Iron and Copper Toxicity in Diseases of Aging, Particularly Atherosclerosis and Alzheimer's Disease

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In this review, we point out that natural selection does not act to lessen human diseases after the reproductive and caregiving period and that normal levels of iron and copper that may be healthy during the reproductive years appear to be contributing to diseases of aging and possibly the aging process itself. It is clear that oxidant damage contributes to many of the diseases of aging, such as atherosclerosis, Alzheimer's disease, Parkinson's diseases, diabetes, diseases of inflammation, diseases of fibrosis, diseases of autoimmunity, and so on. It is equally clear that both iron and copper can contribute to excess production of damaging reactive oxygen species through Fenton chemistry. Here, we examine the evidence that "normal" levels of iron and copper contribute to various diseases of aging. Exp Biol Med 232:323–335, 2007

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Introduction

The concept of normalcy is intrinsic to medicine. We have normal values for everything from blood counts to electrolytes. We make minor concessions for variation due to age or gender with some variables, but for the most part, normal values are normal values throughout the human lifespan.

We assume that normal values are healthy values, but in this regard we fail to consider that natural selection works to optimize health and survival only during the reproductive and early care-giving period, roughly the first 50 years of human life. Thus, what might be healthy during that period may not be optimal in terms of health after age 50, that is, the period when diseases of aging become prevalent. There is no natural selection operating to prevent or mitigate diseases of aging as long as the diseases do not impinge significantly on the reproductive period.

In this essay, I would like to consider the above with respect to iron and copper stores and levels of "free" iron and copper in the body. In general, I wish to point out that "normal" stores of iron and copper during reproductive years provide reserves for such things as hemorrhage or periods of severe dietary restrictions and starvation, thus protecting the individual during their early years, but may contribute in a major way to diseases of aging. Thus, these stores may be in the best interests of the younger person in terms of survival and relative good health. However, both iron and copper are transition elements that fuel generation of damaging reactive oxygen species (ROS) if present in "normal" amounts, and this oxidant damage takes its toll in the later years of life. Normal values for iron and copper variables are summarized in Table 1. Actual values for serum ferritin in adult Americans shown in Table 1 come from Zacharski *et al.* (1).

Oxidative metabolism is a key aspect of life and is the underpinning of energy generation and use in most organisms, including the human. But the tradeoff is generation of ROS and the oxidative damage they can cause. Organisms have developed antioxidant protective mechanisms, but these are not perfect, and some ROS escape and cause damage to various molecules. This can become worse in times of stress, such as inflammation, when generation of ROS increases. The evidence is increasing that the aging process itself, as well as many of the diseases of aging, are caused at least in part by oxidant damage.

One of the first authors to point to oxidant radicals as a cause of aging was Harman (2, 3). Butterfield's group has published extensively on the role of oxidant radicals, generated from Fenton reactions dependent on iron or copper, on protein and lipid peroxidation, brain aging, and

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Table 1. Normal Ranges for Iron, Copper and Ferritin

A. Normal ranges for iron variables in adults		
	Men	Women
Serum iron	33–150 μg/dl	33–150 μg/dl
Transferrin saturation	15%–45%	15%–45%
Serum ferritin	18–320 ng/ml	6–155 ng/ml
B. Actual values for serum ferritin in adult Americans (ng/ml)[a]		
Men	Menstruating Women	Menopausal Women
About 150	About 25–35 (ages 17–49)	Age 50–59, about 60 Ages 60–90+, about 90–100
C. Normal ranges for copper variables in adult men and woman		
Serum copper	100 ± 10 μg/dl	
Serum ceruloplasmin	20–40 mg/dl	

[a] From Zacharski *et al.* (1), compiled from NHANES III; these are actual values observed in a population and are not necessarily indicative of healthy values.

neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (4–8). Their reviews, for example Poon *et al.* (4), are quite instructive as to the underlying mechanisms of oxidant radical damage.

The deleterious effects of oxidant damage from iron and copper are not evident early in life, absent mutations causing very excessive accumulations of iron (hemochromatosis) or copper (Wilson's disease). However, the evidence suggests that these harmful effects gradually accumulate, and, as we age, take their toll in terms of many of the diseases of aging. I will argue and present evidence that lowering the availability of both iron and copper might mitigate these diseases and possibly slow the aging process.

Iron Toxicity

Taking iron first, clearly iron is absolutely vital to life. A good review of iron metabolism is given in the first part of the book by Weinberg (9). A short version is given in the introduction of the paper by Zecca *et al.* (10). Iron is a central element of the heme molecule, which is a critical part of hemoglobin and essential for oxygen transport. Heme is also part of many essential enzymes, such as the cytochrome series. Iron is also a vital component of other enzymes and proteins. Having adequate iron is important, because as iron deficiency sets in, anemia ensues, which, depending on its severity, can lead to fatigability, decreased exercise tolerance, and general inanition. Further, such a person becomes much more susceptible to traumatic episodes with bleeding—simply, their reserves of blood are depleted. Thus, evolution has acted against this during the reproductive years by providing for reserve iron stores in times of plenty to balance periods of relative famine and losses from such things as menstruation and hemorrhage. Even so, it must be noted that premenopausal women are not completely protected from iron deficiency anemia. Intake of adequate bioavailable iron, usually in the form of meat, is necessary to prevent anemia, and iron deficiency anemia is common among premenopausal women due to food choice and bleeding.

