Chapter-II

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I. VIEWS REGARDING OXIDATIVE DAMAGE AND EXERCISE

Exercise is considered to be a “double-edged sword”. Regarding the adverse effects of exercise on health, general belief runs – musculo-skeletal injuries may result from excessive or careless physical activity. Also, adverse cardiovascular events can occur during exercise, particularly if a physically unfit individual is performing strenuous exercise. High levels of exercise may decrease an individual’s resistance to infection. The problem observed most often in individuals who exercise is fluid imbalance, where the individuals simply do not consume sufficient amounts of fluids (primarily water).

Research over the past 2 decades has shown that O$_2$ free radical generation is a major cause for cell and tissue injury associated with rigorous physical exertion (Ji, 2000). Furthermore, certain antioxidants such as GSH may be transported between organs (Deneke and Fanburg, 1989). In general, the cell has adequate antioxidant reserve to cope with mild oxidative stress so that serious and long-term damage does not occur. The protective margin of most antioxidants is relatively small, however. Therefore, when ROS production is excessive, or when antioxidant defense is compromised because of inactivation or nutritional deficiency, extensive cell and tissue damage may occur, leading to various pathogenic disorders (Halliwell and Gutteridge, 1989). The resulting oxidative damage can induce further ROS production, thus forming a vicious cycle. There is increasing evidence that unaccustomed and strenuous exercise may inflict an imbalance between ROS and antioxidant defenses in favour of the former (Sen, 1995). This disturbance of antioxidant homeostasis is implied in numerous physiological disorders occurring during and after exercise, such as fatigue, muscle soreness, myofibril disruption and impairment of immune function (Reid et al., 1992).

The hypothesis that mitochondria comprise a primary site of ROS generation during exercise has been implicated in numerous studies. By using isolated mitochondrial preparations, several authors showed that state 4 respiration is increased in muscle and liver mitochondria and heart mitochondria after exhaustive exercise, suggesting a possible inner-membrane leakage inflicted by ROS (Ji, 2000).
Strenuous physical exercise is capable of inducing oxidative stress (Jenkins, 1993; Sen et al., 1994), a state wherein the production of ROS in the body transcends the antioxidant defense capacity. ROS are known to have a wide variety of pathophysiological implications (Kehrer, 1993). It has also been suggested that a high concentration of ROS may contribute to oxidative skeletal muscle fatigue (Barclay and Hansel, 1991). A large number of recent studies have been directed towards the management of exercise-induced oxidative stress. Antioxidant supplementation studies have revealed a beneficial trend (Jenkins, 1993; Sen et al., 1994).

The hypothesis that increased O₂ influx, which is known to occur during moderate and exhaustive exercise, may be potentially toxic to the body, is authenticated by a large number of studies, some of which have been recently reviewed (Alessio, 1993). The association between exercise intensity and related O₂ toxicity in humans is a fundamental problem that is yet to be pertinently addressed.

Endurance exercise may contribute to a two- to three-fold increase in free radical concentrations of the muscle and liver (Davies et al., 1982), which may result in a considerable amount of histological disintegration. Such high concentration of reactive metabolites may contribute to oxidative skeletal muscle fatigue (Barclay and Hansel, 1991).

Most organisms use O₂ as the terminal electron acceptor to oxidize the various metabolic fuels so that stored energy is released for various biologic activities (Halliwell and Gutteridge, 1989). During the process, which occurs primarily in the mitochondria of eukaryotic cells, most O₂ molecules are reduced to water. However, molecular O₂ cannot accept four electrons required for its complete reduction at once because of the spin-restriction rule; instead, it has to take one electron at a time. This process gives rise to several univalently reduced O₂ intermediates and their protonated derivatives. The main species are superoxide (O₂⁻'), hydrogen peroxide (H₂O₂), and hydroxyl radical (·OH), representing one-, two- and three-electron reductants of O₂, respectively (Halliwell and Gutteridge, 1989). All three ROS have a strong tendency to extract electrons to reach a chemically more stable state and therefore are capable of eliciting serious damage to the cellular components, but their chemical properties and detrimental
potential are different. *OH is the most reactive ROS and attacks all biologic materials at a diffusion-limited rate.

In addition to the mitochondrial respiratory chain, cells generate ROS to assist in the elimination of xenobiotics through phagocytosis that involves a respiratory burst (Cannon and Blumberg, 1994). Although this process is generally considered desirable, it can indiscriminately subject the cell to potential oxidative damage (Meydani and Evans, 1993). In addition, O₂ serves as an electron acceptor for several other metabolic pathways in the cell such as purine nucleotide degradation, D-amino acid oxidation, cytochrome P₄₅₀, and catecholamine autooxidation. It is estimated that a normal cell produces 2 × 10¹⁰ O₂* and H₂O₂ per day, which amounts to 3.3 × 10¹⁴ moles (Ames et al., 1995). The constant contact and reaction of the cellular constituents (including genetic materials) with ROS have been proposed to be a main mechanism for organism aging.

