CHAPTER 12

Copper

Jason L. Burkhead and James F. Collins

CHAPTER OUTLINE

Abbreviations

AO, amine oxidase; **AOC3**, amino oxidase copper-containing 3; **CNS**, central nervous system; **CP**, ceruloplasmin; **Cu**, copper; **MAO**, monoamine oxidase; **SOD**, superoxide dismutase; **WD**, Wilson Disease

Historical Highlights

It was recognized over two centuries ago that plants and lower marine invertebrates contained copper, but it was not firmly established that mammalian tissues also contained copper until 1921, when Bodansky showed that the human brain contains copper.[1] Also around this time, it was noted that copper and iron were necessary to cure experimental anemia in rats,[2] highlighting a specific physiologic role for copper. Direct evidence of copper being involved in human pathophysiology was provided in the early 1900s, with the first description of Wilson disease (WD)[3]; however, it was not appreciated until several decades later that WD was an inborn error of metabolism.[4] An association between low body copper levels and anemia in humans was suspected in the 1930s, but conclusive experimental proof came decades later. Copper deficiency in humans was first described in patients with Menkes disease in 1962,[5] but the underlying physiologic defect was not discovered until a decade later.[6] It is now widely accepted that copper is an essential nutrient for humans and other mammals. Copper is present in body fluids and tissues in the parts per million (μg/g) to parts per billion (ng/g) range. As copper is an essential enzyme cofactor, yet toxic when in excess, mammals have developed complex physiologic systems for regulating copper absorption, transport, storage, utilization, and excretion.

Chemistry

Copper has an atomic mass of ∼63.5 Daltons, with two stable isotopes, ^{63}Cu and ^{65}Cu. Seven radioisotopes of copper also exist. The two with the longest half-lives, ^{67}Cu (∼70 hours) and ^{64}Cu (∼13 hours), and the two stable isotopes, are most frequently used for experimental analyses of copper metabolism. Copper has two predominant oxidation states in human biology, Cu^{2+} (cupric) and Cu^{+} (cuprous), and it commonly shifts back and forth during enzymatic reactions. Cuprous copper (Cu^{+}) is insoluble in aqueous solutions and is, thus, strongly complexed.[7] Very little free, or unbound, copper exists within biological systems; most is bound to proteins via specific interactions with amino acid side chains, thus mitigating its reactivity.

Biochemical and Physiologic Functions

Copper serves a predominant role in mammalian biology as an enzymatic cofactor for a host of cuproenzymes (**Table 12.1**). Most cuproenzymes are oxidases that facilitate single electron transfer reactions between a substrate and molecular oxygen using either oxidized (Cu^{2+}) or reduced copper (Cu^{+}) atoms. Detailed descriptions of these proteins and their physiochemical properties and functions have been published elsewhere.[8,9] Moreover, a host of copper-binding proteins have been identified, including transmembrane transporters and intracellular chaperones, copper transport proteins in blood and a copper storage protein (**Table 12.2**). Select examples of these proteins will be briefly discussed below.

Catalytic Functions of Selected Cuproenzymes

Amine Oxidases (AO)

AOs function in the oxidative deamination of biogenic, primary amines. In the plasma, amine oxidases act on the physiologically active amines histidine, tyramine, and polyamines. AOs may also play a role in intracellular signaling via production of hydrogen peroxide.[10] Included in this family of proteins is amino oxidase copper-containing 3 (AOC3), which is purported to be involved in leukocyte trafficking[11] and metabolic regulation of adipocyte function.[12]

Monoamine Oxidases (MAO)

Two isoforms of MAO have been identified, MAOA and MAOB, each with distinct tissue localization. These copper-containing enzymes are involved in the catabolism of catecholamines such as serotonin, norepinephrine, tyramine, and dopamine. Abnormal regulation of MAOs has been associated with depression, substance abuse, attention deficit disorder and irregular sexual maturation.[13] Additionally, recent evidence suggests that MAOs possess proinflammatory properties, as inhibitors of these proteins have anti-inflammatory effects.[14]

Diamine Oxidases (DO)

One DO is involved in the catabolism of histamine. In the stomach, acid production is inhibited, while, throughout the body, allergic reactions are attenuated, by the inactivation of histamine via a diamine oxidase. These enzymes also inactivate polyamines and, thus, limit excessive cell growth, potentially having relevance to apoptosis and cancer.[15]

Lysyl Oxidase (LOX)

LOX is a copper-dependent, amine oxidase that initiates crosslinking and stabilization of elastin and collagen fibers. LOX is involved in the formation of connective tissues, including bone, blood vessels, skin, lungs, and teeth. LOX has been implicated in diverse pathophysiologic processes including fibrosis, tumor progression and metastasis, and neurodegenerative and cardiovascular diseases. LOX is, in fact, considered a potential therapeutic target for these pathologic conditions.[16] LOX may also regulate transcription and mediate the epithelial-to-mesenchymal transition in response to hypoxia or TGFβ.[17] Moreover, a family of at least four LOX genes has now been identified (termed LOX-like proteins), with all encoded proteins having similar catalytic domains and predicted copper and cofactor binding sites.[18,19]