Serum ferritin provides one measure of iron stores, and a low ferritin is a reliable indicator of iron deficiency. However, as pointed out by Hallberg and Hulthen (11), an elevated ferritin is not always a true indicator of iron stores, because ferritin is an acute phase reactant. Nonetheless, under most circumstances ferritin is a good marker of iron stores, and it is usually higher in men then in women, particularly menstruating women (Table 1). There tends to be recovery towards the male values in menopausal women. Thus, menstruation reduces some of the iron stores, and, at least in current times, this isn't compensated for in men.

As already mentioned, the toxicity of iron is related to its involvement in producing oxidant damage. Through Fenton chemistry and other reactions, iron catalyzes the production of the toxic hydroxyl radical as well as other ROS (12–15). The production of ROS *in vivo*, the role of metals such as iron and copper, and the antioxidant protective mechanisms are thoroughly reviewed in Poon *et al.* (4). Increasingly, it is apparent, as documented in the following sections, that oxidant damage is intimately involved with diseases of aging, such as atherosclerosis, diseases of autoimmunity, AD, Parkinson's disease, diabetes, diseases of fibrosis, diseases of inflammation, and others. Thus, right from the start, the production of ROS by iron has to be one suspect in diseases of aging.

Iron and Atherosclerosis. The evidence that relative iron availability contributes to diseases of aging is strongest with atherosclerotic disease, and is of several types (see Table 2 for a summary). The concept was first proposed by Sullivan (16–18), and his major rationale at the start was that the much lower risk of atherosclerotic cardiovascular disease in menstruating women than in men of the same

Table 2. Evidence on the Question of Whether Relative Iron Availability Contributes to Development of Atherosclerosis

	Positive evidence	Negative evidence
1.	Menstruating women, who have low ferritin levels, are protected against atherosclerotic cardiovascular disease, not explained by differing hormone levels (16–22).	
2.	Epidemiological relationship between atherosclerosis and measures of iron stores in 12 studies (23).	27 studies found no relationship (23).
3.	Correlation of oxidative DNA damage (levels of 8–hydroxydeoxyguanosine) and serum ferritin in Japanese (25).	
4.	Three studies in blood donors, expected to have reduced available iron, showed less cardiovascular disease than controls (23).	One study found no effect (26).
5.	12 positive animal model studies (27).	One negative animal model study (27).
6.	A number of positive molecular studies (see text for details) (28–39).	
7.	Increased risk of atherosclerotic disease in heterozygotes for a hemochromatosis (an iron-loading disorder) gene (40, 41).	There appears to be controversy as to increased risk of atherosclerosis in homozygous hemochromatosis (42, 43).

ages was due to the reduced iron stores in the women (see ferritin levels in Table 1). When women stop menstruating they begin to lose this protective effect. Efforts to show that this protective effect in menstruating women is due to hormonal differences during this period have failed. Data from the Framingham study show that the increased risk of coronary heart disease is equal in women who underwent natural as opposed to surgical menopause, independent of oophorectomy as part of the surgery. This finding suggests a uterine (blood loss) etiology to worsening risk as opposed to an ovarian (estrogen) etiology (19, 20). Additionally, postmenopausal hormone replacement therapy is ineffective in reducing the rate of coronary heart disease events (21, 22).

A second line of evidence is epidemiological, looking at atherosclerotic heart disease risk or some other measure of atherosclerosis, such as carotid artery intimal thickness, and correlating it, usually with serum ferritin but occasionally with some other measure of iron stores, such as transferrin saturation. This area has been recently (2005) reviewed by You and Wang (23). They cite 12 epidemiological studies supporting a relationship between stored iron and cardiovascular disease and 27 studies which are nonsupportive. Citations to the original 39 studies can be found in You and Wang (23). One of the problems with this approach is that it is presumably the "free," "reactive," or "readily available" iron, sometimes called the "labile iron pool," that is toxic, and once ferritin levels have reached some minimum, additional stored iron as measured by ferritin (or transferrin saturation) may not influence levels of free iron very much. I agree with Lee and Jacobs (24) that the contrary epidemiological evidence could very well be due to serum ferritin and transferrin saturation not being a good measure of the labile iron pool. This labile pool of iron, nontransferrin-bound, is much more likely to be more intimately involved with the generation of oxidant stress and is probably only weakly correlated with serum ferritin and transferrin saturation. Given this reasonable explanation for the negative epidemiological studies, possibly a good deal more weight should be given to the positive studies, which found an association in spite of the likely poor correlation between measures of stored iron and the level of free iron. Of course, we must keep in mind that association does not prove cause and effect.