The concept that ROS may play an important role in exercise-induced tissue damage occurred in the late 1970s and early 1980s (Dillard et al., 1978). It is now widely accepted that many of the disorders at the cell, tissue, or organ levels observed either immediately after heavy exercise or during post-exercise recovery may be attributed to ROS generation (Meydani and Evans, 1993). By using electron paramagnetic resonance (EPR) spectroscopy as a tool, several authors have provided direct data showing that exercised muscle and heart tissues produce higher levels of free radicals than do those in rested controls (Davies et al., 1982). By using a synthetic intracellular probe dichlorofluorescin (DCFH), Reid and colleagues (Reid et al., 1994) have demonstrated that contracting diaphragm muscle generates O₂*, which is released from muscle cell. Both muscle and heart homogenates from acutely exercised rats exhibit a higher rate of DCFH oxidation than do those from their rested counterparts (Bejma and Ji, 1999). The biochemical mechanism(s) by which ROS production is enhanced during exercise is still largely hypothetical, however. Several biochemical pathways, which may be activated under different physiological conditions and in different organs, tissues, and cellular locations, have been either identified or postulated.
II. VIEW REGARDING ANTIOXIDANT VITAMINS AND ITS FEEDBACK

Vitamin E, C and β-carotene play a critical role in protecting the cells from ROS-induced oxidative stress (Yu, 1994). Because humans cannot synthesize these vital antioxidants, they are dependent exclusively on dietary intake. Recent research suggests that several other low molecular weight compounds, such as uric acid, also serve important antioxidant functions (Yu, 1994).

Recent research has already ensured that antioxidant supplementation is likely to provide beneficial effect against exercise-induced oxidative tissue damage (Sen, 2001). Again, another study (Sachek and Blumberg, 2001) showed that dietary vitamin E supplementation reduce exercise-induced muscle injury and improvement in performance.

a. Vitamin E

Evans and Bishop first discovered Vitamin E in the early 1920s as a fat-soluble nutritional factor that was found to be essential for normal reproduction in rats. It was later isolated as a closely related family of compounds, designated as tocopherols. Soon afterwards the detection of peroxides in the adipose tissue of animals fed diets deficient in vitamin E and the demonstration that several synthetic antioxidants prevent vitamin E deficiency signs in animals resulted in an "antioxidant" theory of vitamin E function (Deshpande et al., 1996).

Although a short-term deficiency of vitamin E in the human diet does not cause any specific deficiency disease, an increasing body of scientific literature during the past two decades has shown vitamin E to be an essential vitamin in both animals and humans. In biological systems, vitamin E plays an important role against free-radical-mediated cellular injuries and in the enhancement of immune response as well as a preventive role against several cancers, cardiovascular diseases, cataract, and Parkinson’s disease.

Vitamin E is practically insoluble in water but is completely soluble in oils, fats, acetone, alcohol, chloroform, ether, benzene, and other nonpolar solvents (Deshpande
et al., 1996). Any significant structural changes in α-tocopherol, including the addition of a double bond to the side chain, shortening or lengthening of the side chain, masking of the phenolic hydroxyl group as an ether, and loss of any methyl group from or the oxidation of the chromanol ring, result in major loss of its biological activity. However, the substitution of an amine or N-methylamine for the phenolic hydroxyl or that of a dihydrobenzofuran for the chromanol ring appears to enhance the activity of tocopherols.

Regarding the effects of physical exercise and vitamin E supplementation on the tissue oxidative metabolism, Quintanilha et al., 1982, reported three major findings:

1) The nuclear membrane appears to be far more sensitive to in vivo peroxidation than microsomal membranes when animals are made vitamin E deficient.

2) Endurance exercise training seems to lead to an increase in cytoplasmic levels of antioxidative enzymes (SOD, glutathione peroxidase and reductase, catalase) in both the heart and skeletal muscle, but not in lung or liver. It is possible that this may be due to the fact that more oxidative damage is likely to occur in the former tissues and therefore higher protection is required.

3) There is an increased requirement for vitamin E during endurance exercise training. Also, it appears that training induces a protective effect against erythrocyte hemolysis despite vitamin E deficiency.

Again in 1986, Lang et al., showed ionic correlation between tocopherol, ubiquinol and ubiquinone in blood and other tissue fluids, which was a path-finder in analyzing viable antioxidant status.

In 1987, Gohil et al., specifically highlighted the impact of exercise training on tissue vitamin E and ubiquinone content. The results suggested that training causes a substantial decrease in vitamin E concentration in the proliferating muscle mitochondrial membrane, thus depleting muscle mitochondria of their major lipid antioxidant. It is known that ubiquinone is located in the inner mitochondrial membrane and vitamin E is also related to mitochondrial membrane. Since vitamin E is the major cellular lipid-
soluble chain breaking antioxidant, these findings indicated increased free radical reactions in the tissues of exercising muscles.

