# Interaction of Antioxidants and Their Implication in Genetic Anemia (44452)

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*Abstract.* **The generation of reactive oxygen species (ROS) is a steady-state cellular event in respiring cells. Their production can be grossly amplified in response to a variety of pathophysiological conditions such as inflammation, immunologic disorders, hypoxia, hyperoxia, metabolism of drug or alcohol, exposure to UV or therapeutic radiation, and deficiency in antioxidant vitamins. Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, protein, and lipids) and other small antioxidant molecules. A number of major cellular defense mechanisms exist to neutralize and combat the damaging effects of these reactive substances. The enzymic system functions by direct or sequential removal of ROS (superoxide dismutase, catalase, and glutathione peroxidase), thereby terminating their activities. Metal binding proteins, targeted to bind iron and copper ions, ensure that these Fenton metals are cryptic. Nonenzymic defense consists of scavenging molecules that are endogenously produced (GSH, ubiquinols, uric acid) or those derived from the diet (vitamins C and E, lipoic acid, selenium, riboflavin, zinc, and the carotenoids). These antioxidant nutrients occupy distinct cellular compartments and among them, there are active recycling. For example, oxidized vitamin E (tocopheroxy radical) has been shown to be regenerated by ascorbate, GSH, lipoic acid, or ubiquinols. GSH disulfides (GSSG) can be regenerated by GSSG reductase (a riboflavin-dependent protein), and enzymic pathways have been identified for the recycling of ascorbate radical and dehydroascorbate. The electrons that are used to fuel these recycling reactions (NADH and NADPH) are ultimately derived from the oxidation of foods. Sickle cell anemia, thalassemia, and glucose-6-phosphate-dehydrogenase deficiency are all hereditary disorders with higher potential for oxidative damage due to chronic redox imbalance in red cells that often results in clinical manifestation of mild to serve hemolysis in patients with these disorders. The release of hemoglobin during hemolysis and the subsequent therapeutic transfusion in some cases lead to systemic iron overloading that further potentiates the generation of ROS. Antioxidant status in anemia will be examined, and the potential application of antioxidant treatment as an adjunct therapy under these conditions will be discussed.** [P.S.E.B.M. 1999, Vol 222]

The production of reactive oxygen and nitrogen species is a steady-state event in respiring cells, and it is now recognized that uncontrolled production of these reactive species is the primary cause of some major degenerative conditions (1). Cellular defense, targeted against these transient but damaging species, can be grouped under several mechanisms, and collectively they operate to terminate free radical reactions or remove reactive species and their secondary products. To minimize heavy metal–induced catalysis of the Fenton and Haber-Weiss reactions that generate the most reactive hydroxyl radical, expression of several specific metal-binding proteins such as haptoglobin, ceruloplasmin, ferritin, transferrin, lactoferrin, and albumin, ensures that these metals remain cryptic, thereby serving as a key preventive measure in the overall free radical defense system. Antioxidant enzymes, most of which are highly dependent upon exogenous co-factors for their activity, can dismutate superoxide anion (superoxide dismutase) directly into hydrogen peroxide

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which in turn can be removed by catalase or several forms of glutathione peroxidase. Finally, direct quenching of radicals can be achieved by a series of endogenous and exogenous antioxidants (Table I). Functioning as scavengers, these molecules donate electrons, and themselves become free radicals that can either initiate chain reactions or conversely, be regenerated. It is now known that active repair pathways exist in which endogenous antioxidants such as glutathione, ubiquinol and NADH/NADPH, are obligatory in the recycling pathways (Figs. 1 and 2).

The antioxidant status in chronic disorders, particularly those with enhanced oxidative stress, has not drawn sufficient attention in the past. In this article, three major red cell disorders, namely, sickle cell anemia (SCA), α-thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency, are chosen as representative chronic disorders. These hereditary conditions are characterized by well-defined genetic defects with distinct clinical manifestation. However, increased oxidative damage to red cells is commonly associated with all three disorders, and such increased oxidative damage to red cells has been attributed to accelerated red cell destruction in all three cases. Transfusion is standard treatment procedure for acute episodes of hemolytic anemia, but repeated transfusions generate a state of iron-loading that further complicates the clinical manifestation and management of these diseases. Although iron-chelating therapy with desferrioxamine is able to remove excessive iron, the procedure often induces a secondary deficiency of zinc and other divalent cations and creates new problems in mineral nutrition. Another characteristic of genetic anemia is an overall accelerated production of reactive oxygen species (ROS) that tend to deplete cellular antioxidants. This review focuses on the interactions of cellular antioxidants and examines the antioxidant status of these patients with special emphasis on the potential adjunct treatments with antioxidant therapy.

**Table I.** Micronutrients and Endogenous Antioxidants Involved in Free Radical Defense[a]

| Nutrients | Functional role |
|---|---|
| Carotenoids | Hydrophobic antioxidant |
| Vitamin E | Hydrophobic antioxidant |
| Vitamin C | Hydrophilic antioxidant |
| Niacin, tryptophan | Precursors to NADH/NADPH |
| Riboflavin | Cofactor for GSH reductase |
| Dihydrolipoic acid | Antioxidant |
| Selenium | Integral part of GSH perioxidase |
| Zinc/copper | Integral part of superoxide dismutase |
| Manganese | Integral part of superoxide dismutase |
| Plant phenolics | Hydrophobic antioxidant |
| Bioflavinoids | Hydrophobic antioxidant |
| GSH | Endogenous hydrophilic antioxidant |
| Ubiquinol | Endogenous hydrophobic antioxidant |
| NADH/NADPH | Endogenous hydrophilic antioxidants |

[a] Modified from Ref. 121.

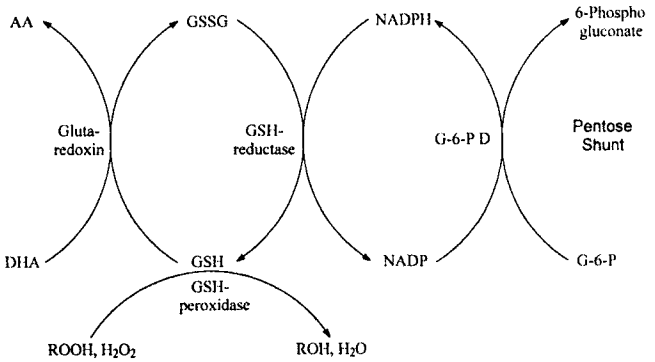


**Figure 1.** Redox cycling of glutathione (GSH) coupled with intracellular reduction of dehydroascorbic acid (DHA) to ascorbic acid (AA) and removal of peroxides by GSH peroxidase. GSSG, glutathione disulfide; G-6-PD, glucose-6-phosphate dehydrogenase; ROOH, organic peroxide; ROH, organic alcohol. Modified from Ref. 8.