Another type of epidemiological evidence is correlation of oxidative damage, in this case oxidative DNA damage measured by circulating 8-hydroxydeoxyguanosine levels, and serum ferritin in a large sample of Japanese men and women. There was a very significant correlation in both sexes (25). Again, this does not prove that higher levels of iron are causing greater oxidation, but it is consistent with that hypothesis.

A fourth line of evidence is the effect of blood donation, expected to reduce available iron, on atherosclerotic heart disease. Three studies of this type cited by You and Wang (23) have been positive but can be criticized on the basis of the "healthy donor effect," namely that volunteer blood donors enjoy better health than nondonors. One study of this type not cited by You and Wang (23) was negative (26).

A fifth line of evidence is various types of animal studies which have generally been supportive of a role of free iron in atherosclerosis and related processes. Meyers (27) cites 12 animal studies which have been supportive and only one nonsupportive. The positive animal model studies range from animals fed iron-deficient diets or given iron chelators and showing less atherosclerosis to animals supplemented with iron showing more atherosclerosis.

A sixth line of evidence is molecular studies which have been supportive of the role of iron in atherosclerosis and provide additional insight into mechanisms. These include findings of high iron deposition in human athero-

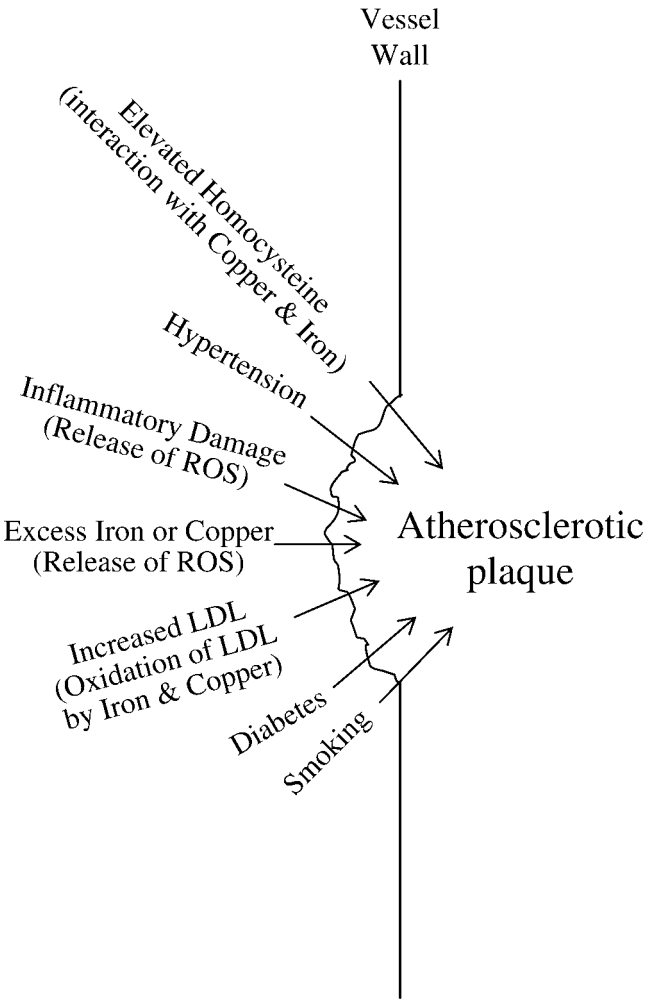

Figure 1. This figure identifies the major risk factors leading to atherosclerosis, and suggest that production of ROS by excessive free iron and copper, oxidation of LDL by iron and copper, and interaction of iron and copper with homocysteine, fits into this scheme. ROS, reactive oxygen species; LDL, low-density lipoprotein.

sclerotic lesions (28–32), demonstration that H- and L-ferritin mRNAs are higher in human and rabbit atherosclerotic vessels than in normal ones (33), the colocalization of iron with ceroid in human atherosclerotic tissue (34), and inhibition of low-density lipoprotein (LDL) oxidation by an iron chelator (35, 36). In other studies, plasma levels of cholesterol oxidation products, thought to be intimately involved in atherosclerosis, were correlated with ferritin levels in Finnish men (37). Another study suggests that homocysteine, another cardiovascular disease risk factor, promotes iron-catalyzed oxidation of LDL (38). As pointed out by Balla *et al.* (39), heme oxygenase and ferritin genes are upregulated in endothelium in the early phase of progression of atherosclerotic lesions, perhaps a response to iron toxicity.

A criticism of some of the molecular studies cited above in which iron and iron-related abnormalities occur in the vessel wall relates to the "chicken and egg" question. That is, other processes, such as inflammation, might initiate the vessel wall abnormality, and accumulation of iron and iron related abnormalities might be secondary phenomena, not important in pathogenesis.

