Chapter 3

The Role of Copper as a Modifier of Lipid Metabolism

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1. Introduction

1.1. Copper homeostasis in mammals

Dietary copper enters the body largely through the small intestine. Two membrane transporters are essential for this process. The high affinity copper uptake protein Ctr1 is responsible for making copper that enters via the apical membrane available in the cytosol for further utilization (1), whereas the copper-transporting ATPase ATP7A facilitates copper exit from the enterocytes into circulation (2) (Figure 1). Complete genetic inactivation of either transporter in experimental animals is embryonically lethal (3-5). However, partial inactivation or tissue specific inactivation of ATP7A or Ctr1, respectively, in either case is associated with copper accumulation in the intestine, impaired copper entry into the bloodstream, and severe copper deficiency in many organs and tissues (1). Copper deficiency, in turn, produces distinct metabolic changes that are discussed in detail in the following sections.

The majority of absorbed dietary copper is initially delivered to the liver. Hepatocytes utilize copper for their metabolic needs (such as respiration and radical defense); they also synthesize and secrete the major copper containing protein in serum, ceruloplasmin, and prevent copper overload in the body by exporting excess copper via the canalicular membrane into the bile (Figure 1). These two important functions of hepatocytes (the production of ceruloplasmin and the removal of excess copper) are performed by another transporter, the copper transporting ATPase ATP7B, which is homologous to ATP7A (6, 7). Inactivation of ATP7B in patients with Wilson's disease and in animal models is associated with marked copper overload in the liver and pathologic changes including marked lipid dysregulation in the liver and the serum (discussed in the later sections).

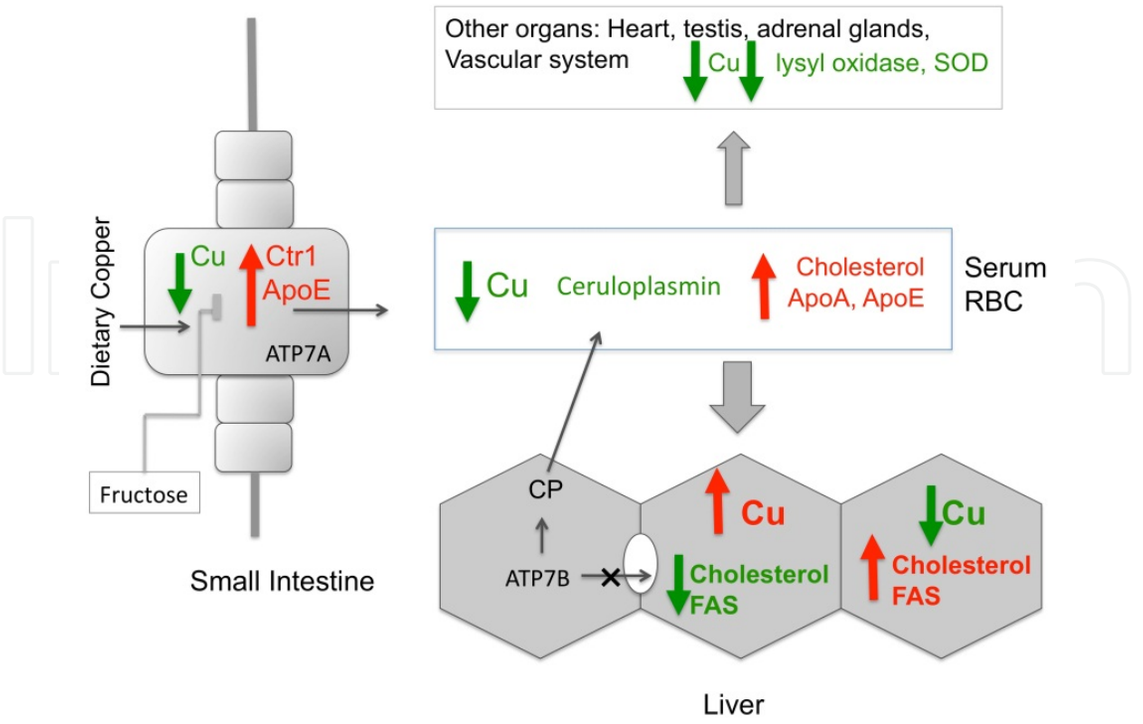

Figure 1. Copper homeostasis and lipid metabolism display functional interactions. Copper enters circulation via small intestine where copper transporters Ctr1 and ATP7A play the major role in the dietary copper absorption. Dietary copper deficiency is associated with a lower level of ceruloplasmin in the serum and increase in cholesterol and lipoproteins. Copper deficiency also upregulates the copper uptake protein Ctr1 in intestine; this compensatory effects is diminished by high fructose. In the liver, copper deficiency is associated with increased synthesis of cholesterol and higher expression of fatty acid synthase (FAS). Also, in the liver, the copper-transporting ATPase ATP7B mediates copper delivery to ceruloplasmin and copper export into the bile. Genetic inactivation of ATP7B in Wilson's disease is associated with copper overload and marked changes in lipid metabolism. Cholesterol biosynthesis is downregulated and both the serum and hepatic lipid profiles are altered.

The level of expression of copper transporters and their regulation varies between various organs (8). For example, ATP7B is the main transporter in hepatocytes, but it is absent from the adrenal gland, whereas opposite is true for ATP7A (9). Most of the tissues such as heart, brain, lung, placenta and kidneys express both copper-transporting ATPases along with the two copper uptake systems Ctr1 and Ctr2. As a result, the consequences of copper deficiency and copper overload are tissue specific, and certain organs such as heart or liver are more profoundly affected (see below). Recent studies also revealed an important homeostatic cross-talk between different organs in either copper deficiency or copper overload. For example, copper overload in the liver is accompanied by functional copper deficiency in the adrenal gland (9), whereas severe copper deficiency in the heart stimulates copper efflux from the liver (10), presumably to compensate for the deleterious effects of copper depletion.

The analysis of available literature also illustrates that variations in copper levels, either through the diet or as a result of genetic copper misbalance, have a profound effect on lipid

metabolism. Significance of these observations is becoming more and more apparent given recent data that dietary influences (such as amount of fat in the diet) could be important modifiers of the course and severity of the disorders associated with copper misbalance (11). Reciprocally, copper deficiency has emerged as a factor in the development of Non-alcoholic Fatty-Liver Disease (NAFLD)(12, 13), although current reports paint complex picture (14) and further studies are needed. Despite ample phenotypic evidence in support of copper-lipid interactions, little mechanistic work has been done so far, and current understanding of this metabolic interaction at the molecular level is very limited. The goal of this review is to illustrate and emphasize the need for such detailed mechanistic investigations.

2. Copper deficiency

2.1. Copper in western diet

Copper deficiency has long been known to alter lipid metabolism; consequently, it has been proposed as a significant factor in human diseases associated with dyslipidemia (15). Copper deficiency is rarely diagnosed in humans, with a notable exception of a growing number of reports pointing to copper and other mineral insufficiencies as unintended consequences of bariatric surgeries (16-18). The under-detection of copper deficiency could be due to limitations of screening using serum or urine samples. Although liver is the main homeostatic organ for copper and has a high copper content, copper levels in serum and urine do not correlate well with a hepatic copper concentration (19), possibly masking deficiency in the liver.