Table 12.1 Selected Mammalian Cuproenzymes

Gene Symbol	Biological Function	Outcomes Associated with Copper Deficiency or Genetic Mutations
AOC1	Inactivation of histamine, putrescine, spermidine	Phlyctenulosis; radiation proctitis
AOC3	Cell adhesion; leukocyte trafficking	Diabetes; microvascular complications of diabetes
CP	Iron release from stores; ferroxidase activity	Aceruloplasminemia; hemosiderosis
CCO	Electron transport chain; ATP production	Decreased capacity for oxidative phosphorylation; hypomyelination
DβH	Neurotransmitter (norepinephrine) synthesis	Dopamine β-Hydroxylase deficiency; orthostatic hypotension
HEPH	Intestinal iron absorption; ferroxidase activity	Low serum iron and iron-restricted erythropoiesis; anemia
HEPHL1	Placental iron efflux; alias-Zyklopen	Cytochrome C oxidase deficiency; hair defects
LOX	Crosslinking of elastin and collagen fibers	Aortic aneurysm, familial thoracic aortic aneurysm and dissection
MAOA/B	Metabolism of neuroactive/vasoactive amines	Atypical depressive disorder; Brunner syndrome; Norrie disease
PAM	Activation of biologically-active peptides	Phaeohyphomycosis; Menkes disease
SOD1	Antioxidant defense; cytoplasm, mitochondria	Motor neuron disease; amyotrophic lateral sclerosis
SOD3	Antioxidant defense; extracellular fluids	Myocardial shunting; abestosis
TYR	Melanin biosynthesis; pigmentation	Albinism, oculocutaneous, types, 1a and 1b

Table 12.2 Selected Mammalian Copper-Interacting Proteins

Gene Symbol	Physiologic Function
A2M	Transport of Cu^{2+} in portal and systemic blood
ALB	Transport of Cu^{2+} in portal and systemic blood
APP	Neurite growth; neuronal adhesion; axogenesis
ATOX1	Copper chaperone for ATP7A and ATP7B; copper-dependent transcription factor
ATP7A	Pumps copper into TGN; copper export; Menkes disease gene
ATP7B	Pumps copper into TGN; copper excretion in bile; Wilson disease gene
COMMD1	Biliary Cu excretion; SOD1 dimerization; endosomal trafficking of ATP7A/ATP7B
CCS	Copper chaperone for cytosolic and mitochondrial SOD1
CTR1	Cu^{+} transporter; plasma membrane
COX11	Mitochondrial chaperone to support CCO biosynthesis
COX17	Mitochondrial chaperone to support CCO biosynthesis
MTI/II	Intracellular copper storage/detoxification (also interacts with zinc and cadmium)
PRNP	Function unknown; possible antioxidant defense or copper transport into the CNS
SCO1	Mitochondrial copper chaperone; supports CCO biosynthesis
SCO2	Mitochondrial copper chaperone; supports CCO biosynthesis
SLC25A3	Copper transporter; mitochondrial inner membrane; supports CCO biosynthesis
XIAP	Ubiquitination of COMMD1 (and CCS) for targeting to proteasome for degradation

Peptidylglycine α-Amidating Monooxygenase (PAM)

PAM is a highly conserved, copper- and ascorbate-dependent enzyme that is essential for the activation of many bioactive peptides. A few examples of peptides amidated by PAM are insulin, glucagon, vasopressin, α-melanocyte stimulating hormone, cholecystokinin, gastrin, neuropeptide Y, and substance P.[20,21] Mice lacking PAM die in mid-gestation,[22] whereas those heterozygous for *PAM* exhibit notable deficits, including the inability to maintain body temperature in cold temperatures, increased anxiety-like behavior, and altered inhibitory synaptic neurotransmission.[20] Moreover, polymorphisms in *PAM* were recently suggested to be associated with increased risk for developing type 2 diabetes.[23]

Multi-Copper Ferroxidases (MCFs)

Three MCFs have been identified to date, ceruloplasmin (CP), hephaestin (HEPH), and hephaestin-like 1 (HEPHL1). CP is a liver-derived, circulating glycoprotein that functions in iron release from some tissues by oxidizing ferrous iron to allow subsequent binding of ferric iron to transferrin for distribution in the blood. Mutations in *CP* cause aceruloplasminemia in humans, which is typified by iron-overload, but copper homeostasis is apparently unaffected. A cell-membrane associated, GPI-anchored CP isoform, which is expressed in hepatocytes, brain, and macrophages, was also recently discovered. GPI-CP may be important for iron release from hepatocytes and also hepatic Kupffer cells.[24]

HEPH is a CP-related protein (50% homology), which was originally described as a membrane-anchored, intestinal ferroxidase. It was originally discovered as the mutant gene causing perturbations in iron homeostasis in the sex-linked anemia (*sla*) mouse.[25] Intestinal HEPH expression may respond to body copper concentration in a manner that modulates its activity and, concomitantly, absorption of dietary iron.[26] Recent evidence demonstrates that HEPH is also expressed in the antrum of the stomach, the enteric nervous system, and pancreatic β-cells,[27] but the function of HEPH in these tissues is currently not understood. HEPH-like 1 (HEPHL1) (or zyklopen) was recently discovered in mice, with a proposed function in placental iron efflux.[28] A recent case report, however, described an adolescent male with compound heterozygous *HEPHL1* mutations that caused an abnormal hair phenotype.[29] HEPHL1-mediated ferroxidase activity may thus be essential for normal hair growth and development. A recent review article provides more detail on the mammalian MCFs.[30]

Cytochrome C Oxidase (CCO)

Mitochondrial CCO, the terminal component of the electron transport chain, reduces molecular oxygen to form water, and ultimately allows ATP to be produced by the generation of a proton gradient. CCO is a large complex, consisting of 13 proteins. It contains two heme groups and three copper ions, which are involved in electron transfer. CCO requires a specific mitochondrial copper delivery system for normal function. Mutations affecting function/activity of this copper delivery system are likely lethal.[31]

Dopamine β-Hydroxylase (DβH)

DβH requires copper in each of its four subunits and ascorbate as a co-factor. It catalyzes the conversion of dopamine to norepinephrine, which functions as a neurotransmitter in the central and peripheral nervous systems. Altered noradrenergic signaling has been associated with the development of nervous system pathologies. Circulating DβH has been evaluated as a biomarker of norepinephrine function in several human diseases.[32] Expression of DβH is highest in the adrenal medulla, sympathetic neurons in the peripheral nervous system, and in noradrenergic and adrenergic neurons in the brain.[21] In mice, *DβH* gene inactivation leads to embryonic lethality, exemplifying its key role in nervous system physiology.[33]