Finally, a seventh line of evidence (Table 2) produces mixed results. Heterozygosity for a hemochromatosis (an iron-loading disorder) gene has produced increased risk of atherosclerotic disease in two separate studies (40, 41). There is a mild increase in iron loading in the heterozygous state. However, there is strong iron overloading in the homozygous state, and there the results are mixed and controversial. Failla *et al.* (42) did find structural abnormalities in the radial arteries of hemochromatosis patients, which largely reverted with iron depletion. However, Niederau (43) has summarized the overall data and concludes beyond any doubt that atherosclerotic coronary heart disease, stroke, and peripheral artery disease are rare clinical features and causes of death in homozygous hemochromatosis. The lack of excess atherosclerosis in hemochromatosis, if this is in fact the case, is negative evidence against the hypothesis that excess available iron is causative in atherosclerosis. Certainly the availability of excess iron is high in this disease, as witness the damage to heart, joints, pancreas, etc. To believe in the excess iron/atherosclerosis hypothesis, one has to postulate that the lack of effect in homozygous hemochromatosis is due to some type of differences or secondary effects which mitigate the atherosclerotic effect.

Figure 1 portrays a scheme in which increased iron could contribute to the pathogenesis of atherosclerosis along with other risk factors. Thus, it is important to keep in mind that the hypothesis is not that iron acts alone, but that it acts in concert with other risk factors. Thus, there is increasing interest in the hypothesis that atherosclerosis is an inflammatory disease. The excess-iron hypothesis fits well with the inflammatory hypothesis in that oxidant damage is a central feature of each, and they would be expected to add to one another. Likewise, increased LDL is a strong risk factor, and it fits with the excess iron hypothesis because iron oxidizes LDL, a central feature of the atherosclerotic process. Similarly, increased serum homocysteine is a risk factor, and there is evidence that homocysteine promotes iron-catalyzed oxidation of LDL.

Admitting that the homozygous hemochromatosis situation is a "fly in the ointment," I nonetheless believe the weight of the rest of the data is sufficient to implicate iron in the atherosclerotic process. Thus, I conclude at this point that Sullivan (16) is likely correct, and that stored iron, or perhaps, more precisely put, ready availability of labile iron, contributes to the disease of aging, atherosclerosis. At the least, there is enough evidence for the involvement of excess iron as a causative factor in atherosclerosis that the hypothesis needs thorough testing, including clinical trials.

Iron and AD. Beyond atherosclerosis, iron has also been implicated in neurodegenerative diseases of aging, including AD. Numerous papers have appeared on this topic (15, 44–48) including a review by Ong and Halliwell (49),

who discuss mechanisms, particularly the interaction of iron and cholesterol in promoting oxidative damage in both atherosclerosis and neurodegeneration. Additional evidence of the involvement of iron in AD is the association of what Zecca *et al*. (10) call "iron management genes" with AD. Thus, mutations in the hemochromatosis gene, HFE, are more common in AD patients than in the general population (50). Patients with the transferrin subtype C2 are also more common in AD than in the general population (51–53). The presence of the C2 variant plus an HFE mutation increased the risk of AD 5-fold (54). A clinical trial of the iron chelator desferrioxamine given for two years to AD patients was very positive in terms of slowing the clinical progression of dementia (55). Dementia was measured by a video recorder home-behavioral assessment, which had three parts and measured 44 tasks, mostly samples of daily living. These videos were scored by two blinded trained raters, who had established a 90% agreement with an "expert rater." The statistically significant better performance of the treated group versus the untreated group means that tasks of daily living were significantly better preserved in the treated group. Desferrioxamine will chelate aluminum as well as iron, so theoretically the benefit could result from reduction of aluminum levels, but aluminum toxicity is not currently believed to play a role in AD.

Other Toxicities of Iron. It is thought that excess iron accelerates the aging process in general (13). A considerable body of literature has built up, with several recent reviews (15, 56, 57), on the concept of iron, free radicals, and mitochondrial injury as keys to the aging process. It is of considerable interest that lowering total body iron has been shown to increase the life span of some organisms. Good examples are the fruit fly (58) and the housefly (59).

There are possibly interesting data relating elevated transferrin saturation to overall mortality from the NHANES 1 study. Individuals with a transferrin saturation greater than 55% (between 1 and 2% of the population) had increased mortality compared with those with lower saturations (60). It was also found that those with elevated transferrin saturation had higher mortality if they had a high iron or red meat intake (61). How many of the people with elevated transferrin saturation might have been heterozygous or even homozygous for hemochromatosis is unclear.