Conversely, regarding the influence of vitamin E on physical performance, Schnass and Pabst, 1987, further pointed out that there are two properties of vitamin E related to better physical performance. On one side it promotes an economical energy metabolism, on the other side it acts as a stabilizing antioxidant in the membrane. Anaerobic threshold and exercise-induced oxidative stress parameters were assessed and they concluded that vitamin E has a beneficial effect on physical performance and on cell protection at least at high altitude.

In 1999, Chatterjee and Bagri showed some positive results of vitamin E supplementation on endurance exercise induced oxidative damage in sedentary females and highlighted its protective role.

In 1999, Sharma et al. conclusively showed the role of vitamin E as a protective factor in experimental atherosclerosis in rhesus monkey. The study suggested that hypercholesterolemia increases the level of lipid peroxidation product (MDA) in platelets, concomitant with the development of atherosclerosis. Vitamin E supplementation at a higher dose can act as a protective agent against the damage. Vitamin E, not only reduces the blood cholesterol level, but it may also act as a chain-breaking antioxidant.

b. Vitamin C

According to Moser and Bendich, nature perhaps gave up ascorbic acid synthesis in higher primates, some fishes and some birds in order to conserve glucose, the precursor of vitamin C. This would mean that a significant amount of glucose, a major source of energy, would be conserved in species that cannot synthesize ascorbic acid.

In 1979, Bodannes and Chan found out the scavenger role of ascorbic acid against singlet O₂. Again in 1989, Frei et al., proved that ascorbate is an outstanding antioxidant in human blood plasma. Of late in the year 2000, Goldenburg et al., clearly
showed the functions of vitamin C as a mediator of transmembrane electron transport in blood cells and related cell culture models. In light of the well-known antioxidant properties of ascorbate (Frei et al., 1989), it is noteworthy that the U.S. recommended daily allowance (RDA) for ascorbate is based exclusively on its function in collagen synthesis (its anti-scorbutic effect) and not on its antioxidant activity.

Under oxidant stress, ascorbate is an outstandingly effective antioxidant: it not only completely protects the lipids from detectable peroxidative damage, but also spares α-tocopherol, urate, or bilirubin (Frei et al., 1989). Plasma ascorbate belongs to the first line of antioxidant defense against lipid-soluble peroxyl radicals.

It has been shown recently (Frei et al., 1989) that in plasma exposed to a water-soluble radical initiator or the oxidants generated by polymorphonuclear leukocytes, no lipid peroxidation can be detected as long as ascorbate is present.

This strongly suggested, yet did not prove, that in plasma ascorbate is capable of completely protecting the lipids against detectable peroxidative damage. During the phase of no detectable lipid peroxidation, not only ascorbate but also part of the plasma proteins thiols were oxidized, suggesting that they also provide very effective antioxidant protection. Plasma ascorbate is indeed an outstandingly effective scavenger of aqueous peroxyl radicals, much more effective than any of the other endogenous antioxidants, and that the protein thiols have only very limited radical-trapping capabilities. Ascorbate proved to be solely protective rather than acting as a prooxidant, providing strictly increased benefit with increased concentrations.

c. β-carotene

β-carotene is a member of the family of carotenoids, which are pigments found in all photosynthesizing organisms including plants and bacteria. Of the approximately 50 carotenoids with provitamin A activity, β-carotene is the carotenoid with the greatest provitamin A activity (Bendich, 1988).

Olson recently classified carotenoids into four distinct types as follows (Deshpande et al., 1996):
Type-1 Biologically and nutritionally active (e.g., \(\beta\)-carotene).

Type-2 Biologically active but nutritionally inactive, at least in mammals (e.g., canthaxanthin, lycopene, violaxanthin).

Type-3 Biologically inactive but nutritionally active (e.g., \(\beta\)-apo-14'-carotenol).

Type-4 Biologically and nutritionally inactive (e.g., phytoene).

The actions are defined in terms of physiological or pharmacological responses to the administration of carotenoids. The response itself may be their beneficial or adverse and may not be essential for physiological well-being. In contrast, the biological associations involve the possibility of a definitive relationship between carotenoids and some physiological or medical events such as the risk of a certain type of cancer. In terms of free-radical pathology, the most important biological functions of carotenoids appear to be their antioxidant nature, their ability to quench singlet \(O_2\), and possible roles in enhancing the immune responses and the inhibition of mutagenesis.

The mechanisms by which \(\beta\)-carotene acts as an antioxidant have been investigated (Deshpande et al., 1996). Unlike antioxidants that prevent the initiation of lipid peroxidation, \(\beta\)-carotene stops the chain reactions by trapping free radicals. It therefore appears to act as a chain-breaking antioxidant, although it does not have the characteristic structural features associated with this class of antioxidants. \(\beta\)-carotene, in fact, has an extensive system of conjugated double bonds that impart a prooxidant character to the molecule. It is therefore very susceptible to attack by peroxyl radicals. In \textit{in vitro} systems, \(\beta\)-carotene interacts irreversibly with peroxyl radicals to form a carbon-centered carotenoid radical.

The reactivity of \(\beta\)-carotene toward peroxyl radicals and the stability of the resulting \(\beta\)-carotene radical give the molecule its antioxidant capability.

