Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity

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Abstract

Lead is an environmentally persistent toxin that causes neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies. The propensity for lead to catalyze oxidative reactions and generate reactive oxygen species has been demonstrated in multiple studies. These reactive oxygen species (ROS) inhibit the production of sulfhydryl antioxidants, inhibit enzyme reactions impairing heme production, cause inflammation in vascular endothelial cells, damage nucleic acids and inhibit DNA repair, and initiate lipid peroxidation in cellular membranes. These wide-ranging effects of ROS generation have been postulated to be major contributors to lead-exposure related disease. Antioxidants – vitamins B6, C and E, zinc, taurine, N-acetylcysteine, and alpha-lipoic acid, either alone or in conjunction with standard pharmaceutical chelating agents – have been studied in lead-exposed animals. The evidence for their use in lead exposure, alone and in conjunction with chelating agents, is reviewed in this article. (Altern Med Rev 2006;11(2):114-127)

Introduction

Lead is a persistent and common environmental contaminant. Like other commonly found, persistent toxic metals – mercury, arsenic, and cadmium – lead damages cellular material and alters cellular genetics. The mechanism all of these toxic metals have in common involves oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. Data now indicate that low-level exposures to lead, resulting in blood lead levels previously considered normal, may cause cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, and renal impairment. The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body.[1]

Recent research examining the etiology of lead toxicity-induced hypertension reveals that the free radical production and lowering of inherent antioxidant reserves resulting from lead toxicity are directly related to vasoconstriction underlying lead-induced hypertension.[2] The mechanisms of lead-related pathologies, many of which are a direct result of the oxidant effect of lead on tissues and cellular components, may be mitigated by improving the cellular availability of antioxidants. N-acetylcysteine (NAC), zinc, vitamins B6, C and E, selenium, taurine, and alpha-lipoic acid have been shown, in a number of animal studies, to interrupt or minimize the damaging effects of lead and improve the effects of pharmaceutical chelating agents.

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Mechanisms of Lead Toxicity: The Effect of Lead on Oxidant/Antioxidant Balance

Lead Binds to Glutathione and Sulfhydryl-Containing Enzymes

Lead toxicity leads to free radical damage via two separate, although related, pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (2) the direct depletion of antioxidant reserves.[1] In any biological system where ROS production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human system's ability to deal with increased oxidant stress occur via independent pathways.

One of the effects of lead exposure is on glutathione metabolism. Glutathione is a cysteine-based molecule produced in the interior compartment of the lymphocyte. More than 90 percent of non-tissue sulfur in the human body is found in the tripeptide glutathione.[3] In addition to acting as an important antioxidant for quenching free radicals, glutathione is a substrate responsible for the metabolism of specific drugs and toxins through glutathione conjugation in the liver.[3]

The sulfhydryl complex of glutathione also directly binds to toxic metals that have a high affinity for sulfhydryl groups. Mercury, arsenic, and lead effectively inactivate the glutathione molecule so it is unavailable as an antioxidant or as a substrate in liver metabolism.[4] Concentrations of glutathione in the blood have been shown to be significantly lower than control levels both in animal studies of lead exposure and in lead-exposed children and adults.[5-8]

Lead also binds to enzymes that have functional sulfhydryl groups, rendering them nonfunctional and further contributing to impairment in oxidative balance. Levels of two specific sulfhydryl-containing enzymes that are inhibited by lead – delta-aminolevulinic acid dehydrogenase (ALAD) and glutathione reductase (GR) – have been demonstrated to be depressed in both animal and human lead-exposure studies.[6,9-12]

In a study of pediatric lead exposure in Lucknow, India, children with blood lead levels of 11.39 μg/dL had significantly depressed levels of ALAD compared to children with levels of 7.11μg/dL or lower.[6] Depressed levels of ALAD in these children correlated with depressed levels of glutathione. In a study of lead-exposed battery plant workers, significant correlations were seen between ALAD activity, blood lead levels, and erythrocyte malondialdehyde (MDA) levels. MDA is a clinical marker of oxidative

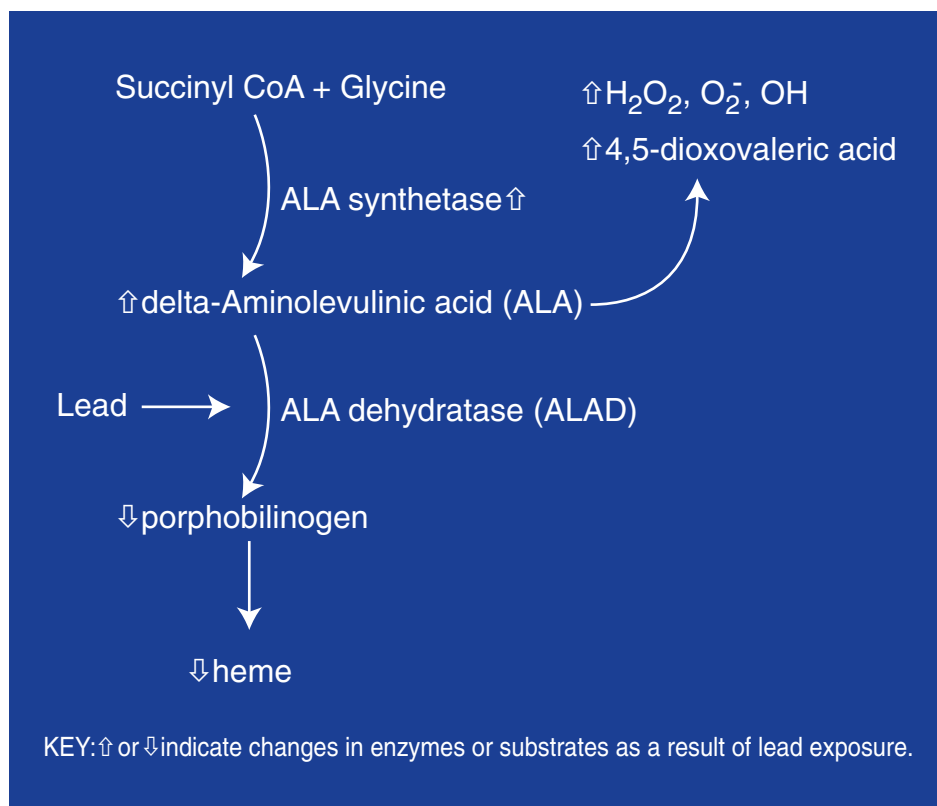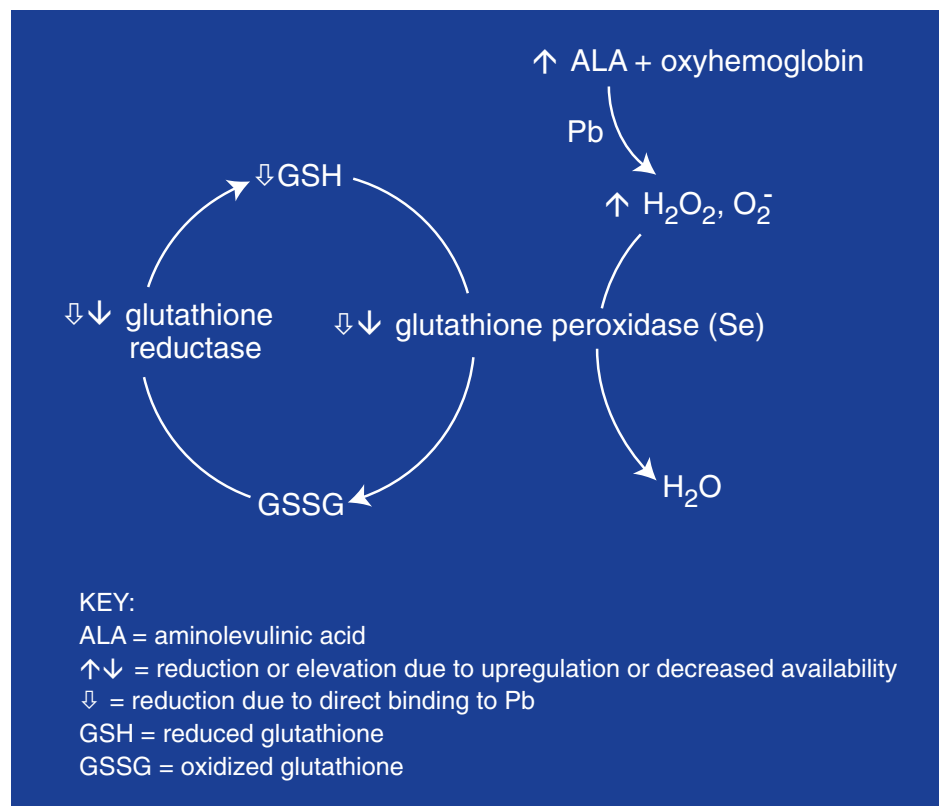**Figure 1. Inhibition of ALAD Results in Elevated ALA**

