, as well as fatty acid composition (Kaya et al., 2018; Skrivan et al., 2000; Solaiman et al., 2006). Excessive copper supply can reduce the lipid content of animal products. The addition of 250 mg/kg of copper to broiler chickens and laying hens resulted in a significant decrease in cholesterol levels in breast muscles and egg yolks compared to the group administered a basal diet (Bakalli et al., 1995; Kaya et al., 2018). Engle et al. (2000) has discovered that copper has the potential to decrease subcutaneous fat thickness at the 12th rib of cattle. Similar effect has been observed in goats by Solaiman et al. (2006), who reported that adding copper at doses of 100 mg/head/day and 200 mg/head/day significantly reduced subcutaneous fat thickness at the 12th rib. However, copper supplementation also decreased fat levels and modified fatty acid composition. Skrivan et al. (2000) demonstrated that the combination of 200 mg/kg copper and 50 g/kg rapeseed oil resulted in a decrease in saturated fatty acids in abdominal fat, an increase in the PUFA/SFA ratio, and a higher content of C18:0 and C18:1 acids in the breast muscles of broiler chickens. Furthermore, the addition of both inorganic and organic copper has been shown to elevate the levels of unsaturated fatty acids and reduce the levels of saturated fatty acids in beef cattle (Correa et al., 2012). Moreover, copper was also shown to significantly increase the unsaturated fatty acid content in the *longissimus dorsi* muscle in goats (Huang et al., 2013). Meanwhile, Zhu et al. (2014) found that high copper concentrations enhanced the expression and activity of carnitine palmitoyl transferase 1

(CPT1) and decreased the expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), thereby reducing fat accumulation in grass carp hepatocytes cultured *in vitro*. These findings suggest that elevated copper levels inhibit lipid synthesis and stimulate fat decomposition. Conversely, low copper levels were found to significantly increase the synthesis of lipids in animals. Research in rats has demonstrated that copper deficiency can induce hypercholesterolemia (Allen and Klevay, 1978). Moreover, studies involving humans have found that a copper-deficient diet leads to elevated plasma cholesterol levels (Klevay et al., 1984). It is currently widely acknowledged that copper deficiency plays a key role in the development of cardiovascular disease and non-alcoholic fatty liver (Olivares et al., 2019; Liu and Miao, 2022). Patients with non-alcoholic steatohepatitis have lower liver copper concentrations than normal groups, as well as patients with chronic hepatitis, hemochromatosis and autoimmune hepatitis (Aigner et al., 2010), indicating that lipid synthesis increases under lowcopper conditions. However, the regulation of lipid metabolism by copper has not yet been fully elucidated. For instance, a key characteristic of atherosclerosis is the accumulation of lipids and cholesterol in macrophages, subsequently leading to the formation of foam cells (Wang et al., 2023).

Utilising DNA microarray analysis of macrophages treated with copper, it was observed that the expression of genes associated with cholesterol synthesis, as well as LDL-R and HMG-CoA reductase, was significantly upregulated (Svensson et al., 2003). The latter study contradicted previous observations wherein excessive copper levels inhibited lipid synthesis, suggesting that cells may exhibit distinct reactions when exposed to short-term administration of adequate copper doses in comparison to chronic high doses. Furthermore, exposure of zebrafish to 16 µg/l of waterborne copper for 60 days resulted in widespread hepatic steatosis, indicating potential adverse effects on lipid metabolism due to chronic exposure to excessive copper levels (Pan et al., 2019). Nonetheless, further investigations are required to fully understand these processes in individual animal models.

High copper levels and lipid metabolism regulation

High levels of copper can originate from both endogenous accumulation and exogenous sources, including dietary intake. Endogenous copper accumulation is mainly linked to the ATP7B gene, which

In Rex rabbits, a high copper diet was shown to suppress *de novo* fat synthesis in the liver by downregulating the expression of the FAS and ACC genes in the liver (Cheng et al., 2010; Li et al., 2021).