\(\beta\)-Carotene, however, does not behave as an antioxidant at normal \(O_2\) concentrations. The antioxidant function of \(\beta\)-carotene therefore complements the action of other antioxidant molecules such as catalase, GSH-Px, vitamin C, and vitamin E,
which are very effective at normal $O_2$ concentrations. Vitamin A, in contrast, is a very weak antioxidant.

The ability of carotenoids to quench singlet $O_2$ is related to the number of double bonds they contain. It was later confirmed that carotenoids with 9, 10 and 11 conjugated double bonds are better quenchers of singlet $O_2$ than those with eight or fewer conjugated double bonds (Deshpande et al., 1996). One molecule of $\beta$-carotene, for example, is estimated to quench up to 1000 molecules of singlet $O_2$.

**Immune Functions**

The antitumor and antioxidant effects of carotenoids, coupled with the fact that vitamin A is a relatively poor antioxidant and cannot quench singlet $O_2$, may very well explain the epidemiological data linking lower carotenoid status with higher incidences of certain cancers (Deshpande et al., 1996).

More definitive studies detailing these *in vivo* functions of carotenoids are being carried out, especially considering the fact that $\beta$-carotene, unlike vitamin A, has proven to be nontoxic to humans when given in high dosages.

Within the last decade, there has been an increasing number of epidemiological studies that have associated low dietary and/or plasma levels of carotenoids with higher incidences of certain cancers. In this perspective, intervention trials, the level of $\beta$-carotene supplementation usually ranges from 15 to 50 mg/day (approx. 10-30 times the usual intake level). It is assumed that the overall utilization of $\beta$-carotene as a source of vitamin A is one-sixth that of retinol (Bendich, 1988).

More recently, there have been strong indications that, in addition to serving as precursors of vitamin A, carotenoids may otherwise be of importance, because humans accumulate carotenoids as well as retinol in serum and tissues. In fact, $\beta$-carotene, but not retinol, can serve as an antioxidant (Burton and Ingold, 1984) as well as a potent quencher of the highly reactive molecular species, singlet $O_2$ (Foote et al., 1970). Carotenes are stored in human fat and are found in most organs and tissues, including the epidermal and dermal layers of the skin and in platelets and red and white blood
cells. The uptake and depletion rates of each tissue were unique, suggesting that certain tissues may have a greater requirement for β-carotene than others (Shapiro et al., 1984). It is also generally recognized as safe both as a dietary supplement and as a nutrient (Bendich, 1988). The administration of large doses of β-carotene to four successive generations of rats had no deleterious effects on the rate of growth, food consumption, white blood cell count, or other blood components or reproductive functions (Bagdon et al., 1960). It was shown that β-carotene had no mutagenic properties (Heywood et al., 1985).

β-carotene has been successfully used to treat people with genetically inherited photosensitivities for more than 15 years. The principal clinical investigator, Dr. M.M. Mathews Roth, stated that "the ingestion of large amounts of pure β-carotene has not produced toxic side effects" (Mathews-Roth, 1981).

In conclusion, daily supplementation with high doses of β-carotene, for over 15 years at levels approximately 60 times the normal intake level, has not been associated with any adverse effects. The only side effect is the hypercarotenodermia, which is reversible when supplementation is discontinued.

III. VIEWS REGARDING LIPID PROFILE, LIPID PEROXIDATION AND EXERCISE STRESS

The effect of exercise on blood-lipids and lipoproteins: a metaanalysis of studies” – by Zung, Vu, Tran, Arthur Weltman, Gene. V. Glass and Dale. P. Mood – a unique paper published in 1983, in "Medicine and Science in Sports and Exercise" Vol.-15, No. 5, pp.393-402 – is considered to be the pathfinder work in this aspect of study, where a cumulative cohesive impact of exercise and smoking and blood-lipid profiles were assessed.

A lot of research have already been performed (Paul, 1973) in this perspective. The story includes a series of meta-analytical studies which indicates the other side of the effect of blood lipids and lipo-proteins. For many years, it has been suggested that elevated levels of serum cholesterol are a primary risk factor in the development of both atherosclerosis and coronary artery diseases (Tran et al., 1983). Recent data (Miller et
 indicate that the manner in which cholesterol is transported in the blood may be critical in the development of CAD than are total blood cholesterol concentration. There is evidence showing LDL-C does this job of transport, while cholesterol carried by HDL-C may be transported from the arterial wall to the liver for catabolism and excretion (Miller et al., 1977) and thus have a protective effect. Several epidemiological studies support this concept which indicate that populations with higher levels of HDL-C and/or decreased level of LDL-C have a lower incidence of CAD (Tran et al., 1983). Based on the above evidences, it has been proposed that the development of atherosclerosis may be more successfully prevented by increasing serum HDL-C and/or decreasing serum LDL-C than by the attempts to decrease the total serum cholesterol (Miller et al., 1977).

Sutherland et al., 1986, have put forward the effect of physical training on plasma lipoproteins and cholesterol in men with hypertriglyceridemia. The relationship with plasma cholesterol, total lipid peroxidation and work have been studied by Ledwoz Y.W. and coworkers, 1983.