Recent work using a categorical regression analysis of copper deficiency and excess shows a U-shaped dose-response curve. Compilation of data on toxicity due to copper excess and deficiency yielded a generalized linear model that was used to estimate adverse responses depending on copper dose or severity of copper limitation, as well as duration of copper misbalance (20). This model indicates that for humans the optimal intake level for Cu is 2.6 mg/day. The current United States Recommended Daily Intake is only 0.9 mg (US Food and Nutrition Board), whereas dietary study indicated that even 1.03 mg of Cu/day may be insufficient for adult men (21). The results of the third National Health and Nutrition Examination Survey (NHANES III, 2003) in the US showed that the mean daily intake of copper, depending on age, was 1.54–1.7 mg/day (±0.05 standard deviation (SD)) for men and 1.13-1.18 mg/d (±0.05 SD) for women. These results imply that a large portion of the population may have insufficient dietary copper intake and mild copper deficiency.

2.2. Low copper and human disease

Current data suggest that copper deficiency may be a common contributing factor in cardiovascular disease (CVD) and non-alcoholic fatty-liver disease (NAFLD) (22-24). As described above, surgical obesity treatment has also been strongly implicated in copper deficiency, likely by causing a diminished absorption of copper after a gastric bypass surgery (16-18). In addition, low copper levels were detected in organs, plasma and tissue of

patients with several chronic diseases including cardiovascular disease, central nervous system, and musculoskeletal disorders (25). In fact, it was suggested that an ischemic heart disease could be largely attributed to copper deficiency (26), and that Cu deficiency and the high sugar consumption characteristic in the Western diet may interact in CVD (27). In a rat model, copper deficiency that reduces plasma levels of copper and ceruloplasmin also reduces copper content of the heart, liver, and testes. Coincidentally, heart zinc is also reduced, whereas hepatic iron levels rise (28), demonstrating the multifaceted effects of copper depletion on the overall body metal homeostasis.

Numerous studies in the rodent and other animal models (discussed below) provide strong indication of a significant link between copper deficiency and dyslipidemia. Specific studies linking human lipid response to copper deficiency are very limited. The available reports clearly illustrate the need for a better mechanistic insight. For example, studies using healthy male volunteers showed severe copper depletion with a diet containing 0.83 mg Cu/day, which is similar to levels in some contemporary diets. In these individuals, along with the diminished copper, the levels of serum copper-depended enzyme ceruloplasmin were reduced, as was copper-zinc superoxide dismutase activity in erythrocytes. In parallel, changes in lipid metabolism were evident. Cholesterol was elevated in the serum, and changes in the cholesterol levels were found to be more sensitive to copper levels than changes in hematology (29). These observations suggest that the dietary copper levels may be significant modifying factors in the disorders associated with lipid misbalance. Indeed, a role for copper deficiency is emerging in NAFLD (discussed below).

2.3. Low copper levels and hypercholesterolemia

Current data indicate that copper deficiency is associated with specific effects on systemic lipid metabolism. Although copper deficiency affects multiple organs (liver, heart, intestine, brain, adipose tissue), the liver and cardiovascular system appear more profoundly affected compared to other tissues. The effects of copper deficiency are partially reversible. In a middle-aged adult population with cardiovascular disease (CVD), copper supplementation was shown to raise the serum copper enzyme activities, but improvement of CVD measures was inconsistent (30). The same report also showed that copper supplementation reduced levels of oxidized serum LDL with statistical significance, but the results were inconsistent necessitating further research.

As described above, plasma cholesterol levels rise in human volunteers consuming a marginally low copper diet (29). In humans, the molecular mechanism behind this phenomenon has not yet been investigated in detail. However, much effort has been made characterizing the influence of copper deficiency on serum cholesterol as well as lipid profiles using animal models. These studies yielded wealth of useful information. Early work feeding rats a copper deficient diet revealed hypercholesterolemia, cardiac hypertrophy, hemorrhage, inflammation, and focal necrosis (31). This work also indicated that some of the cardiac pathology caused by copper deficiency could be linked to the lysyl oxidase deficiency. Lysyl oxidase (LOX) is a copper-dependent enzyme involved in the

cross-linking of collagen and elastin. Aortas of copper-deficient rats showed deformation and loss of elasticity, though myocardial arteries were normal. Since lysyl oxidase is only one of the copper dependent enzymes required for proper heart functions (others include cytochrome C oxidase, cytosolic superoxide dismutase, dopamine beta-mono-oxygenase) the role of low copper in CVD may be realized through multiple mechanisms in addition to the altered lipid metabolism.

The development of cholesterolemia in response to copper deficiency has been explored in some detail in rats. Copper deficiency was found to be associated with the increased HDL and LDL levels (30% increase in HDL in one study). In addition, the plasma volume also increases, thus raising the available pool of cholesterol even more (22, 32). In effect, a 60% increase in total cholesterol may be observed (22). Overall, copper deficiency increases the absolute levels of cholesterol, but there is an argument whether the size of plasma cholesterol pool size may be a more powerful measure of cholesterol elevation. For example, the time course study of serum cholesterol in rats kept for 3-7 weeks on a copper deficient diet found that with time the plasma cholesterol concentration leveled off, but the pool size of cholesterol increased due to increases in the plasma volume (33). Curiously, the rates of HDL catabolism also increased, though the liver and the adrenal gland did not take up additional HDL in copper deficiency, suggesting one possible mechanism for dyslipidemia with respect to the HDL pool (34).

2.4. Serum lipoproteins: changes in structure, composition, and degradation

Rat models have proven to be especially consistent and valuable in studies of the dyslipidemia resulting from copper deficiency. Despite variations in the age, diet composition, and time on a copper deficient diet, an increase in the cholesterol concentration and/or pool was consistently reported in these animals. The lipids and lipoprotein components were analyzed in detail by al-Othman and co-workers and provided valuable data on changes in plasma pool size along with the composition and concentration of lipoproteins (33). These studies demonstrated no change in plasma phospholipid composition in copper deficient rats when compared to controls. In contrast, triglycerides, phospholipids, and cholesterol in LDL and HDL increased 2-fold or more. The VLDL composition of copper deficient animals changed most significantly with a 6-fold increase in triglycerides, 36% reduction in cholesterol and no change in phospholipid. An increase was observed in the VLDL particle size, but not the number of particles. In contrast, an increase in both size and number was seen for the LDL particles, whereas for the HDL particles an increase in the number of particles was observed, but no size change.

The composition of serum lipoproteins in copper deficient animals may also be influenced by shifts in the expression and distribution of apolipoproteins, linking physiological response in the liver to the observed changes in plasma cholesterol. The plasma levels of apolipoproteins A, B, and E increase in rats fed a copper deficient diet, consistent with the

increased particle numbers and sizes (35-39). Remarkably, hepatic apoA1, ApoE and ApoB mRNA levels remain unchanged, suggesting that the alteration of the apolipoprotein levels is not due to an increase in transcription. Indeed, it was found that hepatic synthesis and secretion rates of apoA1 are upregulated (35), whereas the rates of synthesis of ApoB-48 and ApoB-100 are unchanged, despite an increase in secretion (37). Intestinal secretion of apoA-1 was also observed, providing yet another source for increased circulating apolipoproteins and cholesterol (40).

In copper deficiency, plasma HDL rich in apolipoprotein E (ApoE) accumulates and total ApoE binding to liver plasma membranes increases (also reported as a reduction in ApoE-free HDL binding). Interestingly, the cholesterol levels in the liver decrease with copper deficiency, despite an overall increase in hepatic cholesterol synthesis (41, 42). These changes in synthesis, binding properties, and redistribution of lipoproteins suggest some mechanisms through which copper deficiency affects serum cholesterol levels. Thus far, molecular investigations of copper deficiency have identified increased hepatic expression of SREBP1-responsive fatty-acid synthase, along with the increased nuclear localization of the mature SREBP1 transcriptional activator (24). Changes in the expression of other genes involved in the fatty acid synthesis have not been explored in any significant detail, although the mRNA levels for cholesterol 7-alpha hydroxylase were found decreased by 80%.