Succinyl CoA + Glycine

ALA synthetase ⇧

⇧delta-Aminolevulinic acid (ALA)

Lead ⟶ ALA dehydratase (ALAD)

⇩porphobilinogen

⇩heme

⇧H_2O_2, O_2^-, OH
⇧4,5-dioxovaleric acid

KEY: ⇧ or ⇩ indicate changes in enzymes or substrates as a result of lead exposure.

Figure 2. Effect of Lead on Glutathione Metabolism

↑ ALA + oxyhemoglobin

Pb

↑ H_2O_2, O_2^-

⇓GSH

⇓↓ glutathione reductase

⇓↓ glutathione peroxidase (Se)

GSSG

H_2O

KEY:
ALA = aminolevulinic acid
↑↓ = reduction or elevation due to upregulation or decreased availability
⇓ = reduction due to direct binding to Pb
GSH = reduced glutathione
GSSG = oxidized glutathione

Lead Generates Reactive Oxygen Species (ROS)

Erythrocytes have a high affinity for lead, binding 99 percent of the lead in the bloodstream. Lead has a destabilizing effect on cellular membranes, and in red blood cells (RBC) the effect decreases cell membrane fluidity and increases the rate of erythrocyte hemolysis. Hemolysis appears to be the end result of ROS-generated lipid peroxidation in the RBC membrane.[15] Lead can also bind directly to phosphatidylcholine in the RBC membrane, leading to a decrease in phospholipid levels.[16] Lipid peroxidation of cellular membranes has also been identified in tissue from various regions of the brain of lead-exposed rats.[17] Hypochromic or normochromic anemia is a hallmark of lead exposure; it results from ROS generation and subsequent erythrocyte hemolysis.[18] Lead is considered, along with silver, mercury, and copper, to be a strong hemolytic agent, able to cause erythrocyte destruction through the formation of lipid peroxides in cell membranes.[19]

In addition to membrane peroxidation, lead exposure causes hemoglobin oxidation, which can also cause RBC hemolysis. The mechanism responsible for this reaction is lead-induced inhibition of ALAD. ALAD is the enzyme most sensitive to lead's toxic effects – depressed heme formation. As a result, elevated levels of the substrate ALA are found in both the blood and urine of lead-exposed subjects.[10] These elevated levels of ALA generate hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-), and also interact with oxyhemoglobin, resulting in the generation of hydroxyl radicals (OH), the most reactive of the free radicals (Figure 1).[14] As ALA is further oxidized, it becomes 4,5-dioxovaleric acid. The generation of

stress, specifically lipid peroxidation, which occurs in lead exposure.[13] ALAD is a crucial enzyme in lead toxicity because the inhibition of ALAD lowers heme production and increases levels of the substrate delta-aminolevulinic acid (ALA) (Figure 1). Elevated levels of ALA, found both in the blood and urine of subjects with lead exposure, are known to stimulate ROS production.[14]

Glutathione reductase, the enzyme responsible for recycling of glutathione from the oxidized form (glutathione disulfide; GSSG) to the reduced form (reduced glutathione; GSH) is also deactivated by lead.[11] Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase were all found to correlate with depressed glutathione levels in occupationally-exposed workers.[8] Figure 2 demonstrates the effect of lead on glutathione metabolism.

this potentially genotoxic compound is a possible mechanism for the metal-dependent DNA carcinogenicity of lead. By the same mechanism, ALA may be responsible for the increased frequency of liver cancer in acute intermittent porphyria, another condition where elevated levels of ALA occur.[20]

Lead has also been shown to both elevate and suppress blood levels of the antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx).[21-23] Elevations of these enzymes have been seen in lower levels of exposure, while suppression can occur at higher exposure levels over longer periods of time. In one study of 137 lead-exposed workers, those with high blood lead levels (over 40 μg/dL) had significant reductions in blood GPx that correlated with elevated erythrocyte MDA levels.[24] Those with lower exposures (25-40 μg/dL) had elevated levels of GPx, a suggested compensatory reaction for increased lipid peroxidation.

Hypertension: The Role of Lead-induced Oxidative Stress and Nitric Oxide

Multiple studies in animal models and human populations have shown a causal relationship between low-level lead exposure and hypertension.[25] Conversely, one meta-analysis concluded that current data support a weak correlation between lead exposure and incidence of hypertension.[26] Confounding factors, including dietary intake of antioxidants, genetic resistance traits, dietary calcium intake, exposure to other environmental toxins, and dietary excesses of fat, sugar, salt and alcohol complicate the isolation of lead as a risk factor in epidemiological studies.[26,27]

There is evidence, however, that oxidant stress plays a significant role in the etiology of hypertension. Antioxidant supplementation has been shown to ameliorate hypertension in rats.[28] Various studies support the theory that hypertension is the manifestation of ROS production that can occur in many conditions, including lead exposure,[29] experimentally-induced syndrome X,[30] pre-eclampsia,[31] salt-sensitivity,[32] and uremia.[33] Hypertensive patients have lower levels of plasma vitamin C and higher levels of plasma hydrogen peroxide, superoxide anion, and lipid peroxide.[34-36]

Nitric oxide metabolism has been shown to play a central role in the pathogenesis of both oxidative stress-induced hypertension and lead-induced hypertension.[27] Adequate levels of nitric oxide are necessary for endothelium-dependent vasodilation and inhibition of smooth muscle cell proliferation, platelet aggregation, and monocyte adhesion.[37] The role of nitric oxide regulation in vascular function is complex and only briefly summarized here. For a more complete review of the relationship between lead, oxidative stress, and nitric oxide, the extensive review by Vaziri is referenced.[27] Nitric oxide, also known as "endothelium-relaxing factor," is effectively inactivated by ROS.[33] ROS have been shown to oxidize nitric oxide in vascular endothelial cells, creating a nitric oxide deficiency and generating peroxynitrite, a highly active ROS that can damage lipids and DNA.[38] Glutathione depletion and lead exposure have been independently shown to depress nitric oxide and cause hypertension in animal models.[28,29]