Regarding lipid profile and exercise, some recent information showed that LDL-C is known to bind to arterial wall proteoglycan to produce atherosclerosis as a result of cholesterol deposition on arterial walls (Anber et al., 1996).

Again, HDL-C is the acceptor of cellular face cholesterol and acts as a scavenger of this same, and prevents the above process (Sasahara et al., 1997).

Spate et al. (1999) carried out an experiment and the results indicated that there was a significant increase in VO2max in high intensity and moderate intensity exercise group. The results further indicated that moderate intensity exercise was no less than heavy intensity exercise in elevating HDL-C profile.

Oral supplementation of vitamin E and its impact on the level and distribution of different components of lipid profile showed that total LDL-C, TG and chemical compositions of HDL-C, LDL-C and VLDL-C were not altered (Perugini et al., 2000).

Regular exercise appears to produce possible beneficial changes in plasma lipids and lipoproteins (Ballantyne et al., 1981; Moffatt and Gilliam, 1979).
In 1998, R. Niaura and others investigated the effect of exercise, smoking cessation and short term changes in serum lipids in women. Exercise training magnifies the increase in HDL-C that usually occurs with smoking cessation.

In another relevant study by N.E. Miller and others, 1977, it has been found to correlate the relationship of future coronary heart disease (CHD) to the plasma HDL-C concentration.

Grobusch and others, 1999, concluded that the dietary \( \beta \)-carotene, vitamin C and vitamin E (all anti-oxidants) were related to the risk of MI in the elderly population.

Letters et al., 1999, correlated lipid peroxidation in plasma and occurrence of atherosclerosis. Oxidation of lipoproteins is thought to be an early event in atherogenesis.

Manjunatha et al., 2001, showed the effect of Chyawanprash and Vitamin C on glucose tolerance and lipoprotein profile. Vitamin C supplementation had a favourable effect on serum lipid profile.

Lipid peroxidation is mainly studied in isolated microsomes from the liver. Initiation and propagation of lipid peroxidation are catalyzed by iron and microsomal NADPH-cytochrome P-450 reductase (Deshpande et al., 1996).

It is likely that cytochrome P-450 is also involved in this reduction reaction in whole microsomes. However, according to Davis et al., 1982, this is not the case in reconstituted systems and the reductase alone is capable of catalyzing the peroxidation of the lipids in liposomes.

Researchers have yet to ascertain if microsomal lipid peroxidation is induced by hydroxyl radicals derived from the Haber-Weiss reaction or iron-\( \text{O}_2 \) complexes (Halliwell and Gutteridge, 1989).

This difference of opinion appears to be due to the site-specific formation of a reactive species in the membrane or a membrane-like structure. Although xanthine oxidase, which forms superoxide anions, catalyzes lipid peroxidation, the reactive
species initiating lipid peroxidation are unknown. However, the mitochondrial process has not been investigated as systematically as the corresponding microsomal process.

Lipid peroxidation occurs during exercise and that it is attenuated by Vitamin E (Dillard et al., 1978).

The induction of lipid peroxidation by NADPH or ascorbate-reduced iron depends on the presence of free iron, i.e., iron that is not incorporated into protein. Hence, it is pertinent to consider whether such iron exists inside the cell (Horton and Fairhurst, 1987).

The antioxidant vitamin E is presumably present in both membranes unless deficient in diet. Toxic substances could promote lipid peroxidation in mitochondrial membranes by overwhelming or depleting the antioxidant defenses of the cytosol, intermembrane space, and matrix. The outer membrane may become susceptible to peroxidative attack before the inner membrane is affected.

The process of lipid peroxidation can have a variety of detrimental effects on the cell. Cross-linking of protein in this manner inactivates enzymes and produces high molecular weight fluorescent aggregates known variously as ceroid, lipofuscin, or age pigment. A number of reviews of lipid peroxidation in relation to cell damage have been published (Horton and Fairhurst, 1987).

The organism has ways of protecting its membranes from potential peroxidative attacks.

The tocopherols are normal components of most mammalian cell membranes and \( \alpha \)-tocopherol (vitamin E) is the most common form (Horton and Fairhurst, 1987).

It is evident then that the nature of GSH-utilizing antiperoxidation activity and the site of action of the enzyme(s) in the chain of peroxidation reactions are problems that remain to be resolved.

Several hepatotoxic compounds have been shown to deplete GSH and, as a result, enhance lipid peroxidation in mammalian hepatocytes (Horton and Fairhurst,
1987). Lipid peroxidation is believed to arise because of increased peroxisomal $H_2O_2$ generation and decreased peroxidation defenses.

**Wefers and Sies, 1988** suggested that the ascorbate-mediated protection of microsomal membranes against lipid peroxidation is dependent on vitamin E in the membrane. In vitamin E deficiency the pro-oxidant effect of ascorbate was abolished when glutathione (GSH) was present.