Superoxide Dismutases (SOD)

SOD proteins scavenge superoxide free radicals to protect against oxidative damage. Cu/Zn-superoxide dismutase (SOD1) and extracellular SOD (SOD3) require catalytic copper (and structural zinc) for function. Cytosolic SOD1 is a homodimer of ∼32 kDa, while extracellular SOD3 is a tetramer of ∼135 kDa. SOD1 may also function in gene transcription,[32] stabilization of specific RNA species,[32] and in oxygen sensing and nutrient signaling.[34,35] SOD3, the predominant extracellular dismutase, is present in lymph, synovial fluid, and plasma.[23] SOD3 may also be involved in the development of chronic obstructive pulmonary disease in humans,[36] while more recent work suggests broader inflammatory and immunomodulatory functions.[37]

Tyrosinase (TYR)

TYR is required for melanin biosynthesis and, thus, pigmentation. Loss of activity leads to albinism. TYR catalyzes the conversion of tyrosine to dopamine and the subsequent oxidation of dopamine to dopaquinone, steps along the pathway of melanin synthesis. The copper dependency of this process is best exemplified by the achromotrichia observed in copper-depleted domestic and laboratory animals.[21]

Physiologic Functions of Copper

The requirement of copper for several enzymes discussed above gives us clues as to the outcomes of copper depletion. In many cases, symptoms of copper inadequacy can be directly linked to decreased activity of one or more of these cuproenzymes.

Connective Tissue Formation

The copper-dependent enzyme LOX is required for normal formation of connective and bone tissue, as well as the integrity of connective tissue in the heart and vasculature. Copper deficiency may, thus, result in connective tissue disorders, osteoporosis, and bone defects. Skeletal perturbations have been documented in copper-deficient neonates, mirroring the bone abnormalities of scurvy (vitamin C deficiency).[38] Moreover, recent data demonstrated that long-term copper supplementation may decrease bone loss in adult humans,[39] but contradictory results have also been obtained.[40] Although whether copper supplementation positively impacts bone health is unclear, low copper intake is clearly associated with poor bone health,[41] consistent with the connective tissue weaknesses associated with copper transport defects in Menkes disease and other ATP7A-related disorders.[42]

Iron Metabolism

Copper homeostasis is intimately entwined with that of iron.[43] The most obvious link is the multi-copper ferroxidases, CP and HEPH. During copper depletion, HEPH activity in the intestine is impaired and CP activity is extremely low. The net effect is that iron absorption is reduced, and iron efflux from liver (and other tissues) is impaired, leading to reductions in serum iron.[44] This hypoferremia can result in development of an iron-deficiency-like anemia, probably due to reduced iron delivery to the erythron and an inability of erythroid precursors to properly use iron for hemoglobin synthesis.

Central Nervous System Function

Copper plays well known roles in the physiology of the central nervous system (CNS), including in brain development. Copper is deposited in the brain late in gestational development and during the perinatal period and, as such, copper deprivation of pregnant or lactating females results in pathological outcomes in offspring. Many of the effects of copper deprivation can be ascribed to altered expression/activity of cuproenzymes in the CNS, as their activity is impaired by copper depletion.[21] The essentiality of copper in brain development is perhaps best exemplified by the neuropathological phenotype of infants with the genetic copper deficiency disorder Menkes disease, as well as the milder *ATP7A*-related disorders Occipital Horn Syndrome (OHS) and Distal Motor Neuropathy (DMN).[42] The tremors, ataxia, hypo- or demyelination of nerve fibers, and reductions in some neurotransmitters observed during copper deficiency likely result from decreased production of sphingolipids (as mediated by CCO) and decreased activity of DβH and MAOs.[32,45]

Melanin Pigment Production

Copper is necessary for normal pigmentation given the copper dependency of TYR, which is a key enzyme in melanin synthesis. During copper depletion in humans and animals, depigmentation of skin and hair is commonly observed.

Cardiovascular Function

Several pathologic anomalies in the cardiovascular system are noted in severely copper-deficient, young animals. Copper deficiency may also increase chances for developing cardiovascular disease in humans. For example, some clinical trials demonstrated that cardiac arrhythmias developed after consumption of a low-copper diet.[21] Supporting this observation, several observational studies have shown that lower serum copper concentration was associated with increased incidence of coronary heart disease.[46] Copper deficiency may also perturb normal lipid metabolism and result in hypercholesterolemia and hypertriglyceridemia, thus increasing risk for development of atherosclerotic cardiovascular disease.[47]

Immunity

Copper likely plays an important role in immune system function. For example, many studies have shown that systemic copper deficiency is associated with increased risk of infection,[48] probably

because cellular and humoral immune system factors require copper for normal function. Moreover, neutropenia is commonly associated with copper depletion in humans, and macrophage and lymphocyte functions are impaired by even marginal copper deficiency. Studies in adult males demonstrated that in vitro stimulation of T lymphocytes was suppressed by consuming a diet with 0.36 mg copper/day for 42 days (which is well below the RDA of 0.9 mg/d). These individuals further had decreased plasma copper concentration and reduced activity of some copper-dependent enzymes, but their hematologic parameters were normal.[49] Furthermore, at the cellular level, copper may have a direct antimicrobial function, such as in the phagolysosome.[50,51] These, and other recent findings,[52,53] implicate copper in the ability of immune cells to respond to infectious stimuli. Definitive proof, however, is lacking mainly due to the inability to detect marginal copper deficiencies in experimental subjects.