Changes in the plasma lipoprotein composition and structure are expected to influence the lipid content of red blood cells (RBC) through lipid exchange. Consequently, in copper deficiency changes in the RBC lipid composition are likely (43-45). Studies of the lipid profile of plasma and membranes of RBCs in copper deficiency support this view. Both cholesterol and phospholipid levels increase in the RBCs plasma membranes in Cu deficient rats, whereas the molar ratios of cholesterol:phospholipids and cholesterol:membrane protein are reduced (46). The phospholipid profiles change as well, with the increased stearic and docosadienoic acid content, and the lower levels of oleic and linolenic acid (47). A study assessing structural characteristics of the RBC membranes demonstrated a decrease in membrane fluidity and speculated that this could be the cause of hemolysis and anemia (48). Intriguingly, another study reported an increase in the RBC plasma membrane fluidity in copper deficient rats (49). This discrepancy may be due to experimental conditions. Motta and colleagues found that in copper deficiency, increased fluidity in RBCs plasma membrane can be seen alongside with higher rigidity due to enhanced susceptibility of triacylglycerol-rich lipoproteins to lipid peroxidation (50).

2.5. Copper-dependent lipid alteration in tissues

Given significant changes induced by copper deficiency in the serum and the liver, it is not surprising that the lipid and fatty-acid composition of other organs is also affected. Severe copper deficiency can be induced in C57BL mice by feeding dams a copper deficient diet and subsequently weaning pups to the same diet. These animals had lower levels of

phospholipids in the liver and kidney, as well as lower triacylglycerols in kidneys (51). Lower proportion and total amount of di-homo-γ-linoleic acid was observed in all tissues of these mice, though levels of other lipids varied. Severe copper deficiency also induced hepatomegaly, reduced the brain weight, and reduced serum ceruloplasmin to 0.5% of control, indicating profound systemic effects.

The liver is an organ that experiences significant changes in lipid composition and membrane structure in response to copper deficiency. The loss of membrane fluidity in hepatic tissue has been reported and suggested to be caused by changes in the composition of unsaturated fatty acids and triacylglycerols of fatty acids (48). Other observed changes in membrane lipids associated with copper deficiency include a decreased ratio of monounsaturated:saturated C16 and C18 fatty acids in adipose tissue and a decreased fatty acid desaturase activity in liver microsomes (52). Phosphatidylcholine biosynthesis may also be affected, as choline phosphotransferase activity levels are lower both in the heart and liver tissue in copper deficient rats (53).

Concurrent with changes in lipid profiles and synthesis, copper deficiency decreases the total amounts of body fat and shifts metabolic fuel use from carbohydrate to fat. Respiratory quotient is reduced, but total energy intake is the same for animals kept on copper deficient and copper adequate diets (54). Young, weanling rats fed on a copper deficient diet for six weeks are leaner than controls, though they have increased serum cholesterol and triglycerides. Metabolically, whole body respiratory quotient decreases, reflected in a reduction of cardiac and adipose lipoprotein lipase, but not the skeletal muscle lipoprotein lipase (55). The change in fuel use may be related to upregulation of fatty acid synthesis. Copper deficiency does, however, decrease levels of hepatic cytochrome C oxidase (56).

The molecular mechanisms for these changes in lipid metabolism due to copper deficiency are understudied; as a result, current knowledge is limited. Increases in expression of specific apolipoproteins and increased transcription of gene for fatty acid synthase in the liver have been reported (see above), providing first insights into molecular players that are involved in response to low copper. Some key information has also been gained in one gene expression study. A transriptome analysis in the small intestine of copper deficient rats revealed upregulation of mRNA for proteins involved in cholesterol transport including apolipoprotein E and the lecithin:cholesterol acyltransferase providing mechanism for enhanced intestinal cholesterol secretion (57). The study also reported down-regulation of genes in the pathway for fatty acid beta-oxidation (both mitochondrial and peroxisomal). The results suggested a change in cell metabolism that reduced fatty acid oxidation, perhaps as a feedback to the decreased cytochrome C oxidase activity. The specific effects of copper deficiency may differ in tissues and serum. For example, in contrast to intestine, the activity of plasma lecithin:cholesterol acyltransferase (a risk factor for ischemic heart disease) is decreased in rats fed a copper deficient diet (58).

3. Functional interactions between copper, lipid, and other nutrients

3.1. Copper and dietary fat/lipid

Considering that copper deficiency influences systemic lipid metabolism, it would be interesting to know whether interactions between copper and lipid levels are reciprocal. In other words, it is important to determine whether dietary fat consumption, or changes in the type of fat consumed influences the activity or levels of copper-dependent enzymes. Changes in the ratios of saturated and unsaturated fatty acids have been noted in copper deficiency, and a cardioprotective effect of increasing proportion of polyunsaturated fatty acids was proposed (59). Curiously, feeding saturated fat in a copper deficient rat model increased hepatic copper as well as iron levels to a significant degree. Saturated fat consumption, however, did not change copper deficiency-induced lipid peroxidation, despite recovery of some hepatic copper. Copper-zinc superoxide dismutase (Cu/Zn-SOD) in the liver is less active in copper-deficient rats, whereas other hepatic antioxidant enzymes are unaffected by copper deficiency (56). This observation suggests that proper incorporation of copper in Cu/Zn-SOD may be key to preventing lipid peroxidation.

The effect of fatty acids on copper may also be mediated at the level of intestinal absorption. Experiments with the long chain fatty acids palmitate and stearate showed reduced levels of copper absorption from the jejunum (60) in response to treatment. In another study, direct cholesterol feeding of rabbits was used to model hypercholesterolemia and atherosclerosis. Adding cholesterol to 0.5% of diet triggered the redistribution of copper from the liver to plasma, with a 50% increase in plasma Cu and a 74% reduction in liver copper (61). Interestingly, copper supplementation in cholesterol-fed rabbits reduced atherosclerotic lesions (62). Further support for the importance of copper-lipid interactions in cardiac function is indicated by the observation that cardiomyopathy might be exacerbated by combination of high dietary fat and copper restriction. Specifically, when copper restriction and dietary fat supplementation were tested separately and together, the lowest level of cardiac cytochrome C oxidase activity was observed in copper-deficient rats on a high fat diet (63).

3.2. Fructose, lipids, and copper metabolism

The influence of dietary sugar consumption on lipid metabolism may be mediated, in part, by exacerbation of copper deficiency. Copper-deficient rats fed a sucrose-based or starch-based diets all had increased plasma cholesterol and lower plasma ceruloplasmin levels, as observed in copper deficiency alone (64). However, feeding sucrose rather than starch greatly enhanced deleterious effects of copper deficiency, such that those animals showed 60% mortality in the 9-week study. The copper deficient sucrose fed rats had a 3-fold lower hepatic copper level compared to starch-fed copper deficient rats. These results suggested a sucrose-dependent change in copper mobilization or retention within the liver (64). Cardiac abnormalities consistent with copper deficiency were also observed. Follow-up work

indicated that when diets with fructose, glucose or starch were combined with copper deficiency, both glucose and fructose raised plasma cholesterol levels. However, severity of copper deficiency and mortality were much greater with fructose as opposed to glucose feeding (65), suggesting that the exacerbation of copper deficiency by sucrose was due to the fructose component. More recent work (66) indicates that the effect of fructose may be at the level of absorption, whereby copper deficiency induces upregulation of the copper transporter Ctr1, but this effect is eliminated by high fructose feeding (Figure 1).