In the presence of both ascorbate and GSH the chain-breaking activity of Vitamin E is maintained predominantly by ascorbate, while GSH predominantly acts as a preventive antioxidant. Under these conditions, even low amounts of vitamin E together with the reducing capacity of ascorbate seem to provide sufficient chain-breaking activity to protect against lipid peroxidation.

According to Frei *et al.*, 1988, antioxidant defenses mainly Vitamin C supplementation is helpful against lipid peroxidation in human blood plasma.

**Sumida *et al.*, 1989,** investigated the effect of exercise on lipid peroxidation and enzyme leakage due to Vitamin E supplementation and concluded that:

1. Increase of malondialdehyde after exercise before vitamin E supplementation was slight (but statistically significant), however after supplementation with vitamin E, malondialdehyde level after exercise was significantly decreased.

2. Leakage of enzyme was significantly increased after exercise before vitamin E supplementation, but it was lower following exercise after vitamin E supplementation.

3. Lipid peroxidation following a bout of acute heavy exercise can be inhibited by vitamin E supplementation.

**Kanter *et al.*, 1993,** showed that taking ascorbate, α-tocopherol, and β-carotene in clinical doses serves to lower markers of lipid peroxidation at rest and after exercise but does not prevent the exercise-induced increase in oxidative stress.
Alessio and Goldfarb (1988) showed that lipid peroxidation measured by thiobarbituric acid reactive substance (TBARS) correlated with treadmill workload in rats. Ji and colleagues, 1992, showed that the oxidation of GSH to glutathione disulfide (GSSG) in skeletal muscle increased as a function of treadmill speed and incline in rats. Furthermore, vitamin E administration attenuated urinary markers of lipid peroxidation found during the post-exercise period, indicative of the oxidative nature of the injury (Ji, 2000).

IV. VIEWS REGARDING SERUM GLUTATHIONE LEVEL—AN ESSENTIAL STRESS MARKER

Vitamin E is the most important natural antioxidant. It prevents oxidation of glutathione and the protein-SH group during the redox reaction (Costagliola et al., 1985). Glutathione is the main derivative of cysteine and the most abundant cellular thiol. One of the roles of glutathione could be to act as the first line of defense against oxidants. Low levels of glutathione and vitamin E in diabetes can be due to their exhaustion during detoxification of free radicals produced by cell membrane lipid peroxidation. Glutathione and vitamin E, both isolated or in combination, form a formidable defense against free radicals (Salonen et al., 1995). Vitamin E supplementation raised total glutathione levels. Since the use of vitamin E supplementation significantly decreased oxidative stress, a possible role of vitamin E supplementation is suggested in reducing free-radical induced oxidant injury in diabetes mellitus (Sharma et al., 2000).

Glutathione is a cofactor for several enzymes in widely different metabolic pathways including glyoxylase, maleylacetoacetate isomerase, prostaglandin endoperoxide isomerase, and DDT dehydrochlorinase. It also plays an important role in the synthesis of thyroid hormones, in the degradation of insulin in animals, and in the metabolism of herbicides, pesticides, and toxic "foreign" compounds, generally in both animal and plant tissues (Halliwell and Gutteridge, 1989).

However, glutathione is not essential for aerobic life; several aerobic bacteria do not contain it (Deshpande et al., 1996) and a lack of glutathione in animal cells causes haemolysis.
In contrast, the disulfide GSSG can inactivate a number of enzymes including aldehyde cyclase, chicken liver fatty acid synthetase, rabbit muscle phosphofructokinase, and phosphorylase phosphatase, probably by forming mixed disulfides with them. Such a situation occurs when a large flux of \( \text{H}_2\text{O}_2 \) and/or \( \text{HO}^+ \) radicals lowers the GSH/GSSG ratio in the cells. GSSG also inhibits protein synthesis in animals cells *(Halliwell and Gutteridge, 1989)*. Because of this, the cells presumably maintain a high GSII/GSSG ratio under normal conditions.

According to *Bounous, 2000*, glutathione antioxidant system is one of the most important member working against cellular protective mechanisms and also against cancer. 

Paterson *et al.*, 2001, showed that sulfur amino acid deficiency depresses brain glutathione concentration.

In 2001, Gambelunghe *et al.*, elucidated that physical exercise resulted in a dramatic decrease in glutathione plasma levels. During light physical exercise there is a low production of ROS with a low request for antioxidant defense such as oxidation of glutathione. The dramatic decrease observed in glutathione levels would indicate the presence of oxidative stress able to modify blood antioxidant profiles. Glutathione plays a central antioxidant role in blood during intensive physical exercise and that its modifications are closely related to exercise intensity.