Lipid Metabolism

A reciprocal relationship between copper and lipid metabolism has been defined through experimental animal model and human studies over the last 4 decades. For example, elevated liver cholesterol synthesis and serum triglycerides are noted in copper deficiency, while copper excess has the opposite effect. Copper modulation of lipid metabolism may be tissue specific. For example, in white adipose tissue, copper depletion decreases cAMP and lipolysis, while copper excess increases both.[54] Moreover, a recent meta-analysis of eight cross-sectional studies and 27 case-control studies indicated a positive association between elevated serum copper and obesity in children and adults, compared with healthy weight controls and no difference between overweight and control.[55] Copper-dependent amine oxidase, AOC3, regulates adipocyte size, while obese patients have increased circulating AOC3 and amine oxidase activity.[12,56]

Cell Cycle Regulation

Although the primary role of copper in cells is typically thought of as a catalytic cofactor, recent work suggests a signaling role that impacts cell cycle, metabolism and potentially cancer progression and treatment. For example, one investigation concluded that copper is an allosteric regulator, acting via interaction with MEK1 and MEK2, which influences cell proliferation and growth.[57] Chelator-mediated copper depletion has been proposed and is in clinical trials as an anti-cancer therapy.[58] Recent work to investigate how excess copper may selectively kill cancer cells has led to a description of copper toxicity named "cuproptosis," where high intracellular copper damages lipoylated TCA cycle proteins, resulting in a specific copper-dependent mechanism of cell death.[59]

Bioavailability

The relative amount of copper in the diet seems to be the major predictor of absorption. Several factors, including certain amino acids and proteins, iron, zinc, molybdenum, vitamin C, and carbohydrates have been reported to exert adverse effects on the bioavailability of dietary copper.[60] High doses of zinc induce symptoms of systemic copper deficiency, as recently reported in several patients that used excessive amounts of zinc-containing denture cream.[61] The impact of dietary components on copper absorption may be more pronounced in neonates, as digestive function and homeostatic regulation of biliary copper excretion are not yet fully developed.

Nutrient Interactions

Cu metabolism is known to be affected by iron, zinc, and vitamin C. Variations in dietary copper may also affect the homeostasis of other nutrients, a topic that will not be considered in detail here.

Copper-Iron Interactions

Copper and iron interact in numerous ways.[62] Important iron-copper interactions in the gut include the regulation of HEPH by dietary copper intakes and the regulation of ATPase Copper Transporting Alpha (ATP7A; a copper efflux transporter) expression by iron concentration.[63,64] Furthermore, liver copper varies inversely with iron status, for unexplained reasons.[65] Interestingly, an unknown aspect of erythroblast iron use is copper dependent as, during copper deficiency, hemoglobin production is inefficient despite normal serum iron.[66]

Copper-Zinc Interactions

High dietary zinc intake impairs copper absorption. This may, in part, be explained by the induction of metallothionein, a copper-binding protein, in enterocytes. Moreover, copper depletion has been observed in humans consuming supplements containing 50 mg of zinc daily for extended periods of time; this is, in fact, the rationale for the UL for zinc of 40 mg/d for adults.[67] Reciprocally, studies focused on copper toxicity in Wilson Disease suggest that excess copper inhibits specific zinc-dependent biochemical processes.[68]

Copper-Ascorbate Interactions

Ascorbic acid supplementation may induce copper deficiency in experimental animals and could have a similar effect in humans. In premature infants, plasma vitamin C was negatively correlated with serum CP and antioxidant activity.[69] Other human studies have also suggested that ascorbic acid supplementation may perturb serum ferroxidase activity.

Food Sources

The typical diet of an adult in the U.S. supplies slightly more copper than is recommended by the RDA (0.9 mg/d). The richest sources of dietary copper are shellfish, seeds, nuts, organ meats, wheat bran cereal, whole grain products and chocolate containing foods. Vegan diets contain ample copper, but absorption seems to be lower from plant foods than from animal-based sources.[70] Other sources of copper include vitamin and mineral supplements, although copper is often in the cupric oxide form which has low bioavailability.

Metabolism

Genetic Regulation

Expression of transcripts encoding many proteins involved in copper metabolism, including, for example, copper transporters (e.g., CTR1, ATP7A, ATP7B), does not change in response to

variations in dietary copper intake or according to body copper status.[71] Regulation of copper homeostasis, thus, seems to be mostly at a post-transcriptional level, predominantly via protein trafficking mechanisms.[72]

Body Copper Homeostasis

Copper intake from a typical, balanced diet is ~1.2–1.3 mg/d, with around 0.8 mg being absorbed daily in the upper portion of the small intestine (**Figure 12.1**). Excretion occurs predominantly into the bile (~0.4 mg/d), with total fecal losses being ~1 mg/d. In the liver, copper is incorporated into CP and other cuproenzymes. CP is secreted into the blood, along with atomic copper, which binds to serum proteins for copper delivery to cells of the body. Homeostatic control of body copper includes modulation of enteral copper absorption and biliary copper excretion.

Vectorial Intestinal Copper Transport

Dietary and supplemental copper, and endogenous copper from saliva, gastric juices, and pancreatic and biliary secretions, constitute the intestinal copper pool (Figure 12.1). Enteral copper (mainly Cu^{2+}) must be reduced to the Cu^{+} state for absorption (**Figure 12.2**). Three cupric reductases have been identified, cytochrome b [558] ferric/cupric reductase, Steap 2 and Cybrd1, but the relative contribution of each has not been definitively established.[62] Subsequently, copper is transported into enterocytes mainly via copper transporter 1 (CTR1).[73] Within intestinal epithelial cells, copper is bound by chaperones that traffic copper within the cytosol to support SOD1 function (CCS) or deliver it to the *trans*-golgi network (ATOX1) for incorporation into cuproenzymes in the secretory pathway. Copper is also trafficked into mitochondria, but the chaperone involved has not been definitively established. Copper movement within mitochondria involves an additional host of copper chaperones and a copper transporter that moves copper from the inter membrane space into the matrix (Figure 12.2). Excess copper may be bound in cells by metallothionein (MT). Finally, copper can be transported out of enterocytes by ATP7A. Once copper exits enterocytes, the oxidizing environment of the interstitial fluids presumably converts cuprous copper (Cu^{+}) to cupric copper (Cu^{2+}), which binds to albumin or α_2-macroglobulin for delivery in the portal blood to the liver.