Copper Toxicity

Turning to copper, it is also vital to life. Copper is an essential component of countless enzymes and other proteins and is critical for numerous reactions necessary for life (62). Deficiency leads to anemia and bone marrow suppression, followed by a neurologic syndrome called a myelopathy (63). As with iron, the most bioavailable source of copper is in meat. Although almost all foods have some copper, vegetarian diets are much more borderline in providing adequate copper (64, 65). Perhaps to counter gaps in dietary, particularly meat, provision of copper, evolution has provided for copper storage in the liver, primarily bound to metallothionein, a protein which can bind multiple molecules of copper (as well as other divalent cations, such as zinc). A blood protein, ceruloplasmin (Cp), synthesized by the liver and also containing several molecules of copper, is, like ferritin for iron, one marker of body copper status (66). Plasma Cp levels begin to decrease after the readily mobilizable copper stores of the liver are depleted.

As with iron, the reserve stores of copper have no doubt been selected for by natural selection to maintain adequate copper during periods of famine, to maximize reproductive potential. However, just as with iron, copper participates in generation of ROS through Fenton chemistry and can produce oxidative damage in much the same manner.

The copper that participates in producing ROS is called "free" copper, probably a somewhat inappropriate term. This term is used to include the noncovalently bound copper, and thus refers to copper more loosely bound to proteins and other molecules, so for the most part it is not really free. As with iron, a better term might be "labile copper pool," although we will use the term "free" copper to refer to this pool in this review. About 90% of blood copper in humans is covalently bound to Cp, while the remaining 10% is free, loosely bound to albumin and other molecules. In cells of all types, there is a considerable pool of free copper, some of it stored in metallothionein. This amount of free copper produces ROS throughout life, with at least partial protection by antioxidant defense mechanisms. It is our hypothesis that damage gradually accrues from this source and becomes evident in the many diseases of aging.

Copper and Atherosclerosis. One type of evidence that copper contributes to diseases of aging is, again, epidemiological evidence relating copper levels, or in some cases Cp levels, to atherosclerotic disease. (For a summary of the evidence that copper and/or Cp contributes to development of atherosclerosis, see Table 3.) At this point we must clarify the relation of serum copper to serum Cp levels. Cp normally accounts for about 90% of serum copper. Further, Cp is an acute phase reactant, and plasma levels will go up, for example, in the presence of inflammation. Thus, when serum copper is measured and an increase noted, unless both Cp and copper are measured it is not possible to tell whether the increased copper is due to an increased Cp or an increase in the non-Cp serum copper, or both. Since Cp is increased in inflammation, an increase in Cp levels in atherosclerosis could be a marker of the inflammation of the atherosclerotic process, or perhaps is causally involved in the process unrelated to inflammation. The hypothesis that Cp is causally involved is supported by work that shows that Cp can oxidize LDL through an interaction of one of the copper molecules carried by Cp (Table 3; Refs. 67–69).

In the literature there are papers citing an epidemio-

Table 3. Evidence on the Question of Whether Relative Copper Availability Contributes to Development of Atherosclerosis

	Positive evidence	Negative evidence
1.	Ceruloplasmin can oxidize LDL[a] through an interaction of one of its copper molecules (67–69).	
2.	Epidemiologic relationship between serum copper and atherosclerotic disease in seven studies (70–76), and epidemiologic relationship between ceruloplasmin levels and atherosclerotic disease in eight studies (77–84).	One epidemiologic study failed to find a relationship between serum copper and coronary heart disease (85) and another failed to find a relationship between ceruloplasmin and coronary heart disease (86).
3.	Elevated levels of ceruloplasmin and copper in diabetes mellitus (87). Diabetic patients with vascular complications have elevated serum copper (87). Patients with the metabolic syndrome have elevated ceruloplasmin levels (88).	
4.	Rabbit model study in which there was a biphasic response to copper exposure, i.e., there was more atherosclerosis in high and very low copper intakes than at intermediate levels (93).	Rabbit model study in which copper supplementation beyond that in normal rabbit chow caused less blood vessel damage (92).
5.	Molecular studies showing elevated copper in atherosclerotic plaques (32); copper oxidizes LDL; homocysteine interacts with copper to produce oxidant stress according to five studies (95–99).	Mass spectrophotometric assays of markers in atherosclerotic plaques indicate that free metal ions are not involved in LDL oxidation at these sites (100).

[a] LDL, low-density lipoprotein.

logical relationship between serum copper and atherosclerotic disease (70–76) and papers citing an epidemiologic relationship between Cp levels and atherosclerotic disease (Table 3; Refs. 77–83). One group controlled for the inflammatory component by adjusting for such things as protein C levels, and found that a substantial risk from elevated Cp remained (84). We found one paper failing to find a relationship between serum copper and coronary heart disease, at least in patients with moderate coronary heart disease (85), and another paper that observed no relationship between Cp and coronary heart disease when patients with inflammation were excluded (86).

Elevated levels of Cp and copper have been found in type I and type II diabetes mellitus (Table 3), which are risk factors for cardiovascular disease (87). Diabetic patients with vascular complications have higher plasma copper levels than diabetic patients without complications or normal controls (87). Patients with the "metabolic syndrome" (patients having in common risk factors such as obesity, hypertension, glucose intolerance, and dyslipidemia) also have elevated Cp levels (88).