Physiological thiols act as promoters of glutathione oxidation and modifying agents in protein S-thiolation *(Del Corso *et al.*, 2002)*. Glutathione is one of the most relevant antioxidants present in cells. It exerts its scavenging action through the involvement of efficient and ubiquitous enzymes. The study further resolves that glutathione, on the other hand, due to its chemical feature, can scavenge ROS without the involvement of enzymatic systems, rather, by using physiological thiols (i.e. cysteine and cysteinyl glycine) which are far more sensitive than glutathione to oxidative conditions. The entire study showed that there is a possibility that glutathione may be recruited in controlling cellular \( \text{O}_2 \) tension.
V. VIEWS REGARDING SERUM URIC ACID – A UNIQUE ENDOGENOUS ANTIOXIDANT

Uric acid is a by-product of purine metabolism and has usually been thought to be a waste product with no biological function (Halliwell and Gutteridge, 1989). Human tissues do not contain the enzyme urate oxidase. It therefore, accumulates in the body. Its normal blood plasma concentration in humans ranges from 0.25 to 0.45 mM.

Long back in 1977, Adamopoulos et al., showed the unique relationship of sex steroids and uric acid levels in plasma and urine. The study indicated that oestrogens may exert their action through a uricosuric agent in both the sexes, although not conclusively. But sex steroids do affect uric acid metabolism. On the other hand, progesterone exerts an action on plasma uric acid, similar to that of oestrogen in females.

Uric acid also interferes with radical reactions by binding iron and copper ions forms that do not participate in radical reactions. For example, it is a very powerful inhibitor of copper-dependent formation of HO* from H2O, apparently acting by binding the copper ions.

In 1981, Ames et al., hypothesized that uric acid provides an antioxidant defense in human against oxidant and radical-caused aging and cancer. They proposed plasma uric acid to be an important protective mechanism against O2 radicals. Uric acid is a powerful antioxidant and is a scavenger of singlet O2 and radicals. They showed that at physiological concentrations, urate reduces the oxo-haem oxidant formed by peroxide reaction with haemoglobin protects erythrocyte ghosts against lipid peroxidation and protects erythrocytes from peroxidative damage leading to lysis.

Of late, in 2002 Kuzuya et al., showed the effect of aging on serum uric acid level. The study pointed out that serum uric acid level in men and women increased with advancing age despite the changes in drinking and BMI.

Hink et al., 2002, showed the role of uric acid in modulating vascular redox state by preserving the activity of peroxidase and SOD.
Svensson et al., 2002, showed a new relationship between stress-induced glutathione level and uric acid metabolism.

Whiteman et al., 2002, showed that uric acid is a powerful inhibitor of tyrosine nitration induced by peroxynitrite, but fails to prevent antiproteinase inactivation by peroxynitrite. Protection against peroxynitrite mediated inactivation is decreased by ascorbate, glutathione etc. but is enhanced by uric acid.

Shimizu et al., 2002, however, showed that serum uric acid has been proposed as a marker for impaired oxidative metabolism.

Ford et al., 2002, found the complex correlation and kinetics between NO, glutathione, cysteine and uric acid at physiological pH and prominently the study showed that uric acid protects glutathione against depletion by oxidative damage from NO.

VI. VIEWS REGARDING WHOLE BLOOD G-6-P-D LEVEL – ITS CORRELATION WITH GLUTATHIONE AND NADPH. THE NOVEL APPROACH IN STRESS MANAGEMENT

G-6-PD deficiency, a common enzymopathy affecting over 200 million people worldwide, can cause neonatal jaundice, drug or infection-induced haemolytic crisis, favism and less commonly, non-spherocytic hemolytic anemia (Beutler, 1991). The haemolytic nature of all these syndromes is attributed to the inability of erythrocytes under oxidative stress to maintain NADPH in its reduced form. This subsequently causes oxidation of cellular components and eventual removal of the damaged cells from circulation (Ho et al., 2000). Most studies of G-6-PD have explored the pathophysiology of G-6-PD-deficient erythrocytes and the molecular characterization of different G-6-PD variants. In contrast, the chronic effects of G-6-PD deficiency on cells other than erythrocytes remain to be elucidated.

Oxidative damage has been implicated as a causative factor of cellular senescence. ROS produced during metabolism may cause cumulative damage, provoking senescence (Johnson et al., 1999). Exercise also causes serious oxidative damage and ROS production.
Further, regeneration of glutathione by glutathione reductase, role of NADPH and its origin from G-6-PD in RBC and other tissues therefore, are supposed to be highly correlated. Regeneration of glutathione from glutathione disulfide (GSSG) is accomplished by the flavin-containing enzyme glutathione reductase. NADPH is used as the reducing power of this reaction, which is coupled with G-6-PD in erythrocytes and some other tissues (Ji, 2000). When H₂O₂ concentration is increased in the red cells, there is a tendency towards an elevated GSSG, which appears to affect the regulation of HMP shunt in the following manner. First, GSSG activates G-6-PD directly; second, regeneration of glutathione decreases NADPH level, which is normally inhibitory to G-6-PD; and third, consumption of NADPH elevates NADP⁺, which is a substrate and allosteric activator of G-6-PD.

This humble study aims to find that correlation in exercising females.