## Central Role of Endogenous Antioxidants

Cellular redox state has been increasingly recognized as a critical component of stress-induced cellular response in the disease state. A significant portion of the cellular redox state is governed by key endogenous antioxidants: glutathione, ubiquinol (Coenzyme Q), and the pyridine nucleotides (NADH and NADPH) that occupy the cytosol or membrane component of the cell (Table I). Once formed from *de novo* synthesis, they are maintained chiefly by their corresponding recycling pathways. Since the predominant repairing pathways are enzymic, they have requirements for specific co-factors, an adequate supply of which is therefore necessary to restore the cellular redox state. For example, the regeneration of oxidized glutathione disulfide (GSSG) requires both NADPH and glutathione reductase, the activity of which is dependent upon adequate intake of riboflavin and an operative pentose shunt that provides NADPH (Fig. 1 and Table I).

Glutathione (GSH) is an ubiquitous tripeptide that plays a central role in the overall maintenance of the cellular redox state. Its intracellular concentration ranges up to 10 m*M*, with 95% present in the reduced sulfhydryl form (2, 3). Among the roles attributed to GSH, some of which will be discussed in detail, are maintenance of protein sulfhydryl groups; protection of cells against oxidative or radiation-induced damages; detoxification of highly reactive xenobiotic metabolites or peroxides; and regeneration of antioxidant vitamins. Figure 1 depicts the interaction of GSH with other major cellular antioxidants. It is clear that a steady-state GSH level is highly coupled with the energy status of the organism and proper functioning of the pentose shunt pathway. Thus, fasting could reduce the intracellular GSH pool by half.

Ubiquinol is a major endogenous antioxidant that functions in the mitochondrial electron transport chain. Together with tocopherol, they represent two major membrane-associated antioxidants. It is known that a high level of ubiquinol supplementation in the diet of rats can replace the

need for vitamin E (4). Although the semiquinone form of ubiquinone has been implicated in generating superoxide and hence is a pro-oxidant (5), ubiquinol has been shown to repair the tocopheroxyl radical (6, 7).

Pyridine nucleotides are major electron carriers; their levels are in turn governed by adequate intake of niacin, or under extreme conditions, tryptophan can replace niacin to form nicotinamide (Table I). The reduced forms of these pyridine nucleotides are continuously maintained by electrons derived from the oxidation of foods. Therefore, cellular redox-cycling is tightly coupled with the energy status of an organism, and it can be expected that during prolonged energy deficit or deficiencies of niacin or riboflavin, the efficiency of redox cycling will be compromised. Hence, from the point of view of energy balance, the repairing processes are costly, resulting in a net consumption of food-derived electrons. Other nutritional influence on cellular antioxidant defense systems have been reviewed (8).

## Interaction of Antioxidants and Regeneration of Antioxidant Vitamins: Recycling of Vitamin E

A large body of evidence suggests that a high degree of interaction exists among endogenous and exogenous antioxidants. The ability of one antioxidant to regenerate another oxidized species is common, and appears to be more dependent on the pecking order of their corresponding redox potential than their cellular compartments (9). Located in the biomembrane, vitamin E acts as a major peroxyl radical scavenger with powerful chain-breaking properties, and its protective role as a membrane stabilizer is well established. In 1968, Tappel (10) proposed that ascorbate may function to regenerate oxidized vitamin E. Although it is difficult to conceive of this occurring *in vivo* due to distinct cellular locations of these two vitamins, a direct reduction of tocopheroxyl radical by ascorbate was detected in a pure solution (11). Subsequent studies revealed that oxidation of vitamin E can be spared by ascorbate or glutathione in a number of pure or biological systems (12–20). In human platelet homogenate, addition of arachidonic acid caused a rapid oxidation of endogenous vitamin E by free radicals generated from the turnover of arachidonic acid *via* the 12-lipoxygenase pathway (Fig. 2). In this system, distinct chemical and enzymic pathways for vitamin E recycling, afforded by ascorbate and glutathione, have been identified (21).

To what extent does the redox cycling of vitamin E occur *in vivo*? In intact animals, a sparing effect of vitamin C on the nutritional status of vitamin E has been demonstrated in guinea pigs (22), in a genetic rat model defective in ascorbate synthesis (23, 24), and in fish (25). In humans, evidence for the recycling of vitamin E by vitamin C is less clear due to inherent experimental design difficulties in depleting a human subject of vitamin E. In an attempt to demonstrate the interaction in humans, Jacob *et al.* (26) reported a trend toward sparing of tissue vitamin E by vitamin C. More studies will be needed to show conclusively

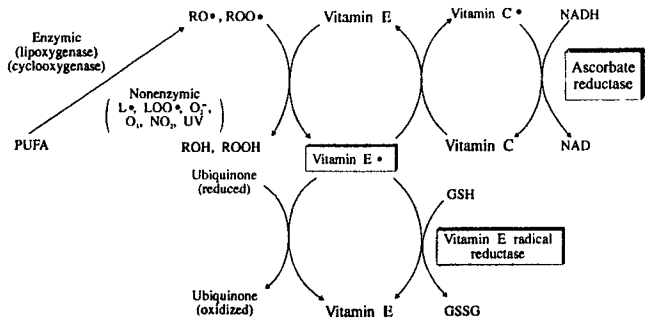


**Figure 2.** Interaction of antioxidants: Pathways for the oxidation and regeneration of vitamin E. Modified from Ref. 121.

whether the recycling reactions are physiologically relevant in man. Whether ascorbate is the key reductant in the recycling reaction or whether endogenous antioxidants such as ubiquinol, glutathione, or dihydrolipoate, which have been shown to reduce tocopherol radical (4, 6, 19, 27), are remains to be elucidated. In intact animals, it is important to note that an adaptive response to oxidative stress may result in upregulating the synthesis of endogenous antioxidants. For example, in a rat model that was subjected to serve oxidative stress from depletion of dietary vitamin E and selenium, there was an increased expression of the ubiquinol-dependent enzymic system in the plasma membrane (28). Hence, an adaptive response to oxidative stress may enrich the total pool of antioxidants of an organism through an increased expression of enzymes that are responsible for the synthesis of endogenous antioxidants. Along the same lines, recent interests in other minor dietary antioxidants such as plant phenolic compounds and bioflavonoids, which exhibit similar pharmacokinetics as vitamin E (i.e., plasma level in the micro molar range), may enter the overall pool of antioxidants that fuels recycling reactions. Their involvement in cellular redox state remains as a new frontier for more investigation.