Some animal model work has been done on copper and atherosclerosis. First, it is clear that severe copper deficiency damages blood vessels, perhaps as a result of deficiencies of copper-dependent enzymes, such as lysyl oxidase, important in collagen cross-linking, and copper/zinc superoxide dismutase (SOD), important in oxidant protection. For example, Dalle Lucca *et al.* (89) find increased neointima thickening in the copper-deficient rat carotid artery and attribute it to the lower SOD levels they also find. Similarly, Saari *et al.* (90) review all the harmful effects on the cardiovascular system of severe copper deficiency and also conclude that these are primarily due to deficiencies of enzymes that depend on copper for activity.

Lamb *et al.* (91) have used the cholesterol-fed rabbit model of atherosclerosis and compared a copper-deficient and copper-adequate diet. They found evidence of more damage in the aortas of the copper-deficient animals. This group also studied this model, comparing a copper-adequate to a copper-supplemented sample of rabbits, and found the copper supplemented-group had significantly smaller intimal lesions (92). Thus, this is evidence against the hypothesis that elevated free copper levels are atherogenic, at least in this model. However, Lamb *et al.* (93) have also done a copper dose response in the rabbit model, and find a biphasic atherogenic response. Thus, at both high and low levels of copper there is greater atherosclerosis than at intermediate levels. Although it is difficult to put these dietary copper levels in this rabbit model into context with human levels of free copper, this latter work is at least mildly supportive of the concept that lowering free copper levels could reduce atherosclerosis. It is clear, of course, that copper levels must not be lowered into the range where the activities of copper-dependent enzymes are affected, because that adversely affects the vasculature.

Molecular studies are generally supportive of a relationship of copper to the atherogenic process and provide possible insights into mechanisms (Table 3). Elevated levels of copper have been found in human atherosclerotic plaques (32). Copper is capable of oxidizing LDL, and oxidized LDL is part of the atherogenic process. One study showed that apolipoprotein E may owe its antioxidant effects to inhibiting copper oxidation of LDL (94). A number of papers have shown that homocysteine, a risk factor for cardiovascular disease, interacts with copper

to produce oxidant stress (95–99). One molecular study with a contrary conclusion is that of Leeuwenburgh *et al*. (100), who on the basis of mass spectrophotometric quantification of markers for protein oxidation in atherosclerotic plaques conclude that free metal ions are not involved in LDL oxidation in the arterial wall.

The role of copper in atherosclerosis has been reviewed by Ferns *et al*. (101). In general, they conclude that the relationship is probably biphasic, with both severe copper deficiency and excess copper causing enhanced atherogenesis. With respect to the latter, they emphasize the role of copper in oxidizing LDL, which they point out is important in the early phases of atherogenesis.

I would like to make it clear that severe copper deficiency, that is, copper deficiency severe enough to cause lessened activity of copper-dependent enzymes, is not relevant to the question I am discussing, which is whether free copper levels are high enough in "normal" people during aging to contribute to diseases of aging, such as atherosclerosis. If so, the proposal would be to bring these levels down mildly, not so much as to affect copper-dependent enzymes.

Turning to a summary of the evidence for copper levels being high enough as people age to contribute to atherosclerosis, the epidemiologic evidence of points 2 and 3 of Table 3 are not as persuasive for copper as were the positive epidemiologic studies for ferritin, in my opinion. The reason has already been discussed, that Cp is an acute phase reactant, and when Cp is elevated, so is serum copper. Thus, the inflammatory component of the atherosclerotic process could be simply elevating Cp levels secondarily. On the other hand, the positive data involving copper and homocysteine are more compelling. This interaction causes production of ROS, known to be involved in atherogenesis, and would be an explanation for homocysteine levels as a cardiovascular risk factor. The evidence suggesting that iron is contributing to atherogenesis should be kept in mind when considering copper, because the two metals are toxic through identical mechanisms, generation of ROS. Thus, if a clinical trial of iron depletion is positive, it would provide weight to the consideration of a clinical trial of copper depletion in atherosclerotic disease.

Copper and AD. Copper may be involved at many steps with the pathogenesis of AD, another disease of aging (see Table 4 for a summary of evidence). It is believed that β-amyloid (Aβ), present in amyloid plaques in AD, is intimately involved with pathogenesis. β-Amyloid is generated from amyloid precursor protein from cleavage by β-secretase. Amyloid precursor protein has a copper-binding domain which reduces copper (II) to copper (I) and then produces oxidative damage (102, 103). β-Secretase itself also binds copper for activity (104). β-Amyloid binds copper and cholesterol, facilitating copper oxidation of cholesterol to 7–0H cholesterol, extremely toxic to neurons (105, 106). One study has shown that amyloid plagues and neurofibrillary tangles, also common in AD brains, are major sites of catalytic redox activity (107). Deferoxamine, an iron chelator, or EDTA, a general metal chelator, abolishes this redox activity, while replenishment with copper or iron restores the activity (107). Tau protein is a major component of neurofibrillary tangles and also binds copper, which appears to be important for its aggregation (108).