Dietary fructose consumption and dietary copper deficiency independently alter fatty-acid metabolism; in combination, the effect is enhanced (66-69). Feeding weanling rats for three weeks on a diet adequate or deficient in copper along with either fructose or corn starch as a carbohydrate source (62% carbohydrate) revealed that fructose feeding enhanced indicators of copper deficiency, such as enhanced heart/body weight ratio and reduced hepatic copper (70). Analysis of hepatic enzymes involved in the lipid and carbohydrate metabolism also indicated that a diet deficient in copper had greatest metabolic effects in combination with fructose, less with glucose and least with starch (71). Glucose-6-phosphate dehydrogenase, malic enzyme, L-alpha-glycerophosphate dehydrogenase and fructose 1-6-diphosphatase were all unaffected by Cu deficiency, but their activities were enhanced most in combination with fructose, suggesting a complementary rather than direct role for fructose in exacerbating copper deficiency (71).

Copper deficiency in combination with fructose feeding alters the fatty acid composition of triacylglycerol in the heart and the liver. Cardiac phosphatidylinositol and phosphatidylserine were shown to increase nearly two-fold and arachidonic acid and docosapentaenoic acid to be elevated more than two-fold in copper deficient, fructose fed animals (70). A change in cardiac phospholipids may explain the increased mortality observed in copper deficient/fructose fed rats and suggest a potentially significant role for these dietary factors in ischemic heart disease. However, the observed changes in the lipid composition could not be correlated with the extent of copper deficiency, illustrating once again that a detailed mechanistic understanding of these dietary interactions could be highly beneficial.

Western diet is characterized by high fructose and high fat. In combination with a likely mild copper deficiency (see above), it stands to reason that these factors may all interact to induce changes in whole body metabolism, especially lipid metabolism, producing deleterious hepatic and cardiovascular effects. When this idea is tested in rats by combining high fat and fructose, or low fat and fructose with copper deficiency, liver metabolism is most significantly affected by the fat and fructose combination (28). Sugars mobilize copper from the liver to other tissues (64), possibly causing a change in liver physiology. Recent work also indicates that the mobilization of copper from the liver may be driven by heart-specific copper deficiency (10). Thus, in addition to interaction during absorption, an important inter-organ communication exists that jointly modulates levels of copper and lipids in various tissues

4. Copper misbalance and lipid metabolism in human disease

4.1. Lipid metabolism in Menkes disease

The studies discussed above were focused on the effects of dietary copper deficiency. In humans and in animals, genetic inactivation of the copper transporting ATPase ATP7A impairs copper export from the intestine, effectively limiting copper supply to many tissues and causing lethal pathology known as Menkes disease, MND (72). Depending on the type of mutation in ATP7A, severity of copper deficiency as well as disease manifestations vary between MND patients. Lipid profiles have been explored in some patients and found affected, but inconsistently, with the exception of a higher neutral lipid content of VLDL in all tested MND patients compared to controls. Interestingly, although ApoB in patients appeared normal, it degraded faster during storage suggesting lower stability (73). Variations in lipid profiles could also be age-related. Studies in animals (including an animal model for Menkes disease) demonstrate that unlike adults, copper deficient young animals do not show elevated cholesterol in the serum (74). The resistance of young mice and rats to hypercholesterolemia could be due to insufficient depletion of copper in the liver, which serves as a major store of copper in neonatal animals, or/and insufficiently progressed functional defects in the liver at young age (74).

ATP7A plays an important role in the development and maintenance of vasculature by supplying copper for functional maturation of lysyl oxidase. Recent studies also suggested the important role for ATP7A in the pathogenesis of atherosclerosis (75). ATP7A was detected in atherosclerotic lesions of mice with genetically inactivated LDL receptor where it colocalized with macrophages. Down-regulation of ATP7A in a macrophage-derived cell culture by siRNA resulted in decreased expression and enzymatic activity of cytosolic phospholipase A(2) alpha, an important enzyme involved in LDL oxidation (75). Furthermore, only cell-mediated LDL oxidation was reduced following down-regulation of ATP7A, whereas conditioned medium from either control or ATP7A down-regulated cells was without such effect (75). This result indicates that the reduced LDL oxidation is not simply due to a diminished copper export from cells with down-regulated ATP7A, but rather due to complex metabolic interactions between copper misbalance and lipid metabolism

4.2. Copper overload in Wilson's disease markedly alters lipid metabolism.

Direct evidence for the important role of copper in modulating hepatic lipid metabolism and serum lipid profiles has been produced by studies using *Atp7b-/-* mice (an animal model of Wilson's disease, WD) and, subsequently, samples from WD patients. Wilson's disease is a severe genetic disorder of copper overload, caused by inactivating mutations of copper transporting ATPase ATP7B and inability to excrete excess copper from hepatocytes (76). Despite massive accumulation of copper in a cytosol, copper incorporation into ceruloplasmin is impaired and serum levels and activity of ceruloplasmin are greatly diminished (phenotypically resembling consequences of copper deficiency, Figure 2). The

genetically engineered knockouts of Atp7b (*Atp7b-/-* mice) accurately reproduce these key features of WD (77). Consequently, these animals have been used to investigate consequences of copper overload at the molecular level.

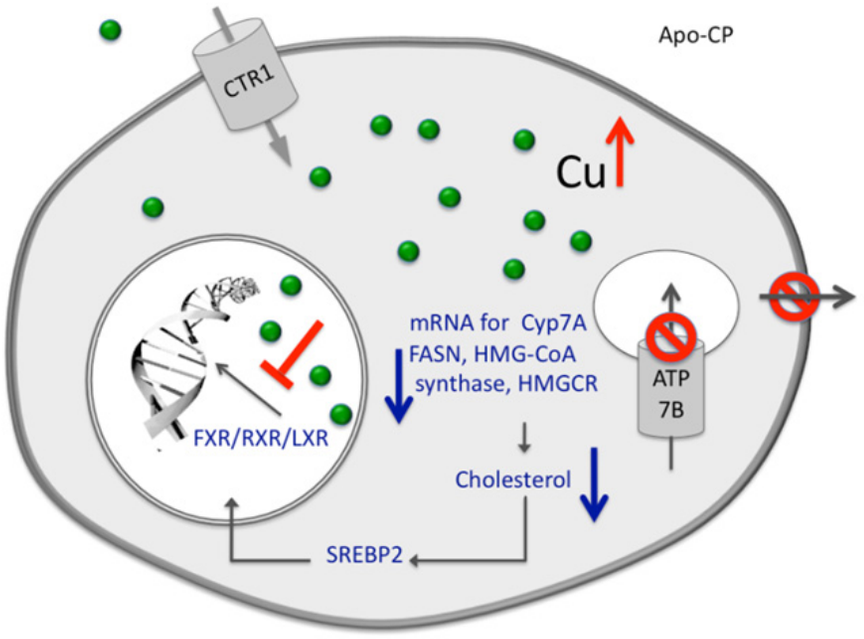

Figure 2. Elevated copper in *Atp7b-/-* hepatocytes inhibits activities of nuclear receptors and dysregulates lipid metabolism. In Wilson's disease, genetic inactivation of the copper transporting ATPase ATP7B results in an impaired copper export from hepatocytes, loss of copper incorporation into ceruloplasmin, CP, (and a secretion of Apo-CP into the serum) along with massive copper accumulation in hepatocytes. Copper concentrates preferentially in the cytosol and nuclei. Cytosolic copper does not prevent sensing of cholesterol levels, allowing SREBP-2 cleavage and entry into the nucleus in response to low cholesterol. However, high copper in the nucleus blocks activity of nuclear receptors, presumably through binding or oxidation. Impaired function of nuclear proteins is associated with a decrease in the mRNA levels for several enzymes, including HMG-CoA reductase (HMGCR) and HMG-CoA synthase, and low total cholesterol in the liver.