## Recycling and Transport of Vitamin C

Pathways for the recycling of ascorbate radical (semidehydroascorbate) as well as the two-electron oxidized product, dehydroascorbic acid, have been reported. A semidehydroascorbate reductase activity that requires NADH as an electron donor has been described by several independent groups (29–31). The existence of this reductase may be significant in specialized tissues that require a higher intracellular concentration of ascorbate. In the adrenal gland, for example, the synthesis of catecholamine generates two ascorbate radicals per each molecule of norepinephrine made. However, the role of this reductase in the overall maintenance of the vitamin C pool in man is unclear at present. Recent studies from Levine *et al.* (32, 33) further reinforce the notion that the maintenance of a high concentration of intracellular ascorbate is of great physiological significance. In neutrophils, distinct ascorbate transporters were identified that actively pump ascorbate from plasma

(μ*M* range) against a 50-fold gradient so that intracellular ascorbate can reach the mM range. The same group also described another major ascorbate transport pathway that requires first an extracellular oxidation of ascorbate into dehydoascorbate followed by its transport into cells by glucose transporters 1 and 3 (34). Once inside the cell, dehydroascorbate was shown to be rapidly reduced back to ascorbic acid by glutaredoxin, with consumption of glutathione (35). Therefore, the recycling reactions of ascorbate are highly dependent on endogenously produced antioxidants such as NADH and glutathione. Figure 1 summarizes the intracellular reduction of dehydroascorbate coupled with the redox cycling of glutathione. For more information on ascorbate transport and recycling, the readers are referred to an excellent recent review (36).

## Pro-Oxidant Effect of Antioxidants

Paradoxical observations with regard to certain pro-oxidant effects of antioxidant compounds (vitamins C, E, and GSH and glucose) have been reported under certain special experimental conditions, indicating the complex interdependency among the pool of physiological relevant cellular antioxidants. It has long been recognized that autoxidation of GSH generates superoxide radicals (37), and addition of GSH to a red cell preparation was shown to induce red cell hemolysis (38). Conditions necessary to demonstrate the pro-oxidant effects of reducing agents include: 1) the induction of oxidative stress by the presence of either a free radical initiator or the involvement of Fenton metal ions; and 2) the severe depletion or absence of one of the key antioxidants. Thus, in low-density lipoproteins (LDL) loaded with vitamin E, Stocker and co-workers have demonstrated that radical initiator-induced oxidation of LDL was potentiated. Addition of ubiquinol, which was low in LDL particles, was able to reverse the pro-oxidant effect of vitamin E loading (39–41). Thus, in a system that is devoid of reductant capable of regenerating the one-electron oxidized product of vitamin E (tocopheroxyl radical), this relatively stable radical may initiate chain reactions to oxidize lipids in the LDL particle. At present, the physiologic relevance of the tocopherol-mediated LDL oxidation remains unclear.

When red cell hemolysis was used as the criterion of oxidative stress, it was shown that addition of vitamin C/dehydroascorbate (42) or GSH (38) could potentiate the rate of hemolysis. In a study designed to evaluate the pro-oxidant potential of antioxidant compounds in a rat model fed different levels of dietary vitamin E, Wang *et al.* (43) reported that red cell GSH content was in direct proportion to the levels of dietary vitamin E, which in turn was inversely related to the rate of $H_2O_2$-induced red cell hemolysis. Interestingly, in the vitamin E-depleted red cells, addition of either GSH, vitamin C, or glucose significantly increased the rate of hemolysis induced by the addition of azobis-radical generator. However, treatment of vitamin E-sufficient red cells with these antioxidants provided considerable protection against hemolysis. Hence, it appears that cellular vitamin E level can influence GSH content directly in cells, and under conditions of low or absent vitamin E, GSH and vitamin C can become pro-oxidants. Based on available evidence, it is concluded that in the presence of a complete profile of antioxidants, they function collectively as antioxidants, but under extreme conditions when one of the antioxidants is absent or low, other reducing agents can have pro-oxidant activity.

## Enhanced Oxidative Stress in SCA, Thalassemia and G6PD-Deficiency

Sickle hemoglobin is a result of a point mutation in the β-*globin* gene leading to a single amino acid substitution in the number 6 position of the β-globin chain. SCA is generally referred to as the homozygous state of the disease because heterozygous state (commonly referred as sickle trait) is usually asymptomatic (44). Since the major function of hemoglobin is to carry molecular oxygen, autoxidation of hemoglobin by oxygen will be an inevitable event. It is estimated that ≈ 3% of oxyhemoglobin is oxidized to methemoglobin daily with the concomitant production of superoxide anion (45–47). Sickle hemoglobin has been reported to exhibit accelerated autoxidation under various conditions (48–51). Hence, enhanced oxidative stress as indicated by increased susceptibility to lipid peroxidation (52), and increased generation of ROS has been found in sickle red cells (53).

The molecular defect in α-thalassemia is due to the deletion of α-globin genes leading to the disproportional presence of globin chains within the red cells of the affected individuals. In terms of clinical presentations, mild to severe hemolytic anemia would be expected from various types of α-thalassemia (44). A preponderance of recent evidence indicates that the enhanced destruction of thalassemic red cells *in vivo* is largely attributed to increased oxidative stress to these cells (54–58). Furthermore, recent studies have pointed out that the enhanced oxidative damage to the cell membrane is largely due to the increased binding of globin chains to the membrane (54–58). Such increased binding of the globin chain to the membrane could lead to the release of heme moiety from the globin chain to the membrane followed by deleterious oxidative reactions initiated by the heme moiety. G6PD-deficiency is by far the most common enzymopathy affecting over 200 million people worldwide and is mainly caused by diverse point mutations in the G6PD gene. G6DP is a key enzyme for the pentose pathway (hexose monophosphate shunt) which is essential for an adequate supply of NADPH. The red cell has limited capacity for reducing power, and in the absence of intracellular organelles, it depends solely on the pentose pathway for the generation of NADPH. Since the production of NADPH in G6PD-deficient red cells is highly compromised, increased oxidative stress in G6PD-deficient cells is well documented (59–61). To add to the intrinsic problems of oxidant load, additional oxidative stress is attributed

to periodic exposure to unprotected hemoglobin resulting from hemolysis. Repeated transfusions, which remain as standard treatment procedure to some of these conditions, generate a state of iron-overloading that further compromises the antioxidant status and complicates the clinical manifestations and management of these diseases. Although iron-chelating therapy with desferrioxamine is able to remove some excessive iron, the procedure induces a secondary deficiency of zinc and other divalent cations leading to major problems in mineral nutrition.