ATP7B knockout mice are commonly employed as models to study copper-related metabolic disorders. Notably, ATP7B knockout has been demonstrated to significantly reduce triglyceride levels in the liver (Krishnamoorthy et al., 2016). Prior to the onset of liver inflammation in ATP7B knockout mice, alterations in lipid metabolism were observed, particularly the inhibition of cholesterol synthesis (Seessle et al., 2011). Similarly, a significant reduction was found in cholesterol and very low-density lipoprotein cholesterol (VLDL-C) levels in liver of this mouse model, which was also accompanied by increased expression of cell cycle-related molecules. Examination of the liver transcriptome revealed a significant downregulation of the expression of genes associated with lipid metabolism, particularly those implicated in cholesterol biosynthesis. Further experiments using ATP7B knockout mice also showed a marked decrease in the expression of HMG-CoA reductase, a pivotal enzyme involved in cholesterol synthesis in the liver (Huster et al., 2007).

Numerous studies have confirmed that the activation of the liver X receptor/retinoid X receptor (LXR/RXR) complex promotes lipid synthesis and metabolism, while concurrently suppressing inflammatory responses (Zelcer and Tontonoz, 2006). Similarly, in the liver of ATP7B knockout mice, Hamilton et al. (2016) observed a significant downregulation of LXR/RXR activity and a significant decrease in mRNA expression of key enzymes involved in fatty acid and cholesterol synthesis, namely FAS and HMG-CoA reductase. Moreover, administration of the LXR agonist T0901317 also significantly increased plasma levels of total cholesterol (LDL and HDL), as well as triglyceride concentrations and FAS synthase expression in the liver of these mice. In addition, the same mouse KO model was employed to demonstrate downregulation of the peroxisome proliferator-activated receptor (PPAR) signalling pathway, promoting the expression of lipogenic genes, and consequently inducing *de novo* fat synthesis (Morán-Salvador et al., 2011; He et al., 2011; Tailleux et al., 2012). He et al. (2011) also reported steroid biosynthesis inhibition in the liver of ATP7B knockout mice. Studies have demonstrated that ATP7B inactivation is associated with copper accumulation in several tissues, particularly the liver and brain

(Lutsenko et al., 2007). Moreover, the overexpression of ATP7B has potentially been shown to alleviate liver damage and apoptosis induced by increased copper levels (Sauer et al., 2010). Therefore, excessive copper intake may suppress lipid synthesis by inhibiting LXR/RXR activity and the PPAR signalling pathway through ATP7B inactivation.

Insulin plays a significant role in stimulating fat synthesis, and copper has been shown to reduce plasma insulin levels in lambs (Cheng et al., 2010), while also increasing Akt (known as protein kinase B) and forkhead box O 1 (FoxO1) phosphorylation, similarly to insulin action. Copper exerts its effects through tyrosine kinases, independently of insulin receptor/insulin-like growth factor 1 receptor (IR/IGF1R) (Hamann et al., 2014). FoxO1, a transcription factor belonging to the Fox family, plays a significant role in lipid metabolism (Matsumoto et al., 2006). FoxO1 overexpression in the liver has been found to induce lipid deposition in hepatocytes, while FoxO1 knockout in the liver significantly attenuated hepatic steatosis and very low density lipoprotein-triglyceride (VLDL-TG) secretion (Kamagate et al., 2008). In their study, Walter et al. (2006) observed that copper could activate the phosphoinositide 3-kinase-Akt (PI3K/Akt) pathway in liver cells, resulting in FoxO1 phosphorylation and its relocation from the nucleus to the cytoplasm. Furthermore, it has been demonstrated that copper can deactivate FoxO1 through Akt activation, leading to a reduction in downstream expression of gluconeogenic enzymes and inhibition of gluconeogenesis (Barthel et al., 2007). Thus, collectively, copper is speculated to affect lipid synthesis by influencing FoxO1 activity, but the specific relationship between copper, FoxO1, and lipid metabolism requires further elucidation in additional studies.