Copper Transport and Transfer

After transport to the liver, Cu^{2+} is reduced and then imported into hepatocytes via CTR1 (**Figure 12.3**).[74] Inside liver cells, copper is bound to chaperones and distributed similarly to what occurs in enterocytes. ATP7B pumps copper into the TGN where it is incorporated into CP and other cuproproteins. Excess copper stimulates the translocation of ATP7B from the TGN to the canalicular membrane of the hepatocyte, facilitating excretion of copper into bile.[75]

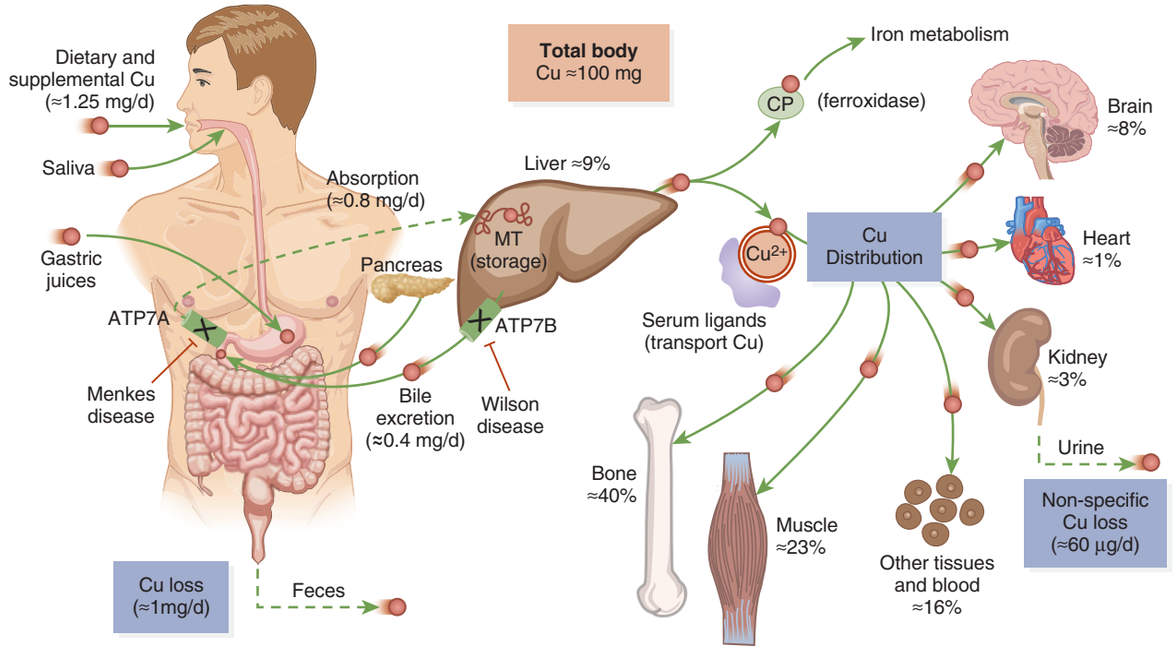

Figure 12.1 Total Body Copper Homeostasis. The major regulatory mechanisms that control body copper levels, including absorption, transport, and excretion are depicted in this diagram. Enteral copper derives from the diet and from endogenous GI tract and pancreatic secretions. The ATP7A copper-transporting ATPase mediates copper efflux from enterocytes. Copper can be bound within MT in the liver, or released and distributed to body cells, tissues, and organs. Numbers under various organs indicate the approximate percentage of body copper that is present in that organ/tissue. Although most copper in the serum is bound to CP, other copper binding proteins must exist as the absence of CP (in aceruloplasminemia) does not cause copper depletion of peripheral tissues. Copper excretion occurs mainly in the bile and is mediated by the ATP7B copper-transporting ATPase. Small amounts of copper are lost in the urine and excess enteral copper is eliminated in the feces. Mutations in *ATP7A* underlie Menkes Disease (MD) and mutations in *ATP7B* cause Wilson Disease (WD).