## Nutritional Problems and Impaired Antioxidant Status in SCA, α-Thalassemia, and G6PD-Deficiency

Children with sickle cell anemia exhibit stunted growth (reduced weight and height) (62–64), and there is evidence that these children have higher requirements for dietary protein and energy due to an accelerated rate of whole-body protein turnover and, consequently, an increased energy expenditure (65). Direct determination of resting energy expenditure has confirmed that adolescents with sickle cell anemia have a higher than normal resting metabolic rate (66). It is clear from these studies that whereas total caloric and protein intake of patients maybe comparable to nondisease controls, the overall accelerated metabolic demand renders the level of intake to be inadequate to maintain normal growth and development.

By far the most severe nutrition problem in the three main anemias discussed is the continuing depletion of antioxidant nutrients in response to chronic oxidative stress. In the sickle cell/thalassemia condition, there is a persistent state of low-grade inflammation as evidenced by the elevated levels of circulating inflammatory proteins such as C-reactive protein and soluble HLA class-I heterodimers (67). Table II summarizes the antioxidant status in genetic anemia. There are gross depletions of hydrophilic and hydrophobic antioxidants, and there is depressed antioxidant enzyme activity that is functionally linked to the increased need for antioxidant defense under these chronic inflammatory conditions. With the exception of a compensatory increase in glutathione peroxidase activity found in thalassemia and glucose-6-phosphate dehydrogenase deficiency, all other parameters reviewed were significantly depressed.

Severe impairment of vitamin E status is a common feature in all three conditions of genetic anemia (Table II), and this is due to higher consumption of this vitamin (63, 68). In sickle cell patients, plasma vitamin E concentrations were negatively associated with vaso-occlusive events (69) as well as a higher number of irreversibly sickled cells. Supplementation with this vitamin has been shown to restore plasma vitamin E levels, improve clinical outcome, and reduce the number of irreversible sickled red cells (70–73). Similar supplemental effect and improved outcome were reported in patients with thalassemia (68) and glucose-6-phosphate-dehydrogenase deficiency in which reduced hemolysis and improved red cell survival were seen after vitamin E supplements (74, 75).

In some of the studies reported, plasma vitamin E concentrations in patients were below 0.6 mg/dl, a cut-off point below which is considered inadequate for vitamin E nutriture in man. In a long-term feeding trial aimed at determining the vitamin E need of man, Horwitt *et al.* (76) depleted human subjects with a low vitamin E diet (3 mg/day) for 6 years. Both plasma vitamin E levels and hydrogen peroxide-induced red cell hemolysis were employed as criteria for vitamin E adequacy. When plasma levels dropped below 0.6 mg/dl, a significant increase in $H_2O_2$-induced red cell hemolysis occurred. Incubation of red cells from depleted subjects with vitamin E *in vitro* further reinforced the notion that a minimum level of plasma vitamin E is needed to protect red cells from peroxide-induced hemolysis (77). Similar findings were reported in patients with Cystic Fibrosis with mal-absorption of lipids. In an extensive study of vitamin E status and peroxide-induced red cell hemolysis in patients with Cystic Fibrosis, Farrell *et al.* (78) confirmed that plasma vitamin E dose-dependently protected red cells from peroxide-induced hemolysis. At present, it is unclear whether a low vitamin E status in plasma and red cell membrane is a direct trigger to the periodic hemolysis seem in genetic anemia. However, available evidence seems to suggest that vitamin E supplementation should be beneficial to patients with genetic anemia. Reduced circulating leukocytes and vitamin C is another common feature in patients with genetic anemia (Table II). In thalassemia, this defect has been linked with biochemical function of vitamin C in collagen formation and bone health to the increased incidence of osteopenia, frequent fractures, and skeletal deformities commonly found in patients with thalassemia (79).

**Table II.** Antioxidant Status in Patients with Genetic Anemia[a]

| Antioxidants | Sickle cell anemia | Thalassemia | G-6-P D deficiency |
|---|---|---|---|
| Vitamin E | ↓ 63, 69–71, 96–98 | ↓ 79, 94, 95, 100–109 | ↓ 74, 75, 99 |
| Vitamin C | ↓ 96, 114, 115 | ↓ 79 | ↓ 99 |
| Carotenoids | ↓ 96, 117 | ↓ 79 | ↓ 99 |
| GSH | ↓ 80, 84 | ↓ 118 | ↓ 2, 83, 118, 119 |
| GSH-peroxidae | ↓ 98, 110, 111 | ↑ 107, 112 | ↑ 74, 112, 113 |
| GSH-reductase | ↓ 80 | — | ↓ 2, 81, 82 |
| Zinc | ↓ 63 | ↓ 120 | — |

[a] Numbers denote reference number reporting the same event.

With the depletion of ascorbate and continuing demand for redox repair, it is not surprising that the glutathione level is also low in genetic anemia in view of the intense interaction of soluble reductants (Fig. 1) and the impaired GSH reductase activity seen in sickle cell (80) and G-6-PD deficiency (2, 81, 82, Table II). The depletion of cellular GSH has been shown to cause higher glycation of hemoglobin in glucose-6-phosphate-dehydrogenase deficiency (83) and an increased number of irreversibly sickled red cells (84).

## Implications of Low Antioxidant Status in Genetic Anemia: Lipid Peroxidation and Atherogenesis

Both hemoglobin and iron are potent catalysts for lipid peroxidation. Peroxidation of lipids and more specifically the oxidation of low-density lipoproteins (LDL) is now recognized as a key cause of atherogenesis (85). Antioxidants play major roles in the process of atherosclerosis, and the protective effects of vitamin E in the atherogenic process has been reviewed recently (86). When LDL was enriched with dietary vitamin E, the rate of LDL oxidation was shown to be significantly reduced in cell-mediated LDL oxidation (87) or when LDL oxidation was induced by addition of copper ion *in vitro* (87–90). Similar inhibition of LDL oxidation by vitamin C has been reported (91–93). The low status of vitamins C and E in genetic anemia will render the LDL more prone to oxidative attack. Indeed, LDL from patients with thalassemia was found to be more susceptible to *in vitro* oxidative modification (94). In another study with thalassemia, the oxidative state of LDL was found to contain significantly lower vitamin E, together with a 3-fold increase in conjugated dienes and a 17% reduction in the lysil residues of Apo B-100 (95). Thus, a higher rate of LDL oxidation in thalassemia patients is due to a lower concentration of vitamin E in the LDL particles. This elevated steady-state oxidation suggests an increased risk of atherogenesis in thalassemia. Since enrichment with vitamins E and C was effective in preventing LDL oxidation, it appears that repletion of antioxidants in genetic anemia may prove to be effective in reducing the risk of developing blood vessel–related disorders.