Lead exposure has also been shown to upregulate monoamine oxidase activity in aortic tissues and elevate plasma norepinephrine.[39] Patients with lead-induced hypertension exhibit neurotransmitter changes that indicate increased sympathetic nervous system activity and activation of the renin-angiotensin system.[40] Nitric oxide has the ability to regulate sympathetic nervous system activation. Although no studies have directly investigated the effect of nitric oxide depression on increased sympathetic activity in hypertension, it has been hypothesized that the potential of ROS to suppress nitric oxide may play a role in this aspect of lead-induced hypertension.[27]

In two hypertensive rat studies, antioxidants increased nitric oxide availability and eliminated hypertension.[28,41] In the first study, rats were made hypertensive by blocking glutathione production and then fed vitamin E (5,000 IU/kg) and vitamin C (3 mMol/L of drinking water).[28] Although the antioxidants were not sufficient to normalize glutathione levels, they were sufficient to eliminate hypertension in all the animals and normalize nitric oxide levels, which had been suppressed by the glutathione-blocking drug. When the glutathione-blocking drug was administered, elevated levels of nitrotyrosine were observed in the tissue, a "footprint" of ROS action on nitric oxide. The researchers then assumed the

hypertension was a result of ROS generated by gluta-thione depletion and reversed by the addition of vitamins C and E to the rat diet.

The second study examined lead-induced hypertension in an animal model and the use of tempol (dimethylthiourea), a drug that mimics superoxide dismutase.[41] The researchers completely eliminated lead-induced hypertension with tempol, which enters the intracellular space and eliminates superoxide radicals. The use of a substance that acts as an antioxidant, quenching the superoxide radical, was based on evidence that lead was causing elevations of ROS (specifically superoxide anion, hydrogen peroxide, and hydroxyl radical), decreasing availability of nitric oxide, and resulting in vasoconstriction. Using the antioxidant enzyme SOD was not effective because it is unable to enter the intracellular space (where mitochondrial superoxide is generated).

Is Oxidative Stress Responsible for the Pathology of Lead Toxicity?

Lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning.[42] Oxidative stress has also been implicated in specific organs with lead-associated injury, including liver, kidneys, and brain tissue. ROS generated as a result of lead exposure have been identified in lung, endothelial tissue, testes, sperm, liver, and brain.[42]

Workers with occupational lead-exposure have a significantly higher frequency of infertility, stillbirths, miscarriages and spontaneous abortion, reduced sperm counts and motility, increased rates of teratospermia, and decreased libido.[43] Studies of ROS in lead-exposed animals have shown alterations in SOD activity in testicular tissue and increased sperm ROS production that have been associated with decreased sperm counts, decreased motility, and decreased sperm-oocyte penetration.[44]

The Role of Antioxidants in Addressing Lead-induced Oxidative Stress
Vitamin C

Vitamin C is a known free-radical scavenger and has been shown to inhibit lipid peroxidation in liver and brain tissue of lead-exposed animals.[45] In lead-exposed rats, a minimal 500 mg/L concentration in drinking water was able to reduce ROS levels by 40 percent.[46] In other animal studies, the toxic effects of lead on heme production were reversed by a vitamin C dose of 100 mg/kg.[47] This vitamin C intervention also normalized blood ALAD levels and resulted in a significant decrease in blood and hepatic lead content.

Other studies indicate vitamin C might have significant chelation capacity for lead. One rat pharmacokinetic study found intravenously administered vitamin C lowered lead tissue levels in rats that were continuously administered lead.[48] A human study, evaluating blood lead levels in pregnant women, found that 1,000 mg vitamin C per day, in addition to a prenatal multivitamin supplement, significantly lowered blood lead levels from a mean of 5.1 to 1.1 μg/dL during the course of pregnancy.[49] The safety of chelating pregnant women, however, and potential exposure of the fetus to lead, was not addressed in this study.

In a study of silver refining (involving lead smelting), workers with mean blood lead levels of 32.84 μg/dL and symptoms of lead toxicity (anemia, muscle wasting, abdominal colic) were given thiamine (vitamin B1) or vitamin C to evaluate the ability of these supplements to affect lead exposure. With continuous lead exposure and either 75 mg thiamine once daily or 250 mg vitamin C twice daily for 30 days, both vitamins significantly lowered blood lead levels. However, only vitamin C was effective in reversing the inhibition of blood ALAD levels, indicating a significant antioxidant effect as well.[50]

The lead-lowering effect of vitamin C has not been proven effective in exposure studies where occupationally-exposed workers had higher blood lead levels. Workers with long-term lead exposure and significant blood lead levels (28.9-76.4 μg/dL; mean >40 μg/dL) were given 1 g vitamin C orally once

daily, five days a week for 20 weeks or 60 mg zinc (as zinc gluconate) once daily, five days a week for eight weeks. Neither treatment affected lead levels or lead metabolism.[51]

The effect of vitamin C on lead levels has been clarified by studies that show ascorbic acid decreases intestinal absorption of lead.[52] By reducing ferric iron to ferrous iron in the duodenum, vitamin C increases the availability of iron, which competes with lead for intestinal absorption.[52]

In a study assessing the mechanism of vitamin C's lead-lowering capacity, 75 male smokers with no known occupational or residential lead exposure were given 1,000 mg vitamin C daily for 30 days. Blood lead levels were effectively lowered from a mean of 1.8 μg/dL to 0.4 μg/dL within one week and remained at that level for the remainder of the study.[53] Urine lead levels did not change during the length of the study, regardless of the vitamin C dosage. The authors concluded that vitamin C was working to inhibit intestinal absorption of lead. This would involve the entero-hepatic recycling of lead through erythrocyte catabolism rather than increasing lead excretion through renal elimination.

In addition to acting as an antioxidant and anti-absorption agent, vitamin C also has an inhibiting effect on lead uptake on a cellular level. Mammalian cell culture studies – using a methodology that assessed lead uptake, lead toxicity, and lead release by chelating agents and nutrients – determined the capacity of these agents to modify the way cells absorb and are damaged by lead.[54] Vitamin C was effective at inhibiting lead uptake and reducing lead cytotoxicity. Lead chelators calcium disodium ethylenediaminetetraacetic acid (CaNa$_2$EDTA; versenate),

meso-2,3-dimercaptosuccinic acid (DMSA; succimer), sodium 2,3-dimercapto-1-propanesulfonate (DMPS), and penicillamine, as well as nutrients thiamine and pyridoxine, were also effective at inhibiting uptake of lead and reducing its cytotoxic effects.

Vitamin C, in combination with silymarin, has also been shown to effectively reduce the hepatotoxic effect of acute lead poisoning.[55] In a rat study using toxic amounts of lead (500 mg/kg diet), vitamin C (1 mg/100 g body weight) and silymarin (1 mg/100 g body weight) were supplemented in an attempt to inhibit genetic damage to hepatocytes and halt the onset of acute hepatitis. The combination of vitamin C with silymarin significantly improved elevated liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP), as well as evidence of histopathological liver damage.