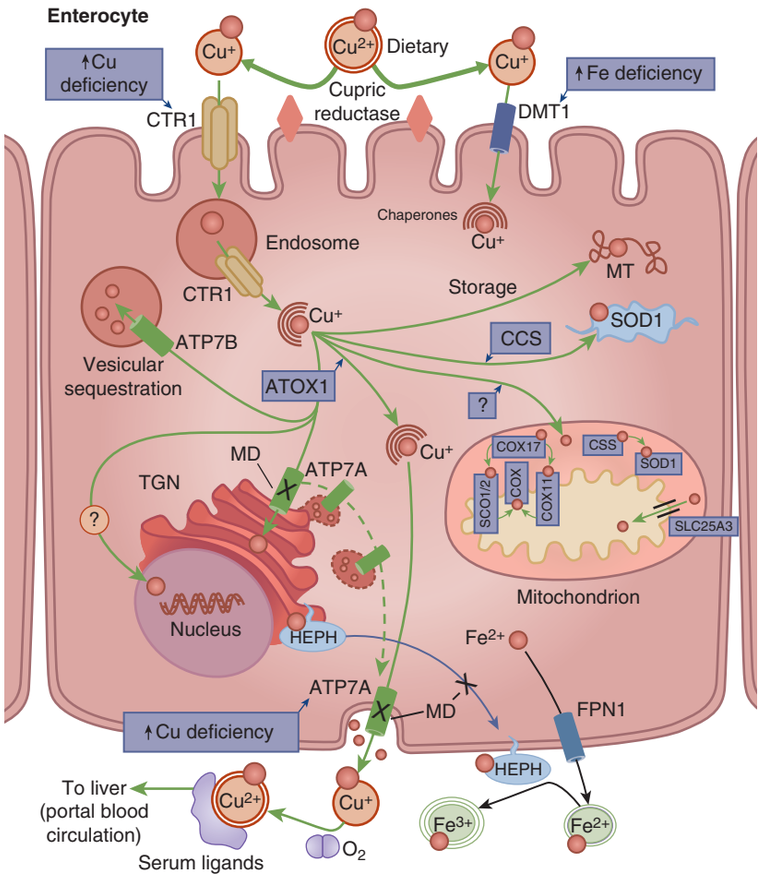

Figure 12.2 Enterocyte Copper Homeostasis. A single enterocyte is depicted in this diagram, which shows the processes that are involved in enteral copper absorption. Dietary Cu^{2+} is reduced to Cu^{+}, probably by three identified cupric reductases, and then transported into enterocytes by CTR1. This process may involve endocytosis followed by copper export from endosomes into the cytosol. DMT1 could mediate copper import as well, but probably mainly during iron deficiency. Once in cells, Cu^{+} interacts with chaperones for intracellular distribution. The CCS chaperone transfers copper to cytosolic SOD1. ATOX1 delivers copper to ATP7A in the TGN and possibly the basolateral membrane, and into the nucleus (where ATOX1 may function as a transcription factor). ATOX1 may also deliver copper to ATP7B contained within intracellular vesicles for storage. Copper transfer to mitochondria is mediated by an unknown chaperone. Within mitochondria, CCS delivers copper to SOD1, while COX17, SCO1/2, and COX11 provide copper to support biosynthesis of CCO. Copper enters the mitochondrial matrix via SLC25A3, also supporting synthesis of CCO. In the TGN, copper is incorporated into cuproenzymes, including HEPH, which functions as a ferroxidase to allow iron release from cells, and subsequent binding to transferrin (which is not shown). When intracellular copper is elevated, ATP7A-containing vesicles traffic to the BLM, thus facilitating copper efflux by vesicular exocytosis. Cu^{+} that exits cells is oxidized by O_2 (to Cu^{2+}) and then bound by ALB and A2M for transport to the liver. CTR1 and ATP7A expression and/or activity may increase during copper depletion. Dysfunctional copper transport by ATP7A underlies MD in humans. Excess intracellular copper can be stored bound to MT.

Copper Excretion

The primary excretory route for endogenous copper is via hepatocytes into bile, as mediated by ATP7B and a host of accessory proteins. Biliary copper and unabsorbed enteral copper are eliminated from the body in the feces. Copper excretion is less effective during fetal and neonatal life, which increases risk for copper toxicosis. Impaired bile secretion, or cholestasis, can also increase liver copper content.

Copper Storage

Total copper content of adult humans ranges from 50–120 mg. In general, copper is not stored in the human body; tissue copper, thus, likely reflects quantities of cuproenzymes.

Copper: Homeostatic Mechanisms

Humans have developed efficient adaptive mechanisms designed to protect against copper deficiency and toxicity. Absorption of dietary copper is regulated, with percent absorption increasing when intakes are low.[75] Under conditions of high copper intake, copper may be sequestered in metallothionein in enterocytes and biliary excretion may also increase. Copper absorption under normal conditions is ~10%, reflecting combined absorption and subsequent excretion of newly absorbed copper.[75] These adaptive mechanisms become inefficient with chronic intake of copper <0.7 mg/d; fortunately, this intake level is well below the estimated average intake in the United States of ~1.2 mg/d.