## Prospective Research

In the preceding review, it is clear that interactions occur among all the antioxidant nutrients regardless of their locations in the cell and that recycling is a dominant pathway for the maintenance of cellular redox potentials. The totality of the evidence suggests a notion of interdependence among antioxidants and that depletion of one will likely lead to a reduction of others. The electrons that fuel these recycling reactions are ultimately derived from the oxidation of foods and as such, the repairing pathways are closely linked to the overall energy status of an individual. It is equally clear that patients with these genetic defects suffer from chronic oxidative stress and have an altered redox state characterized by a gross depletion of antioxidant nutrients (Table II). Most of the clinical events in patients with these disorders were precipitated directly by severe antioxidant depletion resulting in inadequate protection. Therefore, systematic large-scale clinical trials involving the supplementation of combined antioxidant nutrients from Table II may generate useful information from which antioxidant replacement therapy maybe used as adjunct treatment for these disabling conditions. In addition, the altered metabolic state in sickle cell anemia, which raises the need for dietary protein and total energy, suggests that the increased requirements for macronutrients should also be considered.


1. Gutteridge JM, Halliwell B. Antioxidants in Nutrition, Health, and Disease. New York: Oxford University Press, pp111–123, 1994.
2. Anonymous. Genetic disorders of glutathione and sulfur amino-acid metabolism: New biochemical insights and therapeutic approaches. Ann Int Med **93**:330–346, 1980.
3. Meister A, Tate SS. Gultathione and related γ-glutamyl compounds. Ann Rev Biochem **45**:559–604, 1979.
4. Folkers K. Relationship between coenzyme Q and vitamin E. Am J Clin Nutr **27**:1026–1034, 1974.
5. Nohl H, Gille L, Staniek K. The biochemical, pathophysiological, and medical aspects of ubiquinone function. Ann N Y Acad Sci **854**:394–409, 1998.
6. Stotanovsky DA, Osipov AN, Quinn PJ, Kagan VE. Ubiquinone-dependent recycling of vitamin E radicals by superoxide. Arch Biochem Biophys **323**:343–351, 1995.
7. Kagan VE, Tyurina YY. Recycling and redox cycling of phenolic antioxidants. Ann N Y Acad Sci **854**:425–434, 1998.
8. Chow CK. Nutritional influence on cellular antioxidant defense systems. Am J Clin Nutr **32**:1066–1081, 1979.
9. Buttner GR. The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol and ascorbate. Arch Biochem Biophys **300**:535–543, 1993.
10. Tappel AL. Will antioxidant nutrients slow aging processes. Geriatrics **23**:97–105, 1968.
11. Packer JE, Slater TF, Wilson RL. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature **278**:737–738, 1979.
12. Barclay LR, Locke SJ, MacNeil JM. The auto-oxidation of unsaturated lipids in micelles: Synergism of inhibitors vitamins C and E. Can J Chem **61**:1288–1291, 1983.
13. Maguire JJ, Wilson DS, Packer L. Mitochondrial electron transport-linked tocopheroxyl radical reduction. J Biol Chem **264**:21462–21465, 1989.
14. May JM, Qu ZC, Mendiratta S. Protection and recycling of α-tocopherol in human erythrocytes by intracellular ascorbic acid. Arch Biochem Biophys **349**:281–289, 1998.
15. McCay P. Vitamin E: Interaction with free radicals and ascorbate. Ann Rev Nutr **5**:323–340, 1985.
16. Mehlhorn RJ, Sumida S, Packer L. Tocopherol radical persistence and tocopherol consumption in liposomes and in vitamin E-enriched rat liver mitochondria and microsomes. J Biol Chem **264**:13448–13452, 1989.
17. Niki E, Saito T, Kawakami A, Kamiya Y. Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. J Biol Chem **259**:4177–4182, 1984.
18. Scarpa M, Rigo A, Maiorino M, Ursini F, Gregolin C. Formation of α-tocopherol radical and recycling of α-tocopherol by ascorbate during peroxidation of phosphatidylcholine. Biochim Biophys Acta **801**:215–219, 1984.
19. Stoyanovsky DA, Goldman R, Darrow RM, Organisciak DT, Kagan VE. Endogenous ascorbate regenerates vitamin E in the retina directly and in combination with exogenous dihydrolipoic acid. Curr Eye Res **14**:181–189, 1995.