Studies at the early stage of pathology development in *Atp7b-/-* mice (prior to the onset of visible morphologic changes in the liver) were particularly informative. The analysis of liver transcriptome revealed that copper accumulation is associated with distinct metabolic changes: upregulation of genes involved in cell cycle and chromosome maintenance and down-regulation of lipid metabolism, especially cholesterol biosynthesis (78, 79). The mRNA studies were complemented by the analysis of metabolites, which confirmed significant (30-40%) decrease of cholesterol in the liver, and the lower levels of VLDL cholesterol in the serum (79). These early changes in lipid metabolism are maintained throughout the course of the disease as indicated by the analysis of the mRNA levels and serum lipid profiles at the later stages of the disease (80). Follow-up studies revealed that the sterol metabolism in a brain of young *Atp7b-/-* mice was also dysregulated, with cholesterol, 8-dehydrocholesterol, desmosterol, 7-dehydrocholesterol, and lathosterol all

being highly increased (81, 82). It should be noted that the main cholesterol-sensing pathway that involves proteolysis and nuclear localization of SREBP2 transcription factor is not impaired in *Atp7b*$^{-/-}$ hepatocytes (79).

These important observations necessitated further mechanistic studies. Such work has recently been done using systems biology approach and biochemical/biophysical measurements. Direct *in situ* imaging of copper using X-fluorescence revealed the non-uniform distribution of accumulating copper in *Atp7b*-/- hepatocytes with a predominant increase in the cytosol and nuclei (80). Mass-spectrometry in combination with generation of protein networks provided strong evidence that accumulating copper had a significant and specific functional impact on *Atp7b*-/- nuclei, where it remodeled the RNA-processing machinery (83) and altered abundance and activity of nuclear receptors (84). Specifically, using quantitative Multidimensional Protein Identification Technology (MuDPIT), Wilmarth and colleagues found that the ligand-activated nuclear receptors FXR/NR1H4 and GR/NR3C1 were less abundant in nuclear preparations from *Atp7b*-/- liver, whereas the DNA repair machinery and the nucleus-localized glutathione peroxidase SelH were more abundant, consistent with the earlier transcriptome studies (79).

These findings provide important mechanistic insights into a copper-dependent dysregulation of lipid metabolism (summarized in Figure 2). It seems that hepatic nuclei are the primary sites of action for elevated copper. In WD, the local copper concentration in the nuclei can increase up to 50-100 fold (80). Copper is a redox active metal, and such marked elevation is likely to alter the nuclear redox environment, as also suggested by the upregulation of nuclear glutathione peroxidase SelH. Oxidation of sensitive cysteine residues and/or competition between copper and zinc in zinc fingers (which are common structural features in nuclear proteins) are likely mechanisms that impair activity of nuclear factors, such as transcription factors and/or components of the RNA splicing machinery (Figure 2). Current data suggest that neither potential cysteine oxidation nor copper-zinc competition are wide spread (83) and that nuclear proteins regulating lipid metabolism are preferentially affected by elevated copper. Thus, further studies are needed to understand the molecular basis of this increased sensitivity to copper levels.

The identification of FXR/LXR/RXR as important players in pathology development of WD (85) opens a new avenue for studies aimed on better understanding of the role of a diet (especially cholesterol and fat components) in the time-of-onset and severity of WD. It is important to emphasize that the effect of high copper on lipid metabolism is conserved between species, as evidenced by down-regulation of the same key enzymes in the lipid biosynthesis pathways in mice and human liver in response to copper overload (79). Recent studies of serum samples in the cohort of WD patient revealed differences in cholesterol metabolism that diminished with a copper-chelation therapy (82). These observations further supports high metabolic significance of copper:lipid interactions.

A marked effect of elevated copper on lipid metabolism was also reported in Long-Evans Cinnamon (LEC) rat, another murine model of WD (86). Analysis of the liver and serum lipid profiles in these animals showed that copper overload was associated with a higher

content of triglycerides, free cholesterol and cholesteryl ester when compared to controls. The effect is actually opposite to that reported in *Atp7b-/-* mice. At present, the reason for this discrepancy is not clear. In LEC rat, liver disease is more severe compared to *Atp7b-/-* mice, invariably leading to animals death, which is not the case in mice. The mitochondrial function in these rats is more significantly affected (87), perhaps contributing to metabolic differences. Nevertheless, similarly to *Atp7b-/-* mice, the LEC serum is characterized by hypotriglyceridemia, hypocholesterolemia and abnormalities in the composition and size of circulating lipoproteins. Also similarly to *Atp7b-/-* and human liver, the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase is reduced in LEC rats, along with other important enzyme involved in lipid biosynthesis (Figure 2) (86).

4.3. Copper and NAFLD

The earlier studies of copper misbalance were focused mostly on the effects of copper deficiency and resulting dyslipidemia on cardiovascular disease. The liver, however, is the central organ of copper homeostasis and, as discussed above, it is greatly affected in WD. It may also be functionally affected in copper deficiency (88). Caloric excess associated with the modern Western diet is implicated in NAFLD, however caloric excess does not compensate for copper deficiency. Furthermore, copper deficiency can still be experienced by the liver, even when serum copper levels are maintained or increased due to factors such as dietary cholesterol.

Although numerous studies clearly link copper deficiency to altered lipid metabolism in animal models (12, 22, 24, 89-92) and human volunteers (29), only recently has low dietary copper been implicated in liver dyslipidemia pathology, including non-alcoholic fatty-liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). In a recent groundbreaking study, hepatic copper content in biopsy specimens was inversely correlated with the severity of fatty liver disease, and copper deficiency in a rodent model was found sufficient to induce NAFLD and metabolic syndrome (12). Hepatic iron accumulation, a known consequence of copper deficiency, is also observed in NAFLD (93). Iron accumulation likely results from the loss of holoceruloplasmin, a copper-dependent ferroxidase instrumental in iron distribution (94). This, in turn, results in lower levels of ferroportin in copper deficient rats; coincidentally, NAFLD patients show less ferroportin expression than controls (93). Copper supplementation has been suggested as a therapy for NAFLD based on study in which a diet-induced (high carbohydrate fat-free diet) NAFLD in rats was improved by treatment with a Cu(I)-nicotinate complex (95).

Studies of NAFLD also reflect the intersection of copper deficiency, hepatic lipid metabolism and consumption of fructose as causative agents in NAFLD. High dietary sugars, particularly fructose, have been implicated in development of NAFLD and NASH (96, 97). As discussed above, there is evidence that dietary fructose contributes to copper deficiency (66, 69), indicating cross talk between these dietary factors. Sucrose and fructose may have similar effects, as the enzyme sucrase acts in the digestive system to convert the disaccharide sucrose into fructose and glucose for transport in the

bloodstream. High dietary fructose results in decreased CuZnSOD expression (98), lowering resistance of oxidative damage. A diet of 60% fructose affects lipid metabolism as well as antioxidant status, including CuZnSOD, in the liver of rats after 13 weeks of treatment (68). It is clear these high fructose diets induce NAFLD in rodent models, however these diets are typically 60-70% fructose and may not accurately reflect human fructose consumption (for review see (99)).

It is proposed that fructose metabolism induces oxidative stress, and this may trigger NAFLD. A copper-fructose feeding study indicated that lipid peroxidation due to copper deficiency and a 62% fructose diet could be reduced by supplementing vitamin E to 1 g/kg, however copper deficiency remained, indicating that copper deficiency with fructose feeding may not be entirely a result of oxidative stress (100). Nevertheless, hepatic lipid peroxidation is enhanced significantly in copper deficiency with fructose feeding, supporting the role for oxidative stress in liver disease through the impairment of hepatic antioxidant systems (66).