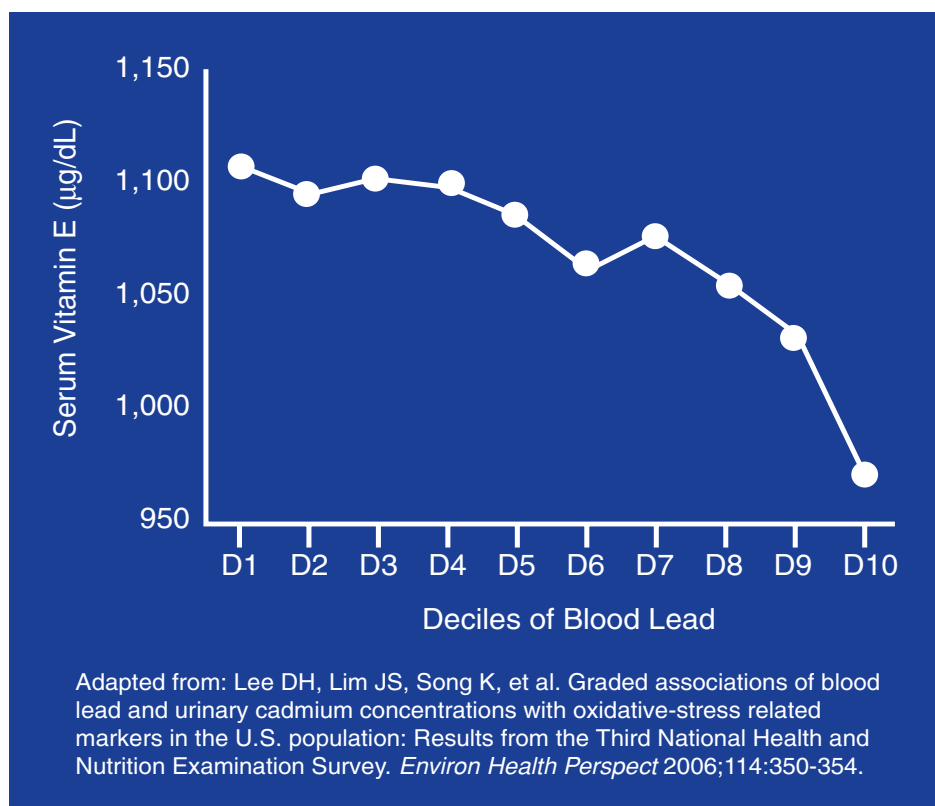**Figure 3. Relationship Between Blood Lead Levels and Serum Vitamin E**

Adapted from: Lee DH, Lim JS, Song K, et al. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress related markers in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. *Environ Health Perspect* 2006;114:350-354.
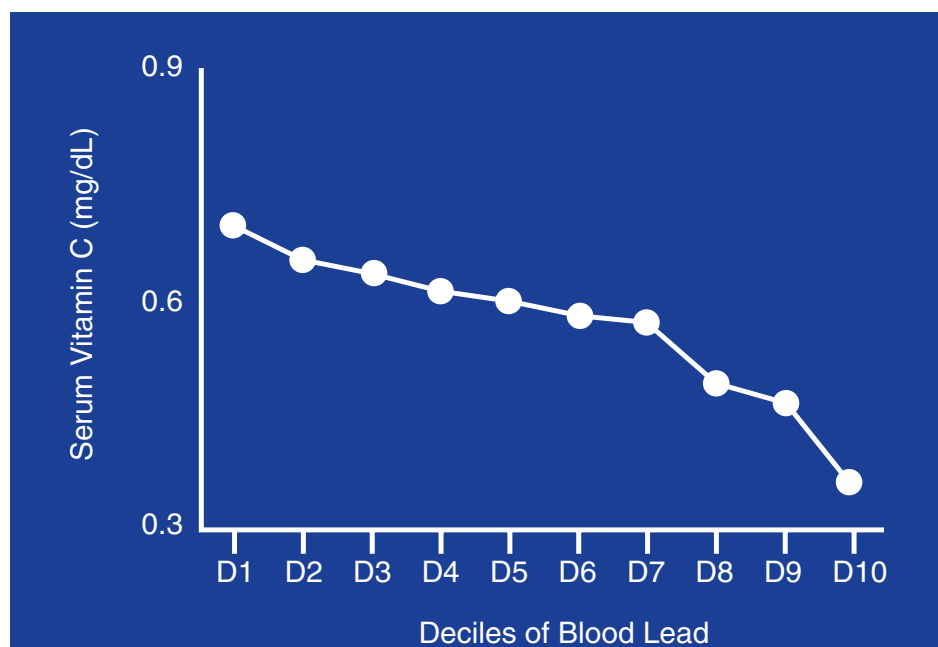
Figure 4. Relationship Between Blood Lead Levels and Serum Vitamin C

Adapted from: Lee DH, Lim JS, Song K, et al. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress related markers in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. *Environ Health Perspect* 2006;114:350-354.

Epidemiological Correlations Between Lead and Vitamin C

Blood lead levels and serum ascorbic acid concentrations in 19,578 subjects in the Third National Health and Nutrition Examination Survey (NHANES) were evaluated in an attempt to find any correlations.[56]After controlling for the effects of age, race, gender, income level, dietary caloric intake, fat, calcium, iron, and zinc, children and adolescents with the highest serum ascorbic acid levels had an 89-percent decreased prevalence of elevated blood lead levels (>15 μg/dL), while adults with the highest serum ascorbic acid levels had a 65- to 68-percent decreased prevalence of elevated blood lead. The authors concluded that these variables (serum ascorbic acid and blood lead) were independently associated and that further research is needed to evaluate vitamin C as a possible therapeutic intervention for lead exposure.

Another evaluation of 10,098 adults from the same NHANES data provides more clarity on the relationship of vitamin C and other antioxidants to lead and oxidative damage. In this analysis, whole blood lead and urinary cadmium levels were analyzed in association with serum vitamins C and E, carotenoids, and serum GGT.[57] In attempting to answer the question, "Is oxidative stress involved in the pathogenesis of lead- or cadmium-related disease in the general population?" the authors explored the cross-sectional associations of lead and cadmium using blood levels of antioxidants and serum GGT as markers of oxidative stress. Normal range variations of GGT have been examined as an early biomarker of oxidative stress in epidemiological studies.[58] GGT has been inversely correlated with serum antioxidant vitamins and positively associated with serum C-reactive protein.[59,60] Mean blood lead levels in this population were 2.8 μg/dL (range 0.7-56 μg/dL). Blood lead was positively associated with serum GGT elevations within normal lab ranges and inversely associated individually with serum vitamin E and vitamin C (Figures 3 and 4). The relationship between serum carotenoids and lead was not as clear, but still maintained an inverse relationship in the upper deciles of lead (Figure 5). Even when the relationship between blood lead levels and GGT values was adjusted for race, gender, smoking status, drinking status, and body mass index, there was still a statistically significant positive correlation between the two (p value <0.01 for most of the confounders).