The endoplasmic reticulum (ER) has crucial functions in cellular physiology, including protein and lipid synthesis, detoxification and calcium storage (Borgese et al., 2006; Görlach et al., 2006). Research has established a significant correlation between ER stress and lipid metabolism, with the former implicated in the development of dyslipidaemia, insulin resistance, cardiovascular diseases, type 2 diabetes and obesity (Basseri and Austin, 2011). Endoplasmic reticulum stress (ERS) is directly involved in promoting the synthesis of cholesterol/triacylglycerol, leading to changes in lipid metabolism, and conversely, alterations in lipid homeostasis and abnormal lipid metabolism can induce ERS. Saturated fatty acids, such as palmitic acid and stearic acid, can also trigger ERS in various

cell types and modulate their survival and apoptosis (Werstuck et al., 2001; Wei et al., 2006; Guo et al., 2007). Excess copper has been shown to result in the production of free radicals, which bind to amino acid residues of proteins and subsequently modify their structure (Linder and Hazegh-Azam, 1996; Tosco et al., 2010). This oxidative stress caused by abnormal protein deposition can also ultimately lead to ERS (Xue et al., 2005). Importantly, copper stimulates the production of a significant quantity of free radicals in liver cells (Oe et al., 2016). Previous studies demonstrated that copper exposure significantly upregulated the expression of genes related to ERS and lipid deposition in the goby liver on day 30 of the trial, whereas higher concentrations of copper led to a decrease in the expression of these genes on day 60 of this study (Huang et al., 2014; Song et al., 2016a; b). A general conclusion can be drawn that excess copper can inhibit lipid deposition by suppressing ERS.

Additionally, growth hormone secreted by pituitary somatotrophs has been linked to reduced fat deposition (Guo et al., 2022). Copper supplementation has been shown to elevate serum growth hormone levels, thereby enhancing production performance in weaned piglets (Wang et al., 2016). Additionally, Yang et al. (2011) have provided evidence supporting the notion that copper can increase the expression of growth hormone-releasing hormone in the hypothalamus of pigs. Nevertheless, further investigations are needed to explain the precise regulatory role of copper in hormone signalling and lipid metabolism.

High copper levels promote lipolysis

In 3T3-L1 adipocytes, the presence of bathocuproinedisulfonic acid (BCS), a specific intracellular copper chelator, was shown to significantly reduce the release of non-esterified fatty acids (NEFA) and glycerol induced by isoproterenol, indicating that copper deficiency hindered fat breakdown. Conversely, the additions of cupric chloride significantly increased isoproterenol-stimulated NEFA and glycerol release, suggesting that copper supplementation promoted lipolysis (Krishnamoorthy et al., 2016). It has been shown that a diet rich in copper stimulates higher activity of carnitine palmitoyltransferase (CPT-1 and CPT-2) and peroxisome proliferator-activated receptor alpha (PPAR- α) in the liver and adipose tissue, while increasing transport protein, fatty acid-binding protein and lipoprotein lipase (LPL) levels in skeletal muscle, thereby enhancing fatty acid oxidation and degradation (Li et al., 2021). Adenosine 5'-monophosphate

(AMP)-activated protein kinase (AMPK) is part of a crucial metabolic cellular pathway. AMPK activation stimulates glucose uptake and transport, thus promoting its utilisation. During energy deprivation, AMPK also increases fatty acid oxidation to ensure cellular homeostasis. Chen et al. (2018) suggested that high copper levels could induce the upregulation of AMPK member genes in intestinal cells, thereby providing energy for the transport and breakdown of lipids. Activation of the AMPK pathway promotes ACC phosphorylation, which affects the normal progression of ACC and triglyceride synthesis (Gybina and Prohaska, 2008).

The role of catecholamines, such as norepinephrine and epinephrine, in stimulating fat breakdown is widely recognised (Qi and Ding, 2016). Engle et al. (2000) posited that copper alters backfat thickness of beef cattle and influences the metabolism of catecholamines. Administration of excess copper to weaned piglets resulted in a significant elevation in dopamine and norepinephrine concentrations in the midbrain and hypothalamus, with a parallel increase in midbrain dopamine- β -hydroxylase activity. These findings underscore the ability of copper to modulate lipid balance by affecting catecholamine metabolism (Yang et al., 2016). A previous study involving humans showed a positive correlation between copper and leptin levels (Olusi et al., 2003). Cheng et al. (2010) demonstrated that copper supplementation significantly increased plasma leptin levels in lambs, reduced lipogenic and enhanced lipolytic enzyme activities. These findings suggest that copper promotes lipolysis by influencing leptin concentrations. However, the specific mechanism involved in these processes requires further research.