Tissue	Increase	Decrease	Other observation
Erythrocytes	cholesterol, phospholipid, stearic acid, docosadienoic acid	cholesterol:phospholipid ratio, cholesterol: mebmbrane protein ratio, linoleic acid, oleic acid	membrane fluidity?
Plasma and serum	triglycerides, phospholipids and cholesterol in lipoproteins; apoA, apoB, apoE; plasma volume	N/A	hematocrit
Liver	apolipoprotein secretion, cholesterol synthesis and secretion, fatty acid syntase expression	apoE-free HDL binding; monounsaturated:saturated fatty acids ratio	no change in apolipoprotein transcript levels
Heart	stearic acid; docosahexaenoic acid; total phospholipid	elastic fibers; palmitic acid; oleic acid	hypertrophy; inflammation; distorted elastic fibers
Small intestine	transcripts in cholesterol transport	transcripts in fatty-acid beta-oxidation	NA
Kidney	NA	triacylglycrol and phospholipid	NA

Table 1. Tissue-specific effects of copper deficiency on lipid metabolism

5. Conclusions

Copper deficiency and copper overload have multiple and significant effects on systemic and cellular lipid metabolism. Recent studies indicate that copper misbalance is an emerging factor in dyslipidemia and/or fatty-liver disease. In turn, lipid metabolism could be an important modifier of the time-of-onset and severity in Wilson's disease. Work over several decades has yielded physiological and biochemical data on the consequences of copper deficiency, particularly with respect to lipid metabolism, in rodent models. Understanding of human disease would greatly benefit from further analysis of copper levels and lipid profiles in human clinical specimens, as well as assessment of the influence of other nutrients at the molecular level. Animal models will remain important. As much as has already been learned, further mechanistic studies are bound to yield molecular level understanding of the important copper:lipid relationship. It is still not clear how copper deficiency alters gene expression and protein expression/function to produce observed pathologies. Transcript profiling, proteomic analysis, and metabolite profiling, in both data-driven and targeted formats, promise to provide more mechanistic details in animal models that can be tested in human pathology.

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6. References

[1] Nose Y, Kim BE, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab. 2006 Sep;4(3):235-44.

[2] Vulpe CD, Packman S. Cellular copper transport. Annu Rev Nutr. 1995;15:293-322.

[3] Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proc Natl Acad Sci U S A. 2001 Jun 5;98(12):6842-7.

[4] Kuo YM, Zhou B, Cosco D, Gitschier J. The copper transporter CTR1 provides an essential function in mammalian embryonic development. Proc Natl Acad Sci U S A. 2001 Jun 5;98(12):6836-41.

[5] Mercer JF, Ambrosini L, Horton S, Gazeas S, Grimes A. Animal models of Menkes disease. Adv Exp Med Biol. 1999;448:97-108.

[6] Lutsenko S, Gupta A, Burkhead JL, Zuzel V. Cellular multitasking: the dual role of human Cu-ATPases in cofactor delivery and intracellular copper balance. Arch Biochem Biophys. 2008 Aug 1;476(1):22-32.

[7] Gupta A, Lutsenko S. Human copper transporters: mechanism, role in human diseases and therapeutic potential. Future Med Chem. 2009 Sep;1(6):1125-42.

[8] Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. Function and regulation of human copper-transporting ATPases. Physiol Rev. 2007 Jul;87(3):1011-46.

[9] Gerbasi V, Lutsenko S, Lewis EJ. A mutation in the ATP7B copper transporter causes reduced dopamine beta-hydroxylase and norepinephrine in mouse adrenal. Neurochem Res. 2003 Jun;28(6):867-73.

[10] Kim B, Turski M, Nose Y, Casad M, Rockman H, Thiele D. Cardiac copper deficiency activates a systemic signaling mechanism that communicates with the copper acquisition and storage organs. Cell Metab. 2010 May 5;11(5):353-63.

[11] Kegley KM, Sellers MA, Ferber MJ, Johnson MW, Joelson DW, Shrestha R. Fulminant Wilson's disease requiring liver transplantation in one monozygotic twin despite identical genetic mutation. Am J Transplant. 2010 May;10(5):1325-9.

[12] Aigner E, Strasser M, Haufe H, Sonnweber T, Hohla F, Stadlmayr A, et al. A role for low hepatic copper concentrations in nonalcoholic Fatty liver disease. Am J Gastroenterol. 2010 Sep;105(9):1978-85.

[13] Lampon N, Tutor JC. Effect of valproic acid treatment on copper availability in adult epileptic patients. Clin Biochem. 2010 Sep;43(13-14):1074-8.

[14] Lampon N, Tutor JC. A preliminary investigation on the possible association between diminished copper availability and non-alcoholic fatty liver disease in epileptic patients treated with valproic acid. Ups J Med Sci. 2011 May;116(2):148-54.

[15] Stemmer KL, Petering HG, Murthy L, Finelli VN, Menden EE. Copper deficiency effects on cardiovascular system and lipid metabolism in the rat; the role of dietary proteins and excessive zinc. Ann Nutr Metab. 1985;29(6):332-47.

[16] Ernst B, Thurnheer M, Schultes B. Copper deficiency after gastric bypass surgery. Obesity (Silver Spring). 2009 Nov;17(11):1980-1.

[17] Gletsu-Miller N, Broderius M, Frediani J, Zhao V, Griffith D, Davis SJ, et al. Incidence and prevalence of copper deficiency following roux-en-y gastric bypass surgery. Int J Obes (Lond). 2012 Mar;36(3):328-35.

[18] Medeiros D. Gastric bypass and copper deficiency: a possible overlooked consequence. Obes Surg. 2011 Sep;21(9):1482-3; author reply 4-5.

[19] Evans J, Newman S, Sherlock S. Liver copper levels in intrahepatic cholestasis of childhood. Gastroenterology. 1978 Nov;75(5):875-8.

[20] Chambers A, Krewski D, Birkett N, Plunkett L, Hertzberg R, Danzeisen R, et al. An exposure-response curve for copper excess and deficiency. J Toxicol Environ Health B Crit Rev. 2010 Oct;13(7-8):546-78.

[21] Reiser S, Smith JJ, Mertz W, Holbrook J, Scholfield D, Powell A, et al. Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am J Clin Nutr. 1985 Aug;42(2):242-51.

[22] Lei K. Alterations in plasma lipid, lipoprotein and apolipoprotein concentrations in copper-deficient rats. J Nutr. 1983 Nov;113(11):2178-83.

[23] Saari J. Copper deficiency and cardiovascular disease: role of peroxidation, glycation, and nitration. Can J Physiol Pharmacol. 2000 Oct;78(10):848-55.

[24] Tang Z, Gasperkova D, Xu J, Baillie R, Lee J, Clarke S. Copper deficiency induces hepatic fatty acid synthase gene transcription in rats by increasing the nuclear content of mature sterol regulatory element binding protein 1. J Nutr. 2000 Dec;130(12):2915-21.

[25] Klevay L. Is the Western diet adequate in copper? J Trace Elem Med Biol. 2011 Oct 5.

[26] Klevay L. Ischemic heart disease as deficiency disease. Cell Mol Biol (Noisy-le-grand). 2004 Dec;50(8):877-84.

[27] Aliabadi H. A deleterious interaction between copper deficiency and sugar ingestion may be the missing link in heart disease. Med Hypotheses. 2008;70(6):1163-6.

[28] Wapnir R, Devas G. Copper deficiency: interaction with high-fructose and high-fat diets in rats. Am J Clin Nutr. 1995 Jan;61(1):105-10.

[29] Klevay L, Inman L, Johnson L, Lawler M, Mahalko J, Milne D, et al. Increased cholesterol in plasma in a young man during experimental copper depletion. Metabolism. 1984 Dec;33(12):1112-8.