20. Waters RE, White LL, May JM. Liposomes containing α-tocopherol and ascorbate are protected from an external oxidant stress. Free Radic Res **26:**373–379, 1997.
21. Chan AC, Tran K, Raynor C, Ganz PR, Chow CK. Regeneration of vitamin E in human platelets. J Biol Chem **266:**17290–17295, 1991.
22. Bendich A, D'Apolito P, Gabriel E, Machlin LT. Interaction of dietary vitamin C and vitamin E on guinea pig immune responses to mitogens. J Nutr **114:**1588–1593, 1984.
23. Igarachi O, Yonekawa K, Fujiyama-Fujihara Y. Synergistic action of vitamin E and vitamin C *in vivo* using a new mutant of Wister-strain rats, ODS, unable to synthesize vitamin C. J Nutr Sci Vitaminol **37:**359–369, 1991.
24. Tanaka K, Hashimoto T, Tokumaru S, Iguchi H, Kojo S. Interactions between vitamin C and vitamin E are observed in tissues of inherently scorbutic rats. J Nutr **127:**2060–2064, 1997.
25. Harme L, Waagbo R, Berge RK, Lie O. Vitamin C and E interact in juvenile Atlantic salmon. Free Radic Biol Med **22:**137–149, 1997.
26. Jacob RA, Kutnink MA, Csallany AS, Daroszewska M, Burton GW. Vitamin C nutriture has little short-term effect on vitamin E concentrations in healthy women. J Nutr **126:**2268–2277, 1996.
27. Podda M, Trischler HJ, Ulrich H, Packer L. α-Lipoic acid supplementation prevents symptoms of vitamin E deficiency. Biochem Biophys Res Commun **204:**98–104, 1994.
28. Navarro F, Burgess NJ, Bello RI, De Cabo R, Arroyo A, Villalba JM. Vitamin E and selenium deficiency induces expression of the ubiquinone-dependent antioxidant system at the plasma membrane. FASEB J **12:**1665–1673, 1998.
29. Diliberto EJ, Dean G, Carter C, Allen PL. Tissue, subcellular and submitochondrial distribution of semidehydroascorbate reductase: Possible role of semidehydroascorbate reductase in cofactor regeneration. J Neurochem **39:**563–568, 1982.
30. Green RC, O'Brien PJ. The involvement of semidehydroascorbate reductase in the oxidation of NADH by lipid peroxide in mitochondria and microsomes. Biochim Biophys Acta **293:**334–342, 1973.
31. Schneider W, Staudinger HJ. Reduced nicotinamide adenine dinucleotide-dependent reduction of semidehydroascorbaic acid. Biochim Biophys Acta **96:**157–159, 1965.
32. Washko PW, Rotrosen D, Levine M. Ascorbic acid transport and accumulation in human neutrophils. J Biol Chem **264:**18996–19002, 1989.
33. Washko PW, Wang Y, Levine M. Ascorbic acid recycling in human neutrophils. J Biol Chem **268:**15531–15535, 1993.
34. Rumsey SC, Kwon O, Xu GW, Burant CF, Simpson I, Levine M. Glucose transporter isoforms GLU1 and GLU3 transport dehydroascorbic acid. J Biol Chem **272:**18982–18989, 1997.
35. Park JB, Levine M. Purification, cloning, and expression of dehydroascorbic acid reduction activity from human neutrophils: Identification as glutaredoxin. Biochem J **315:**931–938, 1996.
36. Rumsey SC, Levine M. Absorption, transport, and disposition of ascorbic acid in humans. Nutr Biochem **9:**116–130, 1998.
37. Misra HP. Generation of superoxide radical during the autoxidation of thiols. J Biol Chem **249:**2151–2155, 1974.
38. Brownlee BR, Huttner JJ, Panganamala RV, Cornwell DG. Role of vitamin E in glutathione-induced oxidant stress: Methemoglobin, lipid peroxidation, and hemolysis. J Lipid Res **18:**635–644, 1977.
39. Thomas SR, Neuzil J, Stocker R. Cosupplementation with coenyzme Q prevents the pro-oxidant effect of α-tocopherol and increases the resistance of LDL to transition metal–dependent oxidation initiation. Arterioscler Thromb Vasc Biol **16:**687–696, 1996.
40. Nruzil J, Thomas SR, Stocker R. Requirement for promotion or inhibition by α-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. Free Radic Biol Med **22:**57–71, 1997.
41. Witting PK, Upston JM, Stocker R. Role of α-tocopheroxyl radical in the initiation of lipid peroxidation in human LDL exposed to horse radish peroxidase. Biochemistry **36:**1251–1258, 1997.
42. Lovstad RA. Ascorbate and dehydroascorbate stimulation of copper-induced hemolysis: Protective action of ceruloplasmin, albumin, and apotransferrin. Int J Biochem **16:**155–159, 1984.
43. Wang HP, Huang CJ, Chow CK. Red cell vitamin E and oxidative damage: A dual role of reducing agents. Free Radic Biol Med **24:**291–298, 1994.
44. Weatherall DJ. Genetic disorders of haemoglobin. In: Hoffbrand AV, Lewis SM, Tuddenham, EGD, Eds. Postgraduate Haematology. Oxford, Boston: Butterworth and Heinemann, pp91–119, 1999.
45. Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. J Biol Chem **247:**6960–6962, 1972.
46. Carrell RW, Winterbourn CC, Rachmilewitz EA. Annotation: Activated oxygen and haemolysis. Br J Haematol **30:**259–264, 1975.
47. Winterbourn CC, McGrath BM, Carrell RW. Reaction involving superoxide and normal and unstable haemoglobin. Biochem J **155:**493–502, 1976.
48. MacDonald VW, Sharaches S. Differences in the reaction sequence associated with drug-induced oxidation of hemoglobin E, S, A, and F. J Lab Clin Med **102:**762–772, 1982.
49. Hebbel RP, Morgan WT, Eaton JW, Hedlund BE. Accelerated autoxidation and heme loss due to instability of sickle hemoglobin. Proc Natl Acad Sci U S A **85:**237–241, 1988.
50. Chiu DTY, Van Den Berg J, Kuypers FA, Hung IJ, Wei JS, Liu TZ. Correction of membrane lipid peroxidation with oxidation of hemoglobin variants: Possibly related to the rates of heme release. Free Radic Biol Med **21:**89–95, 1996.
51. Sheng K, Shariff M, Hebbel RP. Comparative oxidation of hemoglobin A and S. Blood **91:**3467–3470, 1998.
52. Chiu D, Lubin B, Shohet SB. Erythrocyte membrane lipid organization during the sickling process. Br J Haematol **41:**223–234, 1979.
53. Hebble RP, Eaton JW, Balasingam M, Steinberg MH. Spontaneous oxygen radical generation by sickle erythrocytes. J Clin Invest **70:**1253–1261, 1982.
54. Schrier SL, Mohandas N. Globin-chain specificity of oxidation-induced changes in red blood cell membrane properties. Blood **79:**1586–1592, 1992.
55. Shinar E, Rachmilewitz EA. Oxidation denaturation of red blood cells in thalassemia. Semin Hematol **27:**70–91, 1990.
56. Hebbel RP, Leung A, Mohandas N. Oxidation-induced changes in microheological properties of the red cell membrane. Blood **76:**1015–1022, 1990.
57. Yuan J, Bunyaratvej A, Fucharoen S, Fung C, Shinar E, Schrier SL. The instability of the membrane skeleton in thalassemic red blood cells. Blood **86:**3945–3950, 1995.
58. Grinberg LN, Rachmilewitz EA, Kitrossky N, Chevion M. Hydroxyl radical generation in β-thalassemia red blood cells. Free Radic Biol Med **18:**611–615, 1995.
59. Arese P, Deflora A. Pathophysiology of hemolysis in glucose-6-phosphate dehydrogenase deficiency. Semin Hematol **27:**1–40, 1990.
60. Liu TZ, Lin TF, Hung IJ, Wei JS, Chiu DTY. Enhanced susceptibility of erythrocytes deficient in glucose-6-phosphate dehydrogenase to alloxan-, GSH-induced decrease in red cell deformability. Life Sci **55:**PL55–60, 1994.
61. Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L. Targeted disruption of the housekeeping gene encoding glucose-6-phosphate dehydrogenase: G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. EMBO J **14:**5209–5215, 1995.
62. Heyman MB, Vichinsky E, Katz R, Gaffield B, Hurst D, Castillo R, Chiu D, Kleman K, Ammann AJ, Thaler MM, Lubin B. Growth retardation in sickle-cell disease treated by nutritional support. Lancet **1:**903–906, 1985.
63. Reed JD, Redding-Lallinger R, Orringer EP. Nutrition and sickle cell disease. Am J Hematol **24:**441–455, 1987.
64. Williams R, George EO, Wang W. Nutrition assessment in children with sickle cell disease. J Assoc Acad Minor Phys **8:**44–48, 1997.
65. Salman EK, Haymond MW, Bayne E, Sager BK, Wiisanen A, Pitel P, Darmaun D. Protein and energy metabolism in prepubertal children with sickle cell anemia. Pediatr Res **40:**34–40, 1996.
66. Kopp-Hoolihan LE, van Loan MD, Menrzer WC, Heyman MB. Elevated resting energy expenditure in adolescents with sickle cell anemia. J Am Diet Assoc **99:**195–199, 1999.
67. Moore C, Ehlayel M, Inostroza J, Leiva LE, Kuvibidila S, Yu L, Gardner R, Ode DL, Warrier R, Sorensen RU. Increased circulating levels of soluble HLA class-I heterodimers in patients with sickle cell anemia. J Natl Med Assoc **90:**157–163, 1998.
68. Rachmilewitz EA, Shifter A, Kahane I. Vitamin E deficiency in β-thalassemia major: Changes in hematological and biochemical parameters after a therapeutic trial with α-tocopherol. Am J Clin Nutr **32:**1850–1858, 1979.
69. Phillips G, Tangney CC. Relationship of plasma α-tocopherol to index of clinical severity in individuals with sickle cell anemia. Am J Hematol **41:**227–231, 1992.