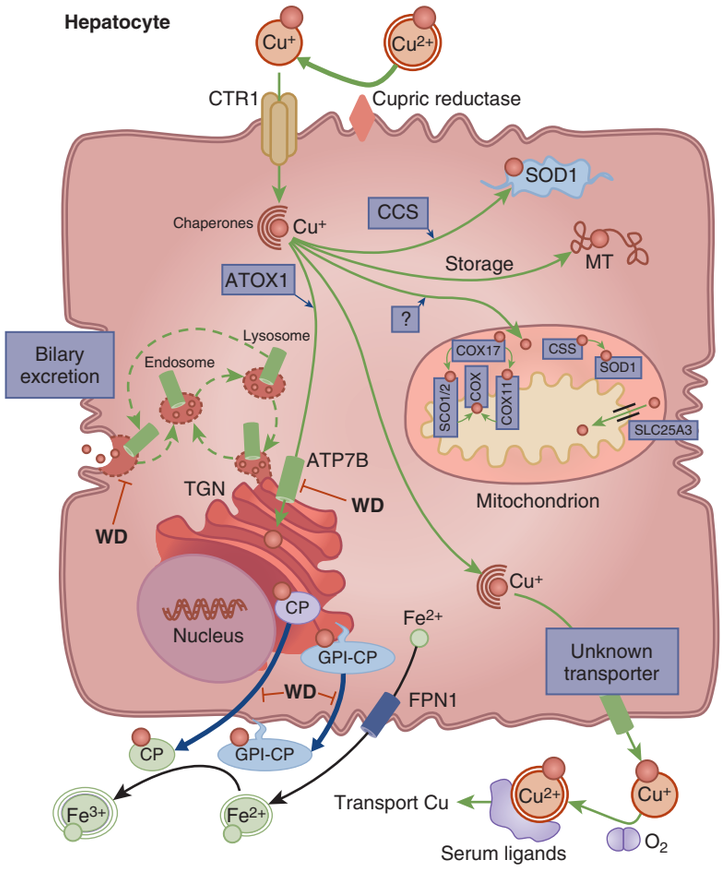

Figure 12.3 Hepatocyte Copper Homeostasis. A single hepatocyte is depicted in this diagram, which shows the major proteins involved in copper metabolism. Cu^{2+} in the portal blood circulation is reduced to Cu^{+} prior to being imported into the cell by CTR1. As in enterocytes, intracellular chaperones distribute copper, and excess copper can be stored in MT. Mitochondrial copper handling is probably the same as that depicted for the enterocyte. The ATP7B copper-transporting ATPase pumps copper into the TGN for incorporation into CP and other cuproenzymes. CP and GPI-CP are ferroxidases that oxidize Fe^{2+} to Fe^{3+} after release by various tissues and cells, including hepatocytes. ATP7B-containing endosomes derived from the TGN fuse with lysosomes, which can then facilitate copper excretion in bile via vesicular exocytosis or cycle back to the TGN. Atomic copper also exits hepatocytes via an unknown transport process, followed by oxidization and binding to various serum ligands (e.g., ALB, A2M) for distribution throughout the body. Dysfunctional ATP7B underlies the copper-loading disorder WD in humans.

Genetic Defects in Copper Metabolism

Copper-related pathologies in humans most frequently arise due to defects in copper exporters encoded by *ATP7A* and *ATP7B*, as detailed below.

Menkes Disease (MD)

MD is an X-linked, recessive disorder of copper metabolism affecting multiple organ systems. As expected, most patients are males. Typical manifestations include progressive neurodegeneration, connective tissue disturbances and unusual "kinky" hair. The disease is usually fatal by the age of 3 years; no cure exists although copper histidine treatment (via subcutaneous injection) of affected individuals early in life has shown promise, particularly in partial amelioration of neurologic symptoms.[76] The prevalence of MD varies in different regions of the world with a lower incidence noted in Japan and Europe (1:300,000–1:360,000), but a much higher frequency in Australia (1:50,000–100,000).[77]

The underlying genetic defect is in the *ATP7A* gene, encoding a copper-transporting ATPase required for copper delivery to cuproenzymes in the secretory pathway and for cellular copper export. Less deleterious *ATP7A* mutations cause Occipital Horn Syndrome (OHS) and Distal Motor Neuropathy (DMN), which present with milder phenotypes than Menkes disease.[42]

Defective copper elimination from cells is the basic physiologic disturbance in MD, with most tissues (except for liver and brain) accumulating excess copper. Copper does not, however, accumulate to toxic levels, at least partially due to an intestinal block to absorption of dietary copper. The lack of copper in some peripheral tissues (e.g., liver and brain), despite accumulation in other tissues (e.g., intestinal mucosa, muscle, spleen, and kidney), leads to signs and symptoms of systemic copper deficiency.[78] These include low serum copper and CP activity, and impaired synthesis of SOD and CCO. LOX activity is also impaired leading to defective artery formation in the CNS, and osteoporosis. Progressive nerve degeneration is noted in the brain, leading to the classic neurologic symptoms of MD.[79]

Wilson Disease (WD)

WD is an autosomal recessive disease in which affected individuals exhibit excessive copper storage.[80] The underlying defect is in the *ATP7B* gene, which encodes a copper-transporting ATPase. The worldwide prevalence of WD was reported to be 1:30,000.[81] In WD patients, copper accumulation in liver, brain, and cornea (Kayser-Fleisher rings) results in multiorgan damage, being particularly severe in brain and liver. Neurological damage and cirrhosis may ensue if left untreated. Acute hepatitis, hemolytic crisis and hepatic failure may also occur. Other observations include abnormally high urinary copper excretion and low CP values. Permanent organ damage can be avoided in WD with proper medical treatment, especially if initiated early in life. Typical treatments use decoppering chelation therapy with penicillamine and/or trientine,[82] with life-long compliance required, or high zinc dosing (which interferes with absorption of dietary copper).

Huppke-Brendel Syndrome

Huppke-Brendel Syndrome is caused by defects in the *SLC33A1* gene and is a lethal autosomal recessive disorder characterized by low or undetectable serum copper and CP, severe developmental delay, congenital cataracts, and significant defects in cerebellar structure. *SLC33A1* encodes a highly conserved acetyl-CoA transporter that is required for acetylation of specific gangliosides and glycoproteins, including CP.[83]

MEDNIK Syndrome

MEDNIK syndrome (Mental retardation, Enteropathy, Deafness, Neuropathy, Ichthyosis, Keratodermia) is caused by mutations in the *AP1S1* gene, which encodes the small subunit of the adapter protein 1 complex (σA1).[84] Lack of AP1S1 activity impacts clathrin coat assembly and subcellular trafficking between the *trans*-Golgi, the endosomal system, and the plasma membrane, which is critical for the copper export functions of ATP7A and ATP7B. Biochemical characteristics include features of both Menkes Disease and Wilson Disease, while treatment is by zinc supplementation, as in Wilson Disease.

Copper Deficiency in Humans and Animals

Severe copper deficiency in animals results in abnormalities in the immune, skeletal, and cardiovascular systems. Further consequences of copper deprivation are hypochromic anemia (which does not respond to iron supplementation), hypopigmentation, thrombocytopenia, and neutropenia.[38] Across many species, other hallmarks of copper deficiency in addition to anemia include neutropenia, and osteoporosis. More specific features include: 1) skeletal abnormalities, fractures, and spinal deformities; 2) neonatal ataxia; 3) depigmentation of hair and wool; 4) abnormal keratinization of hair, wool, and fur; 5) reproductive failure; 6) cardiovascular abnormalities; and 7) impaired immune function. It should be noted here that some of these symptoms have only been observed in one or two species and, further, that manifestations of copper deficiency in experimental and other animals is typically more severe than is what observed in humans (as described in the next paragraphs).