Apolipoprotein E4 is a risk factor for AD, and it has an arginine at position 112 rather than a cysteine, while the other apolipoprotein alleles have cysteine at this position (109). This cysteine may be involved in copper binding and may be related to the diminished antioxidant effect of the E-4 allele (110). Plasma homocysteine levels are a risk factor for AD (111), and copper mediates LDL oxidation by homocysteine (95). β-Amyloid causes copper-dependent inhibition of cytochrome *c*-oxidase, an important enzyme of oxidative metabolism (112). Squitti *et al*. (113) have found a high free copper in the blood of AD patients, and report a high level of serum peroxides, which correlate positively with serum copper (114). Penicillamine (a copper chelator) therapy reduced the level of serum peroxides (114). However, an earlier study (115) did not find a difference between copper levels in AD patients and controls.

In animal studies, Sparks (116) has found that trace amounts of copper added to the drinking water in a rabbit model of AD greatly enhances the accumulation of Aβ in the brains of the rabbits and increased learning deficits. In another study, treatment of the rodent model of AD with clioquinol, a copper/zinc chelator, markedly inhibited Aβ deposition in the brain (117).

However, there is significant controversy over whether an excess of copper is involved in the pathogenesis of AD. Data suggesting otherwise include animal studies in which an increase in brain copper due to amplification of a copper transporter resulted in reduction of Aβ in the brain (118) and supplementation with copper in an AD mouse model lowered Aβ production and increased longevity (119), and human AD studies in which cognitive decline correlated positively with low plasma levels of copper (120).

Thus, at this time the evidence is conflicting as to whether too much copper is involved in the pathogenesis of AD. This will have to be resolved by further experimentation. Similarly, the involvement of copper in other disease of neurodegeneration, suggested by various findings, remains not definitively established.

Other Potential Toxicities of Copper. Intervention data, lowering copper levels with drugs, in diabetes, cancer, diseases of fibrosis, diseases of inflammation, and autoimmune diseases, involving primarily animal studies, are summarized in Table 5 and briefly discussed below.

Cooper and his group (121) have shown that copper metabolism becomes abnormal after induction of diabetes in rats and that the copper chelator trientine, given to these animals, alleviated their heart failure, improved cardiomyocyte structure, and reversed elevations in left ventricular collagen and β_1 integrin without lowering blood glucose.

Table 4. Evidence on the Question of Whether Relative Copper Availability Contributes to Development of Alzheimer's Disease

	Positive evidence	Negative evidence
1.	Amyloid precursor protein binds copper and produces oxidative damage (102, 103).	
2.	β-secretase binds copper (104).	
3.	β-amyloid binds copper and cholesterol, facilitating formation of 7-OH-cholesterol toxic to neurons (105, 106).	
4.	Amyloid plaques and neurofibrillary tangles are major sites of redox activity dependent on copper or iron (107).	
5.	Tau protein, a component of neurofibrillary tangles, binds copper, required for its aggregation (108).	
6.	Copper binding of apolipoprotein alleles correlates inversely with AD[a] risk (109, 110).	
7.	Copper mediates LDL[b] oxidation by homocysteine, whose levels are a risk factor for AD (95, 111).	
8.	β-amyloid causes copper-dependent inhibition of cytochrome C oxidase (112).	
9.	A high free serum copper has been found in AD patients, along with an elevated peroxide level, inhibitable with a copper chelator (penicillamine) (113, 114).	A high free serum copper was not found in one study of AD patients (115).
10.	Trace amounts of copper added to the drinking water greatly enhanced the accumulation of β-amyloid in a rabbit AD model and increased learning deficits (116).	
11.	Treatment of a rodent model with a zinc/copper chelator (clioquinol) markedly inhibited β-amyloid deposition (117).	
12.		Animal model studies in which amplification of a copper transporter increased brain copper and reduced β-amyloid in the brain (118).
13.		Animal model studies in which copper supplementation lowered β-amyloid production and increased longevity (119).
14.		Human AD studies in which cognitive decline correlated positively with low plasma copper levels (120).

[a] AD, Alzheimer's disease.
[b] LDL, low-density lipoprotein.

They followed this up with studies in diabetic patients and showed that trientine therapy decreased left ventricular hypertrophy (121). Others have shown beneficial effects of trientine in animal studies of diabetic neuropathy (122).