70. Bowie LJ, Carreathers SA, Wright AG. Lipid peroxidation, vitamin E, and the generation of irreversibly sickled cells in sickle cell anemia. Clin Chem **25:**1076–1084, 1979.
71. Chiu D, Vichinsky E, Yee M, Kleman K, Lubin BH. Peroxidation, vitamin E, and sickle cell anemia. Ann N Y Acad Sci **82:**323–335, 1982.
72. Leonard PJ, Losowsky MS. Effect of α-tocopherol administration on red cell survival in vitamin E–deficient subjects. Am J Clin Nutr **24:**388–393, 1971.
73. Natta C, Machlin L, Brin M. A decrease in irreversibly sickled erythrocytes in sickle cell anemia patients given vitamin E. Am J Clin Nutr **33:**968–971, 1980.
74. Hafez M, Amar ES, Zedan M, Hammad H, Sorour AH, el-Desouky ES, Gamil N. Improved erythrocyte survival with combined vitamin E and selenium therapy in children with glucose-6-phosphate dehydrogenase deficiency and mild chronic hemolysis. J Pediatr **108:**558–561, 1986.
75. Corash L, Spielberg S, Bartsocas C, Boxer L, Steinherz R, Sheetz M, Egan M, Schlessleman J, Schulman JD. Reduced chronic hemolysis during high-dose vitamin E administration in Mediterranean-type glucose-6-phosphate dehydrogenase deficiency. New Engl J Med **303:**416–420, 1980.
76. Horwitt MK, Harvey CC, Duncan GD, Wilson WC. Effects of limited tocopherol intake in man with relationships to erythrocyte hemolysis and lipid oxidations. Am J Clin Nutr **4:**408–419, 1956.
77. Horwitt MK. Vitamin E and lipid metabolism in man. Am J Clin Nutr **8:**451–461, 1960.
78. Farrell PM, Bieri JG, Fratantoni JF, Wood RE, di Sant'Agnese PA. The occurrence and effects of human vitamin E deficiency: A study in patients with cystic fibrosis. J Clin Invest **60:**233–241, 1977.
79. Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, D'Arpa D, D'Anna R, Bongiorno A. Oxidative stress and antioxidant status in β-thalassemia major: Iron overload and depletion of lipid-soluble antioxidants. Blood **88:**3608–3614, 1996.
80. Lachant NA, Davidson WD, Tanaka KR. Impaired pentose phosphate shunt function in sickle cell disease: A potential mechanism for increased Heinz body formation and membrane lipid peroxidation. Am J Hematol **15:**1–13, 1983.
81. Bozzi A, Parisi M, Strom R. Erythrocyte glutathione determination in the diagnosis of glucose-6-phosphate-dehydrogenase deficiency. Biochem Mol Biol Int **40:**561–569, 1996.
82. Mareni C, Gaetani GF. $NADP^+$ and NADPH in glucose-6-phosphate dehydrogenase-deficient erythrocytes under oxidative stimulation. Biochim Biophys Acta **430:**395–398, 1976.
83. Jain SK. Glutathione and glucose-6-phosphate-dehydrogenase deficiency can increase protein glycation. Free Radic Biol Med **24:**197–201, 1998.
84. Wetterstoem N, Brewer GJ, Warth JA, Mitchinson A, Near K. Relationship of glutathione levels and Heinz body formation to irreversibly sickle cells in sickle cell anemia. J Lab Clin Med **103:**589–596, 1984.
85. Steinberg D. Low-density lipoprotein oxidation and its pathobiological significance. J Biol Chem **272:**20963–20966, 1977.
86. Chan AC. Vitamin E and atherosclerosis. J Nutr **1128:**1593–1596, 1998.
87. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of LDL by endothelial cells involves lipid peroxidation and degradation of LDL phospholipids. Proc Natl Acad Sci U S A **81:**3883–3887, 1984.
88. Dieber-Rotheneder M, Puhl H, Waeg G, Striegl G, Esterbauer H. Effect of oral supplementation with d-α-tocopherol on the vitamin E content of human LDL and resistance to oxidation. J Lipid Res **32:**1325–1332, 1991.
89. Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL. Effect of dietary antioxidant combination in humans: Protection of LDL by vitamin E but not with β-carotene. Arterioscler Thromb Vasc Biol **13:**590–600, 1993.
90. Jialal I, Fuller CJ, Huet BA. The effects of α-tocopherol supplementation on LDL oxidation: A dose-response study. Arterioscler Thromb Vasc Biol **15:**190–198, 1995.
91. Hirano R, Kondo K, Iwamoto T, Igarashi O, Itakura H. Effects of antioxidants on the oxidative susceptibility of LDL. J Nutr Sci Vitaminol **43:**435–444, 1997.
92. Martin A, Frei B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. Arterioscler Thromb Vasc Biol **17:**1583–1590, 1997.
93. Retsky KL, Chen K, Zeind J, Frei B. Inhibition of copper-induced LDL oxidation by vitamin C is associated with decreased copper binding to LDL and 2-oxo-histidine formation. Free Radic Biol Med **26:**90–98, 1999.
94. Tesoriere L, D'Arpa D, Maggio A, Giaccone E, Livrea MA. Oxidative resistance of LDL is correlated with vitamin E status in Italy. Atherosclerosis **137:**429–435, 1998.
95. Livrea MA, Tesoriere L, Maggio A, D'Arpa D, Pintaudi AM, Pedone E. Oxidative modification of LDL and atherogenic risk in β-thalassemia. Blood **92:**3936–3942, 1998.