[30] Disilvestro R, Joseph E, Zhang W, Raimo A, Kim Y. A randomized trial of copper supplementation effects on blood copper enzyme activities and parameters related to cardiovascular health. Metabolism. 2012 Mar 21.

[31] Allen K, Klevay L. Cholesterolemia and cardiovascular abnormalities in rats caused by copper deficiency. Atherosclerosis. 1978 Jan;29(1):81-93.

[32] Carr T, Lei K. High-density lipoprotein cholesteryl ester and protein catabolism in hypercholesterolemic rats induced by copper deficiency. Metabolism. 1990 May;39(5):518-24.

[33] al-Othman A, Rosenstein F, Lei K. Pool size and concentration of plasma cholesterol are increased and tissue copper levels are reduced during early stages of copper deficiency in rats. J Nutr. 1994 May;124(5):628-35.

[34] Carr T, Lei K. In vivo apoprotein catabolism of high density lipoproteins in copper-deficient, hypercholesterolemic rats. Proc Soc Exp Biol Med. 1989 Sep;191(4):370-6.

[35] Hoogeveen R, Reaves S, Lei K. Copper deficiency increases hepatic apolipoprotein A-I synthesis and secretion but does not alter hepatic total cellular apolipoprotein A-I mRNA abundance in rats. J Nutr. 1995 Dec;125(12):2935-44.

[36] Mazur A, Nassir F, Gueux E, Cardot P, Bellanger J, Lamand M, et al. The effect of dietary copper on rat plasma apolipoprotein B, E plasma levels, and apolipoprotein gene expression in liver and intestine. Biol Trace Elem Res. 1992 Aug;34(2):107-13.
[37] Nassir F, Mazur A, Serougne C, Gueux E, Rayssiguier Y. Hepatic apolipoprotein B synthesis in copper-deficient rats. FEBS Lett. 1993 May 3;322(1):33-6.
[38] Nassir F, Giannoni F, Mazur A, Rayssiguier Y, Davidson N. Increased hepatic synthesis and accumulation of plasma apolipoprotein B100 in copper-deficient rats does not result from modification in apolipoprotein B mRNA editing. Lipids. 1996 Apr;31(4):433-6.
[39] Norn M. Pemphigoid related to epinephrine treatment. Am J Ophthalmol: United States; 1977. p. 138.
[40] Wu J, Lei K. Copper deficiency increases total protein and apolipoprotein A-I synthesis in the rat small intestine. Proc Soc Exp Biol Med. 1996 Sep;212(4):369-77.
[41] Hassel C, Lei K, Carr T, Marchello J. Lipoprotein receptors in copper-deficient rats: apolipoprotein E-free high density lipoprotein binding to liver membranes. Metabolism. 1987 Nov;36(11):1054-62.
[42] Yount N, McNamara D, Al-Othman A, Lei K. The effect of copper deficiency on rat hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. J Nutr Biochem. 1990 Jan;1(1):21-7.
[43] Gold J, Phillips M. Effects of membrane lipids and -proteins and cytoskeletal proteins on the kinetics of cholesterol exchange between high density lipoprotein and human red blood cells, ghosts and microvesicles. Biochim Biophys Acta. 1992 Oct 19;1111(1):103-10.
[44] Nikolic M, Stanic D, Baricevic I, Jones D, Nedic O, Niketic V. Efflux of cholesterol and phospholipids derived from the haemoglobin-lipid adduct in human red blood cells into plasma. Clin Biochem. 2007 Mar;40(5-6):305-9.
[45] Schick B, Schick P. Cholesterol exchange in platelets, erythrocytes and megakaryocytes. Biochim Biophys Acta. 1985 Feb 8;833(2):281-90.
[46] Jain S, Williams D. Copper deficiency anemia: altered red blood cell lipids and viscosity in rats. Am J Clin Nutr. 1988 Sep;48(3):637-40.
[47] Abu-Salah K, al-Othman A, Lei K. Lipid composition and fluidity of the erythrocyte membrane in copper-deficient rats. Br J Nutr. 1992 Sep;68(2):435-43.
[48] Lei K, Rosenstein F, Shi F, Hassel C, Carr T, Zhang J. Alterations in lipid composition and fluidity of liver plasma membranes in copper-deficient rats. Proc Soc Exp Biol Med. 1988 Jul;188(3):335-41.
[49] Rock E, Gueux E, Mazur A, Motta C, Rayssiguier Y. Anemia in copper-deficient rats: role of alterations in erythrocyte membrane fluidity and oxidative damage. Am J Physiol. 1995 Nov;269(5 Pt 1):C1245-9.
[50] Motta C, Gueux E, Mazur A, Rayssiguier Y. Lipid fluidity of triacylglycerol-rich lipoproteins isolated from copper-deficient rats. Br J Nutr. 1996 May;75(5):767-73.

[51] Cunnane S, McAdoo K, Prohaska J. Lipid and fatty acid composition of organs from copper-deficient mice. J Nutr. 1986 Jul;116(7):1248-56.

[52] Wahle E, Davies N. Effect of dietary copper deficiency. in the rat on fatty acid composition of adipose tissue and desaturase activity of liver microsomes. Br J Nutr. 1975 Jul;34(1):105-12.

[53] Cornatzer W, Haning J, Klevay L. The effect of copper deficiency on heart microsomal phosphatidylcholine biosynthesis and concentration. Int J Biochem. 1986;18(12):1083-7.

[54] Hoogeveen R, Reaves S, Reid P, Reid B, Lei K. Copper deficiency shifts energy substrate utilization from carbohydrate to fat and reduces fat mass in rats. J Nutr. 1994 Sep;124(9):1660-6.

[55] Wildman R, Mao S. Tissue-specific alterations in lipoprotein lipase activity in copper-deficient rats. Biol Trace Elem Res. 2001 Jun;80(3):221-9.

[56] Lynch S, Strain J. Dietary saturated or polyunsaturated fat and copper deficiency in the rat. Biol Trace Elem Res. 1989 Nov;22(2):131-9.

[57] Tosco A, Fontanella B, Danise R, Cicatiello L, Grober O, Ravo M, et al. Molecular bases of copper and iron deficiency-associated dyslipidemia: a microarray analysis of the rat intestinal transcriptome. Genes Nutr. 2010 Mar;5(1):1-8.

[58] Lau B, Klevay L. Plasma lecithin: cholesterol acyltransferase in copper-deficient rats. J Nutr. 1981 Oct;111(10):1698-703.

[59] Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med. 2010 Mar;7(3):e1000252.

[60] Wapnir R, Sia M. Copper intestinal absorption in the rat: effect of free fatty acids and triglycerides. Proc Soc Exp Biol Med. 1996 Apr;211(4):381-6.

[61] Klevay L. Dietary cholesterol lowers liver copper in rabbits. Biol Trace Elem Res. 1988 Jun;16(1):51-7.

[62] Lamb D, Reeves G, Taylor A, Ferns G. Dietary copper supplementation reduces atherosclerosis in the cholesterol-fed rabbit. Atherosclerosis. 1999 Sep;146(1):33-43.

[63] Jalili T, Medeiros D, Wildman R. Aspects of cardiomyopathy are exacerbated by elevated dietary fat in copper-restricted rats. J Nutr. 1996 Apr;126(4):807-16.

[64] Fields M, Ferretti R, Smith JJ, Reiser S. Effect of copper deficiency on metabolism and mortality in rats fed sucrose or starch diets. J Nutr. 1983 Jul;113(7):1335-45.

[65] Fields M, Ferretti R, Smith JJ, Reiser S. The interaction of type of dietary carbohydrates with copper deficiency. Am J Clin Nutr. 1984 Feb;39(2):289-95.

