REVIEW OF LITERATURE

Relevant literature has been compiled under the following headings:

2.1 Oxygen consumption during exercise

2.2 Oxidative stress and exercise

2.3 Protection against oxidative stress

2.4 Exercise induced oxidative stress and atherosclerosis

2.5 Effect of antioxidant supplementation on oxidative stress markers

2.1 Oxygen consumption during exercise

Exercise increases muscular strength, improves muscular endurance, speed and hence the performance of sportspersons. As exercise intensity increases so does oxygen consumption. However, a point is reached where exercise intensity can continue to increase without the associated rise in oxygen consumption. The point at which oxygen consumption plateaus defines an individual's maximal aerobic capacity abbreviated as VO$_{2}$max. Pollock (1973) defined VO$_{2}$max as "The highest rate of oxygen consumption attainable during maximal or exhaustive exercise". It is generally considered the best indicator of cardio respiratory endurance and aerobic fitness. It is useful as an indicator of a person's aerobic potential or upper limit and as a predictor of success in endurance events (Gaesser and Brooks, 1984).

Wilmore and Costill (2005), in their book “Physiology of Sport and Exercise” mentioned that nonathlete males in the age group 20-29 yrs, has VO$_{2}$max of 43-52 ml/kg/min, while nonathletic females of the same age group has VO$_{2}$max of 33-42 ml/kg/min and within different athletic groups VO$_{2}$max varies. Thus nonathletic female has less VO$_{2}$max then nonathletic males. Exercise levels affect VO$_{2}$max. Pollock (1973) showed that running training at 75% of aerobic power, for 30 minutes, 3 times a week over 6 months increased VO$_{2}$max on average by 15-20%. Hickson et al., (1977) reported that VO$_{2}$max of trainees increased by 40% after 10 week of training that consisted of six
5-min intervals of bicycling at VO₂max for 3 days per week plus 40 min of vigorous running for 3 days per week. Chad and Wenger (1988) demonstrated that oxygen consumption increased with exercise at 70% VO₂max on cycle ergometer for 30, 45 and 60 min. An increase in VO₂max was also observed by Mero et al., (1993) in subjects after 5×3 min treadmill running starting at sub maximal speed of 2.22 m/s and thereafter increasing the speed by 0.56 m/s at each step until the last load of 4.4m/s with the slope of 1 degree at all the time. Helgerud et al., (2007) examined the effects of 8 weeks of running exercise at various intensities in healthy young adult males. One group running at a moderate-intensity at 70% max heart rate (HRmax) for 45 min each session, while another group did vigorous-intensity exercise at 85% HRmax for 24 min per session, third group performed 47 repetitions of 15-s intervals at 90-95% HRmax with 15 s of active resting periods at warm up velocity, corresponding to 70% HRmax between each interval and fourth group using 4×4-min interval running at 90-95% HRmax with 3 min of active resting periods at 70 % HRmax between each interval. Running exercise significantly increased VO₂max in all the participants as compared to their pre exercise values. However third and fourth group showed more increase in VO₂max as compared to groups performing moderate-intensity and vigorous-intensity exercise. Gormley et al., (2008) reported that VO₂max of healthy individuals exercising on a stationary bicycle ergometer for 6 weeks increased with the intensity of exercise. They observed that VO₂max increased by 20.6% after near-maximal exercise (95% VO₂), 14.3% after vigorous (75% VO₂max) and 10.0% after the moderate-intensity exercise (50% VO₂max). Thus the intensity of exercise is directly related to the oxygen consumption.

2.2 Oxidative Stress and Exercise

During exercise, chemical bond energy is converted into mechanical energy. This chemical bond energy comes from ATP which is generated by the aerobic metabolism. Aerobic metabolism involves krebs cycle and electron transport chain of mitochondria and in final step requires molecular oxygen as an electron acceptor. Enhanced utilization of ATP during exercise demand fast oxidation process and finally more requirement of oxygen (Mc cord and Fridovich, 1969). In biological systems the reduction of molecular
oxygen to water requires four electrons and can be carried out in two ways. The main pathway is capable of a tetravalent reduction of oxygen to water and proceeds in the mitochondria with cytochrome oxidase as the final catalyst. This reduction process has been calculated to account for 95 to 98% of the total oxygen consumption. Rest 2-5% of the oxygen consumed by cells is reduced through an alternative univalent pathway in which highly reactive oxygen species are produced (Ernster, 1986).