The authors warn that oxidative stress appears to occur in these populations at low levels of exposure to lead and cadmium and should be considered a mechanism in lead- or cadmium-related disease states. As discussed in Part I of this article, the signs and symptoms of lead toxicity have been well documented in the literature to occur at levels of exposure considered safe by current federal standards.[61]

Vitamin E

Lead has been shown to alter RBC membrane flexibility and to increase RBC fragility, leading to increased risk for hemolysis.[62] Vitamin E has a known protective action in membrane stability and prevents membrane lipoproteins from oxidative damage.[63] Alpha-tocopherol was shown to prevent RBC membrane damage in lead toxicity by lowering lipid peroxide levels and increasing SOD and catalase activity.[64] Animal studies have found vitamin E to effectively prevent lipoperoxide-related lead toxicity in sperm[46] and to be more effective than methionine or vitamin C at decreasing lipoperoxidation in the liver, brain, and kidney of lead-exposed rats when given in doses of 100 IU/kg body weight.[45]

Methionine

Methionine is the preferred substrate for glutathione production by hepatocytes and acts as a precursor for glutathione production in the liver.[65] Lead-exposed rats treated with 100 mg/kg body weight of methionine demonstrated a significant decline in lipid peroxides in the liver.[45] Methionine has been shown to react with ROS to form methionine sulfoxide[66] and to increase ROS-scavenging by improving hepatic glutathione levels.[65] Methionine supplementation also led to increases in thiol molecule groups, sulfur-based protein, and non-protein molecules that act as antioxidants to prevent peroxidation in the liver and kidneys of animals exposed to lead or alcohol.[45,66]
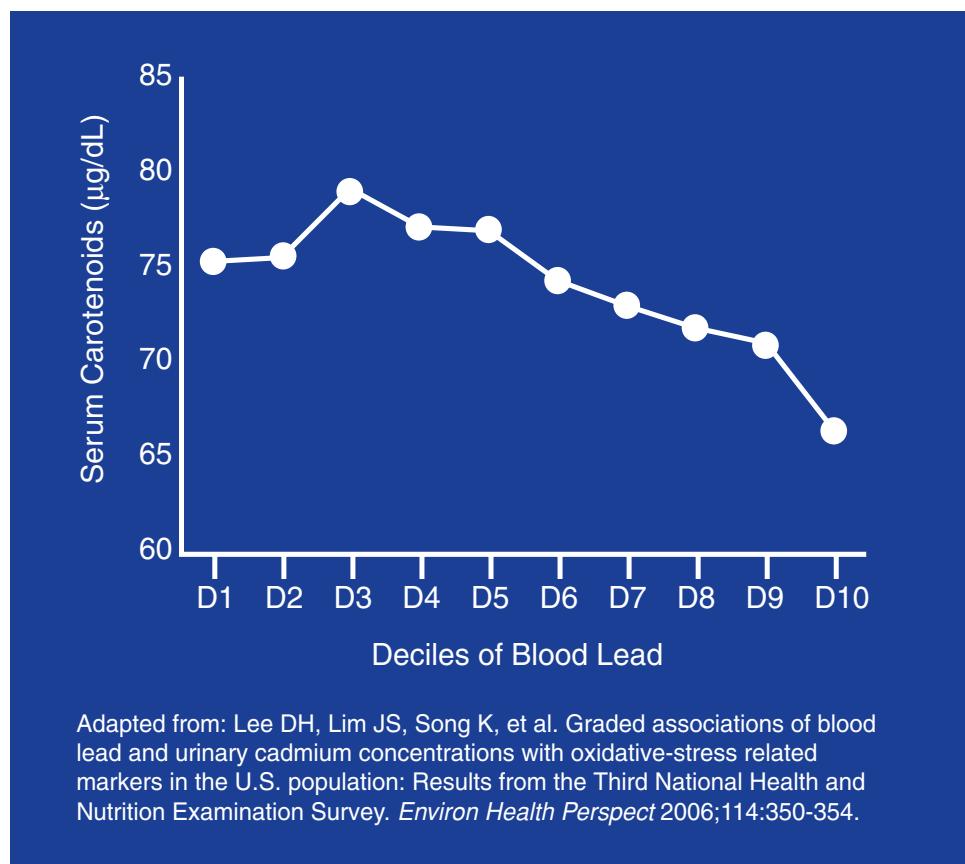
In studies with lead-exposed rats, Flora et al found zinc and thiamine given in addition to methionine normalized ALAD activity and urinary excretion of ALA more effectively than any single nutrient alone.[67,68]

N-acetylcysteine (NAC)

NAC was given to lead-exposed animals with the explicit intention of replenishing reduced glutathione and decreasing oxidant levels.[69] In animals exposed to lead for five weeks prior to treatment, NAC was found to normalize reduced glutathione/oxidized glutathione ratios, improve glutathione status, decrease MDA levels in brain and liver tissue, and increase cell survival rates (which had been significantly reduced by lead toxicity). The antioxidant effect of NAC also appeared to be effective in reducing and actually reversing the oxidant effect of increased levels of aminolevulinic acid, the hallmark of "plumbism" or symptomatic lead toxicity.[70]

Figure 5. Relationship Between Blood Lead Levels and Serum Carotenoids

Adapted from: Lee DH, Lim JS, Song K, et al. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress related markers in the U.S. population: Results from the Third National Health and Nutrition Examination Survey. *Environ Health Perspect* 2006;114:350-354.

Alpha-Lipoic Acid

Alpha-lipoic acid is an antioxidant that functions to replenish glutathione and vitamins C and E, reduce lipid peroxides, and chelate toxic metals.[71] In animal studies exploring its effect in lead toxicity, lipoic acid had no direct chelating ability but was consistent in countering the effects of lead on hepatic glutathione, renal glutathione, and oxidative stress markers.[72,73] In lead-treated cell lines, lipoic acid prevented oxidative stress and increased cell survival rates. Administration of lipoic acid was not effective in decreasing blood or tissue lead levels compared to the well-known chelator DMSA.

Zinc

Zinc is known to compete with lead binding by a metallothionein-like transport protein in the gastrointestinal tract.[74] As a result, zinc appears to have a mitigating effect on lead toxicity. When lead-exposed rats were given zinc, previously depressed levels of SOD in the testes returned to normal and ALAD inhibition was reversed.[75] As mentioned previously, when zinc was given simultaneously with methionine and thiamine in the presence of lead, evidence of lead toxicity (elevated urine ALA and depressed ALAD levels) was significantly improved.[67,68] Although there is no strong evidence that zinc works by a direct antioxidant effect or a chelating effect, the competitive inhibition of lead uptake appears to act indirectly as an antioxidant during ongoing lead exposure.