[66] Song M, Schuschke D, Zhou Z, Chen T, Pierce W, Wang R, et al. High Fructose Feeding Induces Copper Deficiency in Sprague-Dawley rats: A Novel Mechanism for Obesity Related Fatty Liver. J Hepatol. 2011 Jul 19.

[67] Fields M, Lewis C. Dietary fructose but not starch is responsible for hyperlipidemia associated with copper deficiency in rats: effect of high-fat diet. J Am Coll Nutr. 1999 Feb;18(1):83-7.

[68] Girard A, Madani S, Boukortt F, Cherkaoui-Malki M, Belleville J, Prost J. Fructose-enriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats. Nutrition. 2006 Jul-Aug;22(7-8):758-66.

[69] Redman R, Fields M, Reiser S, Smith JJ. Dietary fructose exacerbates the cardiac abnormalities of copper deficiency in rats. Atherosclerosis. 1988 Dec;74(3):203-14.

[70] Cunnane S, Fields M, Lewis C. Dietary carbohydrate influences tissue fatty acid and lipid composition in the copper-deficient rat. Biol Trace Elem Res. 1989 -1990 Winter;23:77-87.

[71] Fields M, Ferretti R, Judge J, Smith J, Reiser S. Effects of different dietary carbohydrates on hepatic enzymes of copper-deficient rats. Proc Soc Exp Biol Med. 1985 Mar;178(3):362-6.

[72] Gasch AT, Kaler SG, Kaiser-Kupfer M. Menkes disease. Ophthalmology. 1999 Mar;106(3):442-3.

[73] Blackett PR, Lee DM, Donaldson DL, Fesmire JD, Chan WY, Holcombe JH, et al. Studies of lipids, lipoproteins, and apolipoproteins in Menkes' disease. Pediatr Res. 1984 Sep;18(9):864-70.

[74] Prohaska JR, Korte JJ, Bailey WR. Serum cholesterol levels are not elevated in young copper-deficient rats, mice or brindled mice. J Nutr. 1985 Dec;115(12):1702-7.

[75] Qin Z, Konaniah ES, Neltner B, Nemenoff RA, Hui DY, Weintraub NL. Participation of ATP7A in macrophage mediated oxidation of LDL. J Lipid Res. 2010 Jun;51(6):1471-7.

[76] Das SK, Ray K. Wilson's disease: an update. Nat Clin Pract Neurol. 2006 Sep;2(9):482-93.

[77] Lutsenko S. Atp7b-/- mice as a model for studies of Wilson's disease. Biochem Soc Trans. 2008 Dec;36(Pt 6):1233-8.

[78] Huster D, Lutsenko S. Wilson disease: not just a copper disorder. Analysis of a Wilson disease model demonstrates the link between copper and lipid metabolism. Mol Biosyst. 2007 Dec;3(12):816-24.

[79] Huster D, Purnat TD, Burkhead JL, Ralle M, Fiehn O, Stuckert F, et al. High copper selectively alters lipid metabolism and cell cycle machinery in the mouse model of Wilson disease. J Biol Chem. 2007 Mar 16;282(11):8343-55.

[80] Ralle M, Huster D, Vogt S, Schirrmeister W, Burkhead JL, Capps TR, et al. Wilson disease at a single cell level: intracellular copper trafficking activates compartment-specific responses in hepatocytes. J Biol Chem. 2010 Oct 1;285(40):30875-83.

[81] Sauer SW, Merle U, Opp S, Haas D, Hoffmann GF, Stremmel W, et al. Severe dysfunction of respiratory chain and cholesterol metabolism in Atp7b(-/-) mice as a model for Wilson disease. Biochim Biophys Acta. 2011 Dec;1812(12):1607-15.

[82] Seessle J, Gohdes A, Gotthardt DN, Pfeiffenberger J, Eckert N, Stremmel W, et al. Alterations of lipid metabolism in Wilson disease. Lipids Health Dis. 2011;10:83.

[83] Burkhead JL, Ralle M, Wilmarth P, David L, Lutsenko S. Elevated copper remodels hepatic RNA processing machinery in the mouse model of Wilson's disease. J Mol Biol. 2011 Feb 11;406(1):44-58.

[84] Wilmarth PA, Short KK, Fiehn O, Lutsenko S, David LL, Burkhead JL. A systems approach implicates nuclear receptor targeting in the Atp7b(-/-) mouse model of Wilson's disease. Metallomics. 2012 Jul 28;4(7):660-8.

[85] Burkhead JL, Gray LW, Lutsenko S. Systems biology approach to Wilson's disease. Biometals. 2011 Jun;24(3):455-66.

[86] Levy E, Brunet S, Alvarez F, Seidman E, Bouchard G, Escobar E, et al. Abnormal hepatobiliary and circulating lipid metabolism in the Long-Evans Cinnamon rat model of Wilson's disease. Life Sci. 2007 Mar 27;80(16):1472-83.

[87] Zischka H, Lichtmannegger J, Schmitt S, Jagemann N, Schulz S, Wartini D, et al. Liver mitochondrial membrane crosslinking and destruction in a rat model of Wilson disease. J Clin Invest. 2011 Apr;121(4):1508-18.

[88] Roberts E, Sarkar B. Liver as a key organ in the supply, storage, and excretion of copper. Am J Clin Nutr. 2008 Sep;88(3):851S-4S.

[89] al-Othman A, Rosenstein F, Lei K. Copper deficiency increases in vivo hepatic synthesis of fatty acids, triacylglycerols, and phospholipids in rats. Proc Soc Exp Biol Med. 1993 Oct;204(1):97-103.

[90] Cunnane S, Horrobin D, Manku M. Contrasting effects of low or high copper intake on rat tissue lipid essential fatty acid composition. Ann Nutr Metab. 1985;29(2):103-10.

[91] Petering H, Murthy L, O'Flaherty E. Influence of dietary copper and zinc on rat lipid metabolism. J Agric Food Chem. 1977 Sep-Oct;25(5):1105-9.

[92] Stemmer K, Petering H, Murthy L, Finelli V, Menden E. Copper deficiency effects on cardiovascular system and lipid metabolism in the rat; the role of dietary proteins and excessive zinc. Ann Nutr Metab. 1985;29(6):332-47.

[93] Aigner E, Theurl I, Haufe H, Seifert M, Hohla F, Scharinger L, et al. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. Gastroenterology. 2008 Aug;135(2):680-8.

[94] Miyajima H, Takahashi Y, Kono S. Aceruloplasminemia, an inherited disorder of iron metabolism. Biometals. 2003;16(1):205-13.

[95] Salama R, Nassar A, Nafady A, Mohamed H. A novel therapeutic drug (copper nicotinic acid complex) for non-alcoholic fatty liver. Liver Int. 2007 May;27(4):454-64.

[96] Alisi A, Manco M, Pezzullo M, Nobili V. Fructose at the center of necroinflammation and fibrosis in nonalcoholic steatohepatitis. Hepatology. 2011 Jan;53(1):372-3.

[97] Sanchez-Lozada L, Mu W, Roncal C, Sautin Y, Abdelmalek M, Reungjui S, et al. Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver. Eur J Nutr. 2010 Feb;49(1):1-9.

[98] Francini F, Castro M, Schinella G, Garcia M, Maiztegui B, Raschia M, et al. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life Sci. 2010 Jun 19;86(25-26):965-71.

[99] Yilmaz Y. Review article: fructose in non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2012 Apr 2.

[100] Silverman S, Fields M, Lewis C. The effect of vitamin E on lipid peroxidation in the copper-deficient rat. J Nutr Biochem. 1990 Feb;1(2):98-101.

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