\[ \text{O}_2 \rightarrow \text{e}^- \rightarrow \text{O}_2^- \rightarrow \text{e}^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+/Cu^+} \rightarrow \text{OH}^- \rightarrow \text{e}^- + \text{H}^+ \rightarrow \text{H}_2\text{O} \]

Superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are the main reactive oxygen species which leads to more oxidation reactions and causes oxidative stress. These reactive oxygen species are discussed in detail as follows.

### 2.2.1 Superoxide Radical

Loschen et al., (1974), first time detected the production of superoxide ions in mitochondrial membrane by the partial reduction of molecular oxygen involving one electron.

\[ \text{O}_2 \rightarrow \text{e}^- \rightarrow \text{O}_2^- \]

Cross and Jones (1991) and Mohazzab and Wolin (1994), reported that oxidative enzymes xanthine oxidase and NADH oxidoreductase were the most important sources of superoxide radical production. These enzymes contain flavin or transition metal ions such as Cu$^+$ and Fe$^{2+}$ which serve as electron donors. Klebanoff (1980) reported that superoxide radical is unstable in an aqueous environment and has a short life span. It spontaneously mutates to produce hydrogen peroxide and oxygen by the following reaction:

\[ 2\text{O}_2^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]
The rate constant for superoxide radical dismutation at pH 7.4 is $2 \times 10^5$ m/s. Because of this relatively rapid rate of dismutation, production of superoxide radical *in vivo* is always accompanied by production of hydrogen peroxide. Pryor, (1986) observed that the life time of $O_2^{--}$ in water cellular environment is $\approx 10^6$ s and dismutation of superoxide radical to hydrogen peroxide is catalyzed by intracellular enzyme Super Oxide Dismutase.

Several investigators have shown that superoxide radical exert cytotoxicity by inactivating a variety of specific enzymes essential to the cell; viz. tRNAase, glyceraldehyde-3-phosphate dehydrogenase, epinephrine and creatine phosphokinase, lactate dehydrogenase, aconitate and 6-phosphogluconate dehydratase (Kellogg and Fridovich, 1977, Motohashi and Mori, 1983, Kono and Fridovich, 1982, McCord and Russell, 1988, Bielski and Chan, 1973 and Gardner and Fridovich, 1992). Gollnick et al., (1990) reported a loss of cytochrome oxidase activity during intensive exercise and correlated it with increased production of superoxide anions. A 40% decrease in cytochrome oxidase activity related to destruction of mitochondrial membrane was demonstrated in rat skeletal muscle after ischemia and reperfusion during intensive exercise (Soussi et al., 1990).

2.2.2 Hydrogen Peroxide:

Oshino et al., (1973) reported that univalent reduction of superoxide anion leads to the formation of hydrogen peroxide, Furthermore, hydrogen peroxide is the most stable reactive oxygen specie.

$$2O_2^{--} + 2 H^+ \overset{SOD}{\rightarrow} H_2O_2 + O_2$$

Decomposition of hydrogen peroxide to water and oxygen is catalyzed by catalase (Michiels et al.,1994).

$$2H_2O_2 \overset{Catalase}{\rightarrow} 2H_2O + O_2$$
Hydrogen peroxide is known to be involved in pathophysiology of a number of diseases. High levels of hydrogen peroxide worsens the cytotoxicity induced by antimycin A, arsenite and tumor necrosis factor alpha (TNF-α) [Quillet et al., (1997), Hagar et al., (1996), Chen et al., (1998) and Sidoti et al., (1998)]. Crapo (2003) suggested that exposure of delicate epithelial surfaces to external oxidative stress caused by hydrogen peroxide leads to acute and chronic lung injury in infants, children and adults. Fink, (2002) reported significantly high levels of hydrogen peroxide in adult patients with acute respiratory distress syndrome (ARDS) as compared to the control group. He observed that hydrogen peroxide induced oxidative stress lead to increased permeability of endothelial