Lipolytic hormones, including adrenaline and glucagon, show binding affinity towards receptors located on target cell membranes, triggering adenylate cyclase activation, resulting in an increase in intracellular cAMP levels (Rogne and Taskén, 2014). Consequently, protein kinase A is activated, leading to the phosphorylation and subsequent activation of lipase, thus stimulating lipolysis (Daval et al., 2006). A recent study has highlighted the role of copper in regulating lipid metabolism via the cAMP signalling pathway (Krishnamoorthy et al., 2016). The latter authors observed that ATP7B knockout mice had reduced copper levels in abdominal fat. Meanwhile, isoproterenol, a β -adrenergic receptor agonist, injected to white adipose tissue led to decreased glycerol release and cAMP levels, suggesting that inhibition of lipid breakdown was closely associated with copper status. In 3T3-L1 adipocytes, the

inclusion of bathocuproinedisulfonic acid (BCS), a specific intracellular copper chelator, significantly diminished the release of NEFA and isoproterenol-induced glycerol, indicating that copper deficiency impeded fat breakdown. Conversely, the inclusion of cupric chloride markedly enhanced the release of NEFA and glycerol induced by isoproterenol, suggesting that copper supplementation promoted lipolysis (Krishnamoorthy et al., 2016). Furthermore, the same study demonstrated that the incorporation of the copper chelator BCS significantly hindered the release of isoproterenol-induced NEFA, mediated by forskolin, an adenylate cyclase activator, and 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, in 3T3-L1 cells (Krishnamoorthy et al., 2016). Similarly, in the same cell line, the protein kinase A (PKA) inhibitor H89 and the hormone-sensitive lipase (HSL) inhibitor CAY10499 significantly inhibited ISO-induced NEFA breakdown. A fluorescence detection technique with copper-sensitive reagent 1 (CSR1) demonstrated that ISO stimulation reduced and stabilised intracellular copper levels. However, this effect became insignificant following the inhibition of PKA. In contrast, inhibiting HSL resulted in a similar fluorescence intensity of the copper pool compared to ISO stimulation, indicating that alterations in unstable intracellular copper levels occurred downstream of PKA and upstream of HSL during the initiation of fat degradation (Krishnamoorthy et al., 2016). Additionally, varying copper levels in ISO-stimulated 3T3-L1 cells also affected cAMP levels. The inclusion of copper increased intracellular cAMP levels, whereas the addition of BCS-chelated copper led to a decrease in cAMP levels. This suggests that copper primarily modulates the breakdown of fat by regulating intracellular cAMP levels. Additionally, phosphodiesterase (PDE) plays a significant role in the regulation of intracellular cAMP levels. When cells were treated with cAMP analogues, specifically the easily hydrolysable 8-bromo-cAMP and the relatively less hydrolysable dibutyryl-cAMP, it was observed that NEFA release decreased after copper chelation with BCS in the 8-bromo-cAMP treatment group. This indicates that copper mediates intracellular lipid degradation by altering cAMP levels by modulating PDE activity (Krishnamoorthy et al., 2016).

Effect of copper deficiency in lipid metabolism regulation

Copper deficiency can arise from both exogenous and endogenous factors, including inadequate