G-6-PD deficiency is characterized by the loss of NADPH and enhanced erythrocyte oxidant sensitivity. Historically, it has been theorized that the elevated oxidant sensitivity of G-6-PD-deficient erythrocytes arises as the direct consequence of decreased intracellular glutathione (and hence increased GSH) concentrations. The effects of altered GSH and NADPH concentrations were examined in normal and G-6-PD-deficient erythrocytes. This study demonstrated that glutathione depletion, by 1-chloro-2, 4-dinitrobenzene (CDNB), had no effect on hemoglobin oxidation in response to hydrogen peroxide (H₂O₂) generating systems (phenazine methosulfate) in either normal or G-6-PD-deficient cells (Scott et al., 1991).

Maintaining glutathione in the reduced state has been thought to be the most important function of G-6-PD. In the absence of this enzyme, erythrocytes can have very low concentrations of GSH and, consequently, lack the cofactor necessary for normal glutathione peroxidase activity. This functional loss of glutathione peroxidase activity has been believed to be responsible for the enhanced sensitivity of G-6-PD-deficient red blood cells (RBCs) to H₂O₂-generating redox active drugs (Scott et al., 1991). Decreased glutathione concentration is not responsible for the increased oxidant sensitivity observed in G-6-PD deficiency. The enhanced oxidant sensitivity arises as a
direct consequence of decreased NADPH concentration and is independent of the steady-state glutathione status.

Oxidative damage has been implicated as a causative factor of cellular senescence. ROS produced during metabolism may cause cumulative damage, provoking senescence. Exercise also causes serious oxidative damage and ROS production (Johnson et al., 1999). As G-6-PD is indispensable to maintenance of the cellular redox balance and detoxification of ROS (Pandolfi et al., 1995), it is likely that the G-6-PD activity regulates cell growth, and any change related to it alters the course of cellular senescence, and thus performance at large.

The effect of G-6-PD deficiency on cells other than the anucleated erythrocytes has remained largely unexplored. G-6-PD deficiency instigates a premature senescence like growth arrest in human primary fibroblasts.

Normal cellular metabolic activities such as oxidative respiration generate stresses in cellular milieu. As a major source of stress, ROS are known to cause damage to organelles and cellular macromolecules. To deal with the oxidative stress, the cells possess a battery of antioxidants that include enzymes and such low-molecular-weight components as glutathione, ascorbic acid, and α-tocopherol. G-6-PD provides the ultimate reducing power for many of these antioxidant pathways. Deficiency in G-6-PD activity results in lowered NADPH/NADP⁺ ratio as well as diminished glutathione level. The importance of G-6-PD in antioxidant defense is evident, as cells with G-6-PD deficiency suffered from increased oxidative stress.

Clinically, findings suggest that the manifestation of G-6-PD deficiency may not be restricted only to erythrocytes. Though not as severe as in erythrocytes, G-6-PD deficiency in nucleated cells still affects cellular physiology (Ho et al., 2000).

Ascorbic acid, or Vitamin C, generally functions as an antioxidant by directly reacting with reactive O₂ intermediates and has a vital role in defenses against oxidative stress. Dehydroascorbate, the oxidized form of vitamin C, stimulates the antioxidant defenses of cells, preferentially importing dehydroascorbate over ascorbate. Puskas et al., 2000 revealed a novel mechanism for increasing glutathione levels through
stimulation of the pentose phosphate pathway and identified dehydroascorbate as an antioxidant.

Bagchi et al., 2001, investigated and suggested that antioxidant and G-6-PD level form a key combination in oxidative stress management in trained females.

Hashida et al., 2002, suggest the importance of G-6-PD in the antioxidant function of brain and pathogenesis of the oxidative stress-related diseases.

Jollow and McMillan, 2001, showed that oxidative stress, G-6-PD dehydrogenase and the red cell are interrelated biochemically.

It has been suggested by Zhang et al., 2001, that high glucose inhibits G-6-PD via cAMP in aortic endothelial cells. G-6-PD plays an essential role in the regulation of oxidative stress by primarily regulating NADPH, the main intracellular reductant. Changes in G-6-PD activity play an important role in high glucose-induced cell damage/death. Although it is known that decreased G-6-PD functionality can result in increased susceptibility to oxidative stress, the molecular targets of this stress are not known (Ayene et al., 2002).

Ingrosso et al., 2002, showed that the increased susceptibility of G-6-PD-deficient erythrocytes to membrane protein aspartate damage in response to oxidative stress suggests the involvement of protein deamidation/isomerization in the mechanisms of cell injury and haemolysis.

To the best of our knowledge (Tavazzi et al., 2002), AMP-deaminase is the first example of an enzyme, fundamental for the maintenance of the correct red blood cell energy metabolism, that is activated (rather than inhibited) by the interaction with ROS.

When G-6-PD is inhibited, GSH levels are not restored because of impaired glutathione reductase activity. Thus Leopold and Loscalzo, 2000, suggested that G-6-PD is a critical determinant of the response to oxidant stress in vascular smooth muscle cells.
VII. REFERENCES


61. Quintanilha AT and Davies KJA. Effects of physical exercise and / or vitamin E on tissue oxidative metabolism. *FEBS Lett.*, 1982; 139: 241-244.