Selenium

Selenium is a required mineral for the metalloenzyme glutathione peroxidase. GPx plays a key role in recycling glutathione and is effective in reducing free radical damage in specific disease states.[76] Selenium supplementation has been shown to have a protective effect when given prior to lead exposure in animals. Increased levels of SOD, glutathione reductase, and reduced glutathione occurred in both liver and kidney tissue after intramuscular injection of sodium selenite.[77] These effects continued even after exposure to lead in rats that had been pre-treated with selenium. Lead can bind to selenium and form highly bonded selenium-lead complexes, which have been proposed as a mechanism for selenium's protective effect in lead toxicity.[74]

Pyridoxine (Vitamin B6)

Supplementation with pyridoxine in lead-exposed rats improved ALAD activity.[78] Blood, kidney, and liver levels of lead were also reduced, while brain levels remained stable. The proposed mechanism of pyridoxine in lead toxicity relates to its role in the metabolic trans-sulfuration pathway, which allows for the metabolism of cysteine from methionine. Methionine is the main dietary source of cysteine, the rate-limiting amino acid in glutathione production. Lead-exposed rats who had diet-induced vitamin B6 deficiencies had significantly lower glutathione levels than lead-exposed rats with normal vitamin B6 levels.[79]

Taurine

Taurine, a semi-essential amino acid, is known to have antioxidant membrane-stabilizing effects. It acts as a potent inhibitor of lipid peroxides, inhibits cellular apoptosis in ischemic reperfusion injury, and prevents glycation injury in erythrocytes.[80-82] In cell cultures exposed to lead, taurine treatment significantly improved cell survival, increased glutathione levels, and decreased MDA levels.[81] Lead-exposed rats given dietary taurine (1.2 g/kg/day) had the same results in RBCs, brain, and liver tissue.[81] Taurine had no direct chelating effect on lead, and the improvement in oxidative status in both studies was believed to be related to the membrane-stabilizing and free-radical scavenging effect of the amino acid.
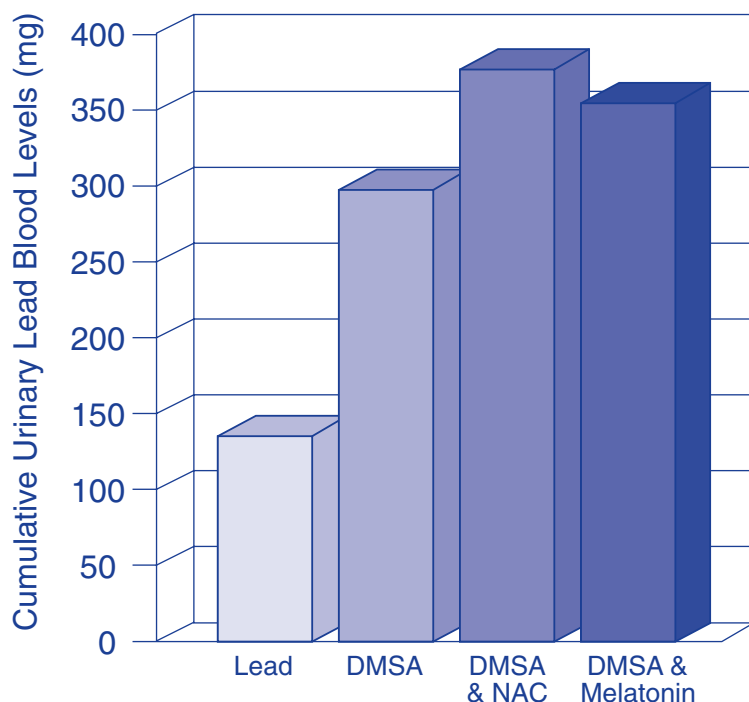
Antioxidants Used in Combination with Lead Chelators

Multiple studies have assessed the effect of antioxidants used in conjunction with the lead chelators $CaNa_2EDTA$ and DMSA. Both $CaNa_2EDTA$ (used intravenously) and DMSA are chelating agents used in pediatric and adult treatment of lead toxicity. Chelating agents have antioxidant properties; DMSA has been shown to lower ROS levels in erythrocytes and D-penicillamine has been shown to act as a free radical scavenger.[69,83] The majority of chelation studies have assessed the role of antioxidants in the presence of DMSA (Table 1).[83-88]

Table 1. Antioxidants in Combination with Chelating Agents

Antioxidants	Treatment	Tissue	Results	Reference
NAC	800 mg/kg/day with DMSA	RBC	Both NAC and DMSA increased RBC GSH	83
NAC	50 mg/kg intraperitoneal injection alone and with DMSA 50 mg/kg	Blood, liver, kidney, brain	More effective than DMSA alone at reversing oxidative lead toxicity; more effective than DMSA alone or DMSA + Melatonin at increasing urinary lead excretion	84
Melatonin	50 mg/kg intraperitoneal injection alone and with DMSA 50 mg/kg	Blood, liver, kidney, brain	More effective than NAC + DMSA at increasing GSH and reducing elevated liver enzymes	84
Vitamin E	5 IU/kg intramuscularly with DMSA 50 mg/kg	Blood, liver, kidney, brain	Vitamin E + DMSA more effective in reducing GSSG and TBARS than either alone	85
Vitamin C	25 mg/kg orally with DMSA 50 mg/kg	Blood, liver, kidney, brain	Effective in reducing GSSG and TBARS; more effective than vitamin E or DMSA for increasing hepatic GSH; no additive chelating effect	85
Alpha-lipoic acid	25 mg/kg intraperitoneal injection alone and with DMSA 20 mg/kg	Liver	Lipoic acid + DMSA effectively reversed lead-induced liver enzyme and ROS damage; neither alone was effective	86
Alpha-lipoic acid	25 mg/kg intraperitoneal injection alone and with DMSA 20 mg/kg	Blood, liver, kidney, brain	Lipoic acid was effective in reducing ROS-related kidney inflammation; lipoic acid + DMSA more effective at decreasing ROS than either alone	72
Zinc	10 mg/kg, 25 mg/kg, or 50 mg/kg (oral) with CaNaEDTA	Blood, liver, kidney, brain	At 10 mg/kg and 25 mg/kg, zinc improved CaNaEDTA mobilization of lead from tissue and blood; at 50 mg/kg it reduced CaNaEDTA effects	87

Figure 6. Cumulative Urinary Lead Excretion: DMSA plus NAC or Melatonin

Adapted from: Flora SJ, Pande M, Kannan GM, Mehta A. Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. *Cell Mol Biol* 2004;50:543-551.

In a study of lead-exposed animals, vitamin E was found to have a synergistic effect with DMSA in reducing levels of oxidized glutathione and preventing free radical damage.[85] While vitamin C was more effective than vitamin E at improving hepatic glutathione levels, there was no evidence vitamin C had a direct lead-chelating effect.[85]

A combination of alpha-lipoic acid and DMSA in lead-exposed animals was more effective than either used alone in preventing oxidative damage as measured by alterations in erythrocyte membrane enzyme levels.[86] When used without a chelator, alpha-lipoic acid had a significant ability to prevent lipid peroxidation in the kidneys of lead-exposed rats, but when DMSA was added, the combined effect was even greater.[72]

Zinc, at dosages of 10 or 25 mg/kg body weight, was effective at increasing the chelating ability of $CaNa_2EDTA$, although a higher dosage of 50 mg/kg actually reduced the chelating capacity of the drug.[87] From the above data, it appears that antioxidants can be used in standard doses in conjunction with lead chelating agents with beneficial effects.

Conclusion

Lead affects mammalian systems by directly lowering antioxidant reserves and generating ROS, specifically hydroperoxides and lipoperoxides. These ROS alter cellular membranes and tissue, resulting in vascular, neurological, and genetic damage. Hypertension is a lead-induced condition where the pathway between exposure and pathology is most clearly understood through nitric oxide metabolism. Antioxidants, specifically vitamins C, E, and B6, taurine, methionine, selenium, zinc, alpha-lipoic acid, and N-acetylcysteine have been shown to lower ROS-generated cellular damage. The literature also indicates that specific antioxidants have chelating capacities, although limited, and that a synergism exists between antioxidants and pharmaceutical chelating agents.