96. Tangney CC, Philips G, Bell RA, Fernandes P, Hopkin R, Wu SM. Selected indices of micronutrient status in adult patients with sickle cell anemia. Am J Hematol **32:**161–166, 1989.
97. Natta C, Machlin L. Plasma levels of tocopherol in sickle cell anemia subjects. Am J Clin Nutr **32:**1359–1362, 1979.
98. Chiu D, Lubin B. Abnormal vitamin E and glutathione peroxidase levels in sickle cell anemia: Evidence for increased susceptibility to lipid peroxidation *in vivo*. J Lab Clin Med **94:**542–548, 1979.
99. Chiu DT-Y, Chang M-L. Blood vitamin C in human glucose-6-phosphate dehydrogenase deficiency. In: Packer L, Fuchs J, Eds. Vitamin C in Health and Disease. New York: Marcel Dekker Inc., pp323–331, 1997.
100. Rachmilewitz EA, Shohet SB, Rubin BH. Lipid membrane peroxidation in β-thalassemia major. Blood **47:**495–505, 1976.
101. Rachmilewitz EA, Kornberg A, Acker M. Vitamin E deficiency due to increased consumption in β-thalassemia and in Gaucher's disease. Ann N Y Acad Sci **393:**336–347, 1982.
102. Zannos-Mariolea L, Yzortzatou F, Dendaki-Svolaki K, Katerellos C, Kavaliari M, Matsaniotis M. Serum vitamin E levels with β-thalassemia major: Preliminary report. Br J Haematol **26:**193–199, 1974.
103. Zannos-Mariolea L, Papagregoriou-Theodoridou M, Costantzas N, Matsaniotis N. Relationship between tocopherols and serum lipid levels in children with β-thalassemia major. Am J Clin Nutr **31:**259–262, 1978.
104. Giardini O, Cantani A, Donfrancesco A. Vitamin E therapy in homozygous β-thalassemia. New Engl J Med **305:**644, 1981.
105. Giardini O, Cantani A, Donfrancesco A, Martino F, Mannarino O, D'Eufemia P, Miano C, Ruberto U, Lubrano R. Biochemical and clinical effects of vitamin E administration in homozygous β-thalassemia. Acta Vitaminol Enzymol **7:**55–60, 1985.
106. Chantharaksri U, Tonsuwonnont W, Fucharoen S, Wasi P. Pathogenesis of hypoxemia. Southeast Asian J Trop Med Public Health **23**(Suppl 2):32–35, 1992.
107. Suthutvoravut U, Hathirat P, Sirichakwal W, Tassaneeyakul A, Feungpean B. Vitamin E status, glutathione peroxidase activity, and the effect of vitamin E supplementation in children with thalassemia. J Med Asso Thailand **76**(Suppl 2):146–152, 1993.
108. Kahane I, Rachmilewitz EA. Alterations in the red blood cell membrane and the effect of vitamin E on osmotic fragility in β-thalassemia major. Israel J Med Sci **12:**11–15, 1976.
109. Miniero R, Canducci E, Ghigo D, Saracco P, Vullo C. Vitamin E in β-thalassemia. Acta Vitamin Enzymol **4:**21–25, 1982.
110. Natta C, Chen LC, Chow CK. Selenium and glutathione peroxidase levels in sickle cell anemia. Acta Haematol **83:**130–132, 1990.
111. Zimmerman CP, Natta C. Glutathione peroxidase activity in whole blood of patients with sickle cell amenia. Scand J Haematol **26:**177–181, 1981.
112. Beutler E, Matsumoto F, Powars D, Warner J. Increased glutathione peroxidase activity in α-thalassemia. Blood **50:**647–655, 1997.
113. Gerli GC, Beretta L, Bianchi M, Agostoni A, Gualadri V, Orsini GB. Erythrocyte superoxide dismutase, catalase, and glutathione peroxidase in glucose-6-phosphate-dehydrogenase deficiency. Scand J Haematol **29:**135–140, 1982.
114. Jain SK, Willaims DW. Reduced levels of plasma ascorbate in sickle cell patients: Its possible role in oxidative damage to sickle cells *in vivo*. Clin Chim Acta **149:**257–264, 1985.
115. Chiu D, Vichinsky E, Ho SL, Liu T, Lubin BH. Vitamin C deficiency in patients with sickle cell anemia. Am J Pediatr Hematol-Oncol **12:**262–267, 1990.
116. Michelson J, Cohen A. Incidence and treatment of fractures in thalassemia. J Orthop Trauma **2:**29–32, 1988.
117. Natta C, Stacewiecz-Sapuntakis M, Bhagavan H, Bowen P. Low

serum levels of carotenoids in sickle cell anemia. Eur J Haematol **41:**131–135, 1988.
118. Magnani M, Stocchi V, Canestrari F, Cucchiarini L, Stocchi O, Coppa GV, Felici L, Giorgi PL, Fornaini G. Redox and energetic state of red blood cells in G6PD deficiency, heterozygous β-thalassemia and the combination of both. Acta Haematol **75:**211–214, 1986.
119. Johnson RM, Ravindranath Y, el-Alfy M, Goyette G. Oxidative damage to erythrocyte membrane in glucose-6-phosphate-dehydrogenase deficiency: Correlation with *in vivo* reduced glutathione concentration and membrane protein oxidation. Blood **83:**1117–1123, 1994.
120. Kajanachumpol S, Tatu T, Sasanakul W, Chuansumrit A, Hathirat P. Zinc and copper status of thalassemic children. Southeast Asian J Trop Med Public Health **28:**877–880, 1997.
121. Chan AC. Partners in defense: Vitamin C and vitamin E. Can J Physiol Pharmacol **71:**725–731, 1993.