dietary intake (Lynch and Strain, 1989), impaired absorption due to internal diseases (Braga et al., 2015), and restricted utilisation (Yang et al., 2018). In contrast to high copper levels, its reduced concentration leads to a significant increase in lipid synthesis in animals (Figure 2). Studies in rats have demonstrated that copper deficiency can cause hypercholesterolaemia (Allen and Klevay, 1978), with similar effects observed in humans, where copper deficiency was associated with elevated plasma cholesterol levels (Klevay et al., 1984). The role of copper deficiency in the onset of cardiovascular diseases and non-alcoholic fatty liver disease is widely acknowledged (Olivares et al., 2019; Liu and Miao, 2022). Individuals with non-alcoholic fatty liver disease often exhibit lower liver copper levels compared to healthy individuals, as well as those with chronic hepatitis, hemochromatosis, and autoimmune hepatitis (Aigner et al., 2010), suggesting increased lipid synthesis at low copper levels. Semicarbazide-sensitive amine oxidase (SSAO) is a copper-dependent enzyme involved in adipocyte differentiation and glucose uptake (Mercader et al., 2010). Deletion of one allele of ATP7A, a copper transport gene, was shown to cause copper deficiency, which suppressed SSAO activity and increased cellular fatty acid uptake, resulting in triglyceride accumulation (Yang et al., 2018). Additionally, copper deficiency was observed to upregulate hepatic FAS expression, promoting *de novo* lipogenesis, while simultaneously suppressing CPT1 activity, a rate-limiting enzyme in fatty acid oxidation (Song et al., 2012).

Sterol regulatory element-binding protein-1 (SREBP-1), a nuclear transcription factor, is a key regulator of lipid metabolism. Tang et al. (2000) observed that copper deficiency increased the expression of mature SREBP-1 protein in the nuclei of liver cells, while simultaneously reducing membrane-bound SREBP-1 protein levels. Moreover, copper deficiency elevated mRNA expression of liver FAS and decreased mRNA expression of cholesterol 7- α hydroxylase. Transcriptome analysis revealed that low copper diets significantly downregulated genes related to mitochondrial and peroxisomal fatty acid beta-oxidation in rat intestines, while concurrently upregulating the expression of genes encoding plasma cholesterol transfer proteins (apolipoprotein E and lecithin-cholesterol acyltransferase) (Tosco et al., 2010). Furthermore, low copper concentration has been linked to decreased plasma activity of lecithin-cholesterol acyltransferase (Lau and Klevay, 1981), potentially due to the tissue specificity of copper deficiency.

This implies that SREBP-1 can be activated under copper deficiency conditions, leading to increased fat and cholesterol synthesis, impaired cholesterol elimination, and consequently inhibition of fatty acid breakdown.

Diet composition may also affect copper homeostasis and lipid metabolism. Rats fed copper-deficient diets supplemented with sucrose or starch showed increased cholesterol concentrations and reduced plasma Cp levels compared to the control groups (Fields et al., 1983). Moreover, hepatic copper concentration in sucrose-fed rats was nearly threefold lower compared to starch-fed rats. These rats also showed increased liver weight, reduced epididymal fat pad and lower blood haemoglobin levels, as well as significant heart hypertrophy with gross deformities (Fields et al., 1983). Further, Fields et al. (1983) demonstrated that the fructose moiety of sucrose was responsible for the exacerbated copper deficiency in sucrose-fed rats compared to starch-fed rats. In the context of copper deficiency, the fructose component of sucrose was found to promote hepatic lipid synthesis as a result of reduced mitochondrial beta-oxidation and upregulated expression of genes associated with hepatic lipid synthesis (Song et al., 2012). Thus, consumption of a copper-poor fructose diet accelerates the progression of hepatic steatosis

Conclusions

Copper plays a vital role as a trace element in the development of livestock and poultry, offering the potential to enhance animal growth through appropriate supplementation. However, excessive administration of copper can lead to hepatic impairment, mitochondrial damage and disruptions in lipid metabolism. Despite numerous studies on the effect of copper on lipid metabolism, the precise mechanisms through which copper modulates these processes remain elusive. Further research should be aimed at exploring the correlation between copper-induced endoplasmic reticulum stress and lipid metabolism, as well as the tissue-specific regulation of copper in fat biochemical processes. Furthermore, it is crucial to consider the health consequences of copper deficiency, including non-alcoholic fatty liver disease, particularly in the context of contemporary high-sugar diets, where copper deficiency may be associated with diseases such as non-alcoholic steatohepatitis. Therefore, future research efforts should aim to uncover the exact mechanisms underlying copper's effects on lipid metabolism and its

associations with health, providing deeper insights and novel strategies for the prevention and treatment of related diseases.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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