The studies that assess the effect of antioxidants as chelating agents do not show that antioxidants act as effectively as conventional chelating agents in reducing lead tissue burden.[83,85] Although lead-exposed rats treated with NAC did show a reduction of blood lead from a baseline of 34.8 μg/dL to 25.3 μg/dL after one week of treatment, DMSA alone reduced blood lead levels to 2.5 μg/dL.[83]

When antioxidants were combined with chelating agents, one trial clearly showed a synergism that improved chelating ability. In an animal trial utilizing DMSA plus melatonin or NAC, the combination of DMSA and NAC resulted in significantly higher urinary lead excretion than either treatment alone or the combination of melatonin and DMSA (Figure 6).[84]

References

1. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress. Part 1. Mechanisms involved in metal-induced oxidative damage. *Curr Top Med Chem* 2001;1:529-539.
2. Vaziri ND, Ding Y, Ni Z. Nitric oxide synthase expression in the course of lead-induced hypertension. *Hypertension* 1999;34:558-562.
3. Meister A, Anderson MD. Glutathione. *Annu Rev Biochem* 1983;52;711-760.
4. Christie NT, Costa M. *In vitro* assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: interaction with membranes, glutathione, metallothionein, and DNA. *Biol Trace Elem Res* 1984;6:139-158.
5. Hsu JM. Lead toxicity as related to glutathione metabolism. *J Nutr* 1981;111:26-33.
6. Ahamed M, Verma S, Kumar A, Siddiqui MK. Environmental exposure to lead and its correlation with biochemical indices in children. *Sci Total Environ* 2005;346:48-55.
7. Garcon G, Leleu B, Zerimech F, et al. Biologic markers of oxidative stress and nephrotoxicity as studied in biomonitoring of adverse effects of occupational exposure to lead and cadmium. *J Occup Environ Med* 2004;46:1180-1186.
8. Hunaiti A, Soud M, Khalil A. Lead concentration and the level of glutathione, glutathione S-transferase, reductase and peroxidase in the blood of some occupational workers from Irbid City, Jordan. *Sci Total Environ* 1995;170:95-100.
9. Lachant NA, Tomoda A, Tanaka KR. Inhibition of the pentose phosphate shunt by lead: a potential mechanism for hemolysis in lead poisoning. *Blood* 1984;63:518-524.
10. Farant JP, Wigfield DC. Biomonitoring lead exposure with delta-aminolevulinate dehydratase (ALA-D) activity ratios. *Int Arch Occup Environ Health* 1982;51:15-24.
11. Sandhir R, Julka D, Gill KD. Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. *Pharmacol Toxicol* 1994;74:66-71.
12. Gurer-Orhan H, Sabir HU, Ozgunes H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. *Toxicology* 2004;195:147-154.
13. Yiin SJ, Lin TH. Lead-catalyzed peroxidation of essential unsaturated fatty acid. *Biol Trace Elem Res* 1995;50:167-172.
14. Bechara EJ. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. *Braz J Med Biol Res* 1996;29:841-851.
15. Lawton LJ, Donaldson WE. Lead-induced tissue fatty acid alterations and lipid peroxidation. *Biol Trace Elem Res* 1991;28:83-97.
16. Shafiq-ur-Rehman S, Abdulla M. Impact of lead on phospholipid metabolism in human erythrocyte membranes. *Bull J Toxicol Occup Environ Health* 1993;2:35.
17. Flora GJ, Seth PK. Alterations in some membrane properties in rat brain following exposure to lead. *Cytobios* 2000;103:103-109.
18. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radic Biol Med* 2000;29:927-945.
19. Ribarov SR, Benov LC. Relationship between the hemolytic action of heavy metals and lipid peroxidation. *Biochim Biophys Acta* 1981;640:721-726.
20. Hiraku Y, Kawanishi S. Mechanism of oxidative DNA damage induced by delta-aminolevulinic acid in the presence of copper ion. *Cancer Res* 1996;56:1786-1793.
21. Sugawara E, Nakamura K, Miyake T, et al. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. *Br J Ind Med* 1991;48:239-242.
22. Chiba M, Shinohara A, Matsushita K, et al. Indices of lead-exposure in blood and urine of lead-exposed workers and concentrations of major and trace elements and activities of SOD, GSH-Px and catalase in their blood. *Tohoku J Exp Med* 1996;178:49-62.
23. Han SG, Kim Y, Kashon ML, et al. Correlates of oxidative stress and free-radical activity in serum from asymptomatic shipyard welders. *Am J Respir Crit Care Med* 2005;172:1541-1548.
24. Kasperczyk S, Kasperczyk A, Ostalowska A, et al. Activity of glutathione peroxidase, glutathione reductase, and lipid peroxidation in erythrocytes in workers exposed to lead. *Biol Trace Elem Res* 2004;102:61-72.
25. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for lead. (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2005:43-59.
26. Staessen JA, Roels H, Lauwerys RR, Amery A. Low-level lead exposure and blood pressure. *J Hum Hypertens* 1995;9:303-328.
27. Vaziri ND, Sica DA. Lead-induced hypertension: role of oxidative stress. *Curr Hypertens Rep* 2004;6:314-320.
28. Vaziri ND, Wang XQ, Oveisi F, Rad B. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* 2000;36:142-146.

29. Vaziri ND, Ding Y, Ni Z, Gonick HC. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. *Kidney Int* 1997;52:1042-1046.

30. Roberts CK, Vaziri ND, Liang KH, Barnard RJ. Reversibility of chronic experimental syndrome X by diet modification. *Hypertension* 2001;37:1323-1328.

31. Roggensack AM, Zhang Y, Davidge ST. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. *Hypertension* 1999;33:83-89.

32. Swei A, Lacy F, DeLano FA, Schmid-Schonbein GW. Oxidative stress in the Dahl hypertensive rat. *Hypertension* 1997;30:1628-1633.

33. Vaziri ND, Oveisi F, Ding Y. Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int* 1998;53:1748-1754.

34. Tse WY, Maxwell SR, Thomason H, et al. Antioxidant status in controlled and uncontrolled hypertension and its relationship to endothelial damage. *J Hum Hypertens* 1994;8:843-849.

35. Lacy F, O'Conner DT, Schmid-Schonbein GW. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *J Hypertens* 1998;16:291-303.

36. Kumar KV, Das UN. Are free radicals involved in the pathobiology of human essential hypertension? *Free Radic Res Commun* 1993;19:59-66.

37. Boger RH, Ron ES. L-arginine improves vascular function by overcoming the deleterious effects of ADMA, a novel cardiovascular risk factor. *Altern Med Rev* 2005;10:14-23.

38. Halliwell B. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrate formation *in vivo*? *FEBS Lett* 1997;411:157-160.

39. Tsao DA, Yu HS, Cheng JT, et al. The change of beta-adrenergic system in lead-induced hypertension. *Toxicol Appl Pharmacol* 2000;164:127-133.

40. Chang HR, Chen SS, Chen TJ, et al. Lymphocyte beta2-adrenergic receptors and plasma catecholamine levels in lead-exposed workers. *Toxicol Appl Pharmacol* 1996;139:1-5.

41. Vaziri ND, Ding Y, Ni Z. Compensatory up-regulation of nitric-oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. *J Pharmacol Exp Ther* 2001;298:679-685.

42. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. *Toxicology* 2002;180:33-44.

43. Levin SM, Goldberg M. Clinical evaluation and management of lead-exposed construction workers. *Am J Ind Med* 2000;37:23-43.