In a series of mouse studies with the copper-lowering agent tetrathiomolybdate (TM), being developed for Wilson's disease (123), our group has shown (summarized in Table 5): i) an antiangiogenic effect, useful in inhibiting cancer growth (124); ii) an antifibrotic effect in lung and liver (125, 126); iii) an anti-inflammatory effect (127, 128); and iv) an inhibitory effect on autoimmune diseases (126, 129). In another study with TM, it was shown that neointimal vascular thickening after balloon injury in the rat was inhibited by this drug (130). Another group has shown that TM inhibits adjuvant-induced arthritis in the rat (131). Clinical studies in cancer have been promising (132), and clinical trials in diseases of fibrosis are just beginning. TM is capable of producing a greater degree of copper depletion than other drugs and of doing so safely, as long as Cp levels are monitored and kept in an intermediate range. At this level of copper depletion, copper-dependent enzymes are not affected. The various disease processes affected by these intervention studies—cancer, fibrosis, inflammation, autoimmunity, diabetes—are all diseases associated with aging.

Summary, Recommendations, and Conclusion

Summarizing, at this point I think the data with iron are compelling enough to warrant a well-designed clinical trial of iron depletion on atherosclerosis. This could be done most safely by phlebotomy. Since the blood donor data have been mostly positive but have been criticized on the basis of nonrandomness of the donors, it seems reasonable to design a randomized study in which a unit of blood is taken at periodic intervals from half of the subjects. These should be subjects who are at risk for atherogenesis, and the endpoints could be carotid artery intimal thickening as well as cardiovascular events. Alternatively this could be done with oral iron chelation therapy, using the recently approved deferasirox (Exjade, Novartis). If this drug proves to be safe enough, it might be preferred by patients to periodic phlebotomy.

Table 5. Evidence on the Question of Whether Relative Copper Availability Contributes to a Variety of Diseases of Aging

	Positive evidence	Negative evidence
1.	Therapy with a copper chelator (trientine) improves cardiac function in diabetic animals and humans (121).	
2.	Therapy with trientine is beneficial in animals with diabetic neuropathy (122).	
3.	A copper complexing agent (TM)[a] is therapeutically effective in:	
	An antiangiogenic, anticancer effect in animal models (124).	
	An antifibrotic effect in lung and liver in animal models (125,126).	
	An anti-inflammatory effect in a variety of animal models (127,128).	
	An inhibitory effect on autoimmune diseases in animal models (126,129).	
	Inhibition of neointimal vascular thickening after balloon injury in an animal model (130).	
	Inhibition of adjuvant induced arthritis in the rat (131).	

[a] TM, tetrathiomolybdate.

A study of the effect of phlebotomy or deferasirox on AD progression should also be undertaken, in view of the increasingly obvious role of oxidant damage in this disease and the one study showing a beneficial effect of the iron chelator desferrioxamine (55). Particularly, if these studies are positive, the effects of iron depletion on other diseases of aging should be evaluated. As mentioned above, phlebotomy is probably safer for iron depletion than currently available iron chelators, but it has the disadvantage that it doesn't involve a pharmaceutical product, and thus there is no pharmaceutical company financing. Thus, if phlebotomy is the method of choice, these studies will have to be undertaken by academic investigators, presumably with financing from federal agencies, such as the National Institutes of Health. These studies should include the best measures of the labile iron pool, as well as other measures of iron status. A pilot study on the feasibility of phlebotomy to reduce iron stores (as measured by serum ferritin) to predictable levels has already been done (133).

Regarding copper, it is probably premature to plan a major clinical intervention study to evaluate copper-lowering effects on atherosclerosis. A good way to proceed would be to carry out animal model studies to see if copper depletion mitigates atherogenesis and if so, to what degree the copper depletion need be carried out. Trientine has shown effects in diabetes, as discussed, but is not generally as capable of producing the degree of copper depletion that TM can produce. TM is not yet commercially available, although it is expected to be approved for Wilson's disease within about a year. Other anticopper drugs on the market are not ideal. Penicillamine is too toxic, and zinc may be too slow-acting and mild to effect the kind of copper depletion readily available with TM. If animal studies are positive, and once TM comes on the market, for example for Wilson's disease, an intervention study should be undertaken using either trientine or TM, looking at effects on atherogenesis, with similar endpoints to the proposed iron study. If both iron and copper studies are positive, a study of the combined effects of iron and copper depletion should then be undertaken.

Regarding the other diseases in which copper depletion may be beneficial, research extending the animal work into the clinic should continue.

It has been hypothesized that even mild copper deficiency might be atherogenic (134). This theory is based in part upon observations that the higher the ratio of zinc to copper, the higher the blood cholesterol (135, 136). It should be noted that zinc levels are unchanged with copper depletion by TM. Further, in our various studies of lowering copper levels with TM, we have not seen an adverse effect on lipid levels or on development of heart disease, as suggested by Klevay (134).

In conclusion, oxidant stress is now viewed as a major culprit in the aging process and in many diseases of aging. Iron and copper are extremely redox-active and are constantly involved in generation of ROS, which may be important in the aging process and in the pathogenesis of many diseases of aging. Evolution has resulted in extra stores of iron and copper, because they are so vital to life and health during the reproductive period. However, these stores may be contributing to diseases as we age, and it is time to begin evaluating what levels of these metals are optimal during the latter part of life.

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