Systemic copper deficiency in humans, which is rare, can result from ineffective absorption of dietary copper or excessive copper loss through the endogenous biliary excretion system. Several groups of individuals are susceptible to copper deficiency, including: 1) individuals chronically receiving total parenteral nutrition without proper copper supplementation; 2) premature infants consuming milk-based formulas lacking adequate copper; 3) neonates experiencing chronic diarrhea or malnutrition; 4) hospitalized patients undergoing long term peritoneal dialysis; 5) severe burn patients; 6) renal dialysis patients; and 7) persons consuming large doses of supplemental zinc, antacids or copper chelators.[19] Malabsorption may also result in copper deficiency. Moreover, recent evidence suggests an association between surgical bowel resection in the management of morbid obesity and acquired copper deficiency.[85]

In humans, moderate to severe copper deficiency is accompanied by low serum copper concentration, and reduced serum ferroxidase (e.g., CP) activity. Usual pathophysiologic features include anemia, leukopenia, and neutropenia. During periods of rapid growth, osteoporosis is a common feature. Furthermore, mild, or subclinical, copper deficiency because of long-term low copper intakes is a possibility in humans. This may result in additional manifestations, including increased risk for developing arthritis, arterial disease, depigmentation, myocardial disease, and neurological abnormalities.[75] Possible additional effects of marginal copper deficiency include cardiac arrhythmias, increased serum cholesterol and glucose intolerance.[86] It is also important to note that these observations were not duplicated in all human studies, indicating that future experimental work is necessary.

Dietary Considerations and Requirements

Dietary Requirements and Recommendations

Dietary reference intakes for copper were established almost 2 decades ago[87] and are listed in **Table 12.3**. Based on a lack of experimental data, Adequate Intake (AI) levels for copper have been established for infants 0–6 months-of-age and for those between 7 and 12 months. The RDA increases throughout childhood and adolescence and is increased from adult levels during pregnancy and lactation. Upper tolerable intake levels (ULs) have also been established for copper (also listed in Table 12.3).

Evaluation of Copper Status

Analytical Methods

The most widely used methods to quantify copper are inductively coupled plasma optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS).[13] For AAS, samples are atomized with a graphite furnace for electrothermal ionization (GFAAS) or with an air-acetylene flame (flame AAS) for

Table 12.3 Copper Dietary Reference Intakes

Copper DRIs (μg/d)			
AGE (years)	RDA[a] (males/females)	AI[b] (males/females)	UL[c]
0–0.5	—	200	ND[d]
0.5–1.0	—	220	ND
1–3	340		1,000
4–8	440		3,000
9–13	700		5,000
14–18	890		8,000
19–30	900		10,000
31–50	900		10,000
51–70	900		10,000
>70	900		10,000
Pregnancy			
≤18	1,000		8,000
19–50	1,000		10,000
Lactation			
≤18	1,300		8,000
19–50	1,300		10,000

[a]RDA = Recommended Dietary Allowance.
[b]AI = Adequate Intake (mean intake of healthy infants receiving human milk).
[c]UL = Tolerable Upper Intake Level (total intake from food, water, and supplements should not exceed this value).
[d]Not Determinable (the source of intake should be from food only for these age groups).

electrothermal ionization. ICP-OES is often used when more than one mineral is being quantified. Studies to determine the distribution of copper in living animals typically use stable isotopes of copper to track the absorption, utilization, excretion, and turnover of copper in biological systems.[75] The most common method to measure the copper isotope ratios is mass spectrometry (MS), with ICP-MS and thermal ionization mass spectrometry (TIMS) being the most commonly used techniques, including more recent capacity for single-cell analysis and spatial localization of metals in tissue sections.[88] X-ray fluorescence microscopy (XFM) is capable of high-resolution spatial quantitation of metals in tissues and cells.[89]

Assessment of Copper Status

Much effort has recently gone into identifying biomarkers of copper status that are sensitive to even marginal deficiency, and are noninvasive and consistently reliable.[78] The most frequently utilized method in humans has been to quantify copper concentration and the activity of various cuproenzymes in blood.[90] Reductions in plasma copper and CP activity are noted in severely copper-deficient humans; intakes in experimental settings of 0.6 mg/d or less for at least a month-and-a-half are required to consistently see these decreases. Observed reductions in serum Cu and CP activity are, however, complicated by the fact that several (patho)physiologic alterations can increase copper content and CP activity in blood, including the acute phase response to infection and inflammation, pregnancy and other hormonal perturbations, and some carcinogenic phenotypes.[90] Other utilized copper biomarkers, including SOD1 activity in erythrocytes, CCO activity in platelets and mononuclear cells, and copper content of various circulating blood cells, have shown limited usefulness in determining the copper status of humans. Erythrocyte CCS expression, however, was shown to be responsive to dietary copper depletion and repletion.[91] Finally, studies in rats revealing alterations in serum and tissue PAM activity[92] have been correlated with similar observations in copper-deficient humans.[93] Additional experimentation with these promising, new biomarkers, is, however, required.

Copper Toxicity and Upper Limits

Copper toxicity is rare, as humans and other mammals have evolved adequate homeostatic mechanisms to regulate body copper content, since free (unbound) copper is potentially toxic (as it can potentiate the generation of reactive oxygen species). Free copper in cells and in the body is, thus, extremely low; copper almost always exists in biological systems bound to proteins. Ingestion of high copper levels may, however, override the innate checkpoints designed to regulate overall body copper, leading to enhanced intestinal absorption in the absence of a physiological demand for copper. Due to possible adverse consequences of high copper ingestion and potential liver damage, a UL of 10 mg/d has been established for adults.

Copper is often included in complete nutrition and micronutrient supplements without consequence. In a recent study, adults were supplemented with 10 mg of cupric gluconate daily for 12 weeks without evidence of liver damage or gastrointestinal distress.[94] Copper toxicity risks are, however, higher for neonates and infants given an immature biliary excretion system and an enhanced intestinal absorption apparatus. Copper loading is observed clinically today in the setting of Wilson disease and other disorders in which biliary copper excretion is impaired such as biliary cirrhosis and biliary atresia.

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