44. Hsu PC, Liu MY, Hsu CC, et al. Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm. *Toxicology* 1997;122:133-143.

45. Patra RC, Swarup D, Dwivedi SK. Antioxidant effects of alpha tocopherol, ascorbic acid, and L-methionine on lead-induced oxidative stress to the liver, kidney and brain in rats. *Toxicology* 2001;162:81-88.

46. Hsu PC, Hsu CC, Liu MY, et al. Lead-induced changes in spermatozoa function and metabolism. *J Toxicol Environ Health A* 1998;55:45-64.

47. Vij AG, Satija NK, Flora SJ. Lead induced disorders in hematopoietic and drug metabolizing enzyme system and their protection by ascorbic acid supplementation. *Biomed Environ Sci* 1998;11:7-14.

48. Dalley JW, Gupta PK, Hung CT. A physiological pharmacokinetic model describing the disposition of lead in the absence and presence of L-ascorbic acid in rats. *Toxicol Lett* 1990;50:337-348.

49. West WL, Knight EM, Edwards CH, et al. Maternal low level lead and pregnancy outcomes. *J Nutr* 1994;124:981S-986S.

50. Tandon SK, Chatterjee M, Bhargava A, et al. Lead poisoning in Indian silver refiners. *Sci Total Environ* 2001;281:177-182.

51. Lauwerys R, Roels H, Buchet JP, et al. The influence of orally-administered vitamin C or zinc on the absorption of and biological response to lead. *J Occup Med* 1983;25:668-678.

52. Suzuki T, Yoshida A. Effectiveness of dietary iron and ascorbic acid in the prevention and cure of moderately long-term lead toxicity in rats. *J Nutr* 1979;109:1974-1978.

53. Dawson EB, Evans DR, Harris WA, et al. The effect of ascorbic acid supplementation on the blood lead level of smokers. *J Am Coll Nutr* 1999;18:166-170.

54. Fischer AB, Hess C, Neubauer T, Eikmann T. Testing of chelating agents and vitamins against lead toxicity using mammalian cell cultures. *Analyst* 1998;123:55-58.

55. Shalan MG, Mostafa MS, Hassouna MM, et al. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. *Toxicology* 2005;206:1-15.

56. Simon JA, Hudes ES. Relationship of ascorbic acid to blood lead levels. *JAMA* 1999;281:2289-2293.

57. Lee DH, Lim JS, Song K, et al. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress-related markers in the U.S. population: results from the Third National Health and Nutrition Examination Survey. *Environ Health Perspect* 2006;114:350-354.

58. Lee DH, Blomhoff R, Jacobs DR Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radic Res* 2004;38:535-539.

59. Lee DH, Jacobs DR Jr. Association between serum gamma-glutamyltransferase and C-reactive protein. *Atherosclerosis* 2005;178:327-330.

60. Lim JS, Yang JH, Chun BY, et al. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? *Free Radic Biol Med* 2004;37:1018-1023.
61. Patrick L. Lead toxicity, a review of the literature. Part I. Exposure, evaluation, and treatment. *Altern Med Rev* 2006;11:2-22.
62. Levander OA, Morris VC, Ferretti RJ. Filterability of erythrocytes from vitamin E-deficient lead-poisoned rats. *J Nutr* 1977:107:363-372.
63. Packer L. Protective role of vitamin E in biological systems. *Am J Clin Nutr* 1991;53:1050S-1055S.
64. Chaurasia SS, Kar A. Protective effects of vitamin E against lead-induced deterioration of membrane associated type-1 iodothyronine 5'-monodeiodinase (5'D-I) activity in male mice. *Toxicology* 1997;124:203-209.
65. Reed DJ, Orrenius S. The role of methionine in glutathione biosynthesis by isolated hepatocytes. *Biochem Biophys Res Commun* 1977;77:1257-1264.
66. Jurczuk M, Moniuszko-Jakoniuk J, Brzoska MM. Involvement of some low-molecular thiols in the peroxidative mechanisms of lead and ethanol action on rat liver and kidney. *Toxicology* 2006;219:11-21.
67. Flora SJ, Singh S, Tandon SK. Thiamine and zinc in prevention or therapy of lead intoxication. *J Int Med Res* 1989;17:68-75.
68. Flora SJ, Kumar D, Das Gupta S. Interaction of zinc, methionine or their combination with lead at gastrointestinal or post-absorptive level in rats. *Pharmacol Toxicol* 1991;68:3-7.
69. Ercal N, Treeratphan P, Hammond TC, et al. *In vivo* indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-acetylcysteine. *Free Radic Biol Med* 1996;21:157-161.
70. Neal R, Yang P, Fiechtl J, et al. Pro-oxidant effects of delta-aminolevulinic acid (delta-ALA) on Chinese hamster ovary (CHO) cells. *Toxicol Lett* 1997;91:169-178.
71. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995;19:227-250.
72. Sivaprasad R, Nagaraj M, Varalakshmi P. Lipoic acid in combination with a chelator ameliorates lead-induced peroxidative damages in rat kidney. *Arch Toxicol* 2002;76:437-441.
73. Pande M, Flora SJ. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. *Toxicology* 2002;177:187-196.
74. Flora SJ, Jain VK, Behari JR, Tandon SK. Protective role of trace metals in lead intoxication. *Toxicol Lett* 1982;13:52-56.
75. Batra N, Nehru B, Bansal MP. The effect of zinc supplementation on the effects of lead in the rat testis. *Reprod Toxicol* 1998;12:535-540.
76. Ryan-Harshman M, Aldoori W. The relevance of selenium to immunity, cancer, and infectious/inflammatory diseases. *Can J Diet Pract Res* 2005;66:98-102.
77. Othman AI, El Missiry MA. The role of selenium against lead toxicity in male rats. *J Biochem Mol Toxicol* 1998;12:345-349.
78. Tandon SK, Flora SJ, Singh S. Influence of pyridoxine (vitamin B6) on lead intoxication in rats. *Ind Health* 1987;25:93-96.
79. McGowan C. Influence of vitamin B6 status on aspects of lead poisoning in rats. *Toxicol Lett* 1989;47:87-93.
80. Wright CE, Tallan HH, Lin YY, Gaull GE. Taurine: biological update. *Annu Rev Biochem* 1986;55:427-453.
81. Selvaraj N, Bobby Z, Sathiyapriya V. Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an *in vitro* study on human erythrocytes. *Clin Chim Acta* 2006;366:190-195.
82. Oriyanhan W, Yamazaki K, Miwa S, et al. Taurine prevents myocardial ischemia/reperfusion-induced oxidative stress and apoptosis in prolonged hypothermic rat heart preservation. *Heart Vessels* 2005;20:278-285.
83. Gurer H, Ozgunes H, Neal R, et al. Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. *Toxicology* 1998;128:181-189.
84. Flora SJ, Pande M, Kannan GM, Mehta A. Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. *Cell Mol Biol (Noisy-le-grand)* 2004;50:OL543-OL551.
85. Flora SJ, Pande M, Mehta A. Beneficial effect of combined administration of some naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead intoxication. *Chem Biol Interact* 2003;145:267-280.
86. Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. *J Nutr Biochem* 2004;15:18-23.
87. Flora SJ, Bhattacharya R, Sachan SR. Dose-dependant effects of zinc supplementation during chelation of lead in rats. *Pharmacol Toxicol* 1994;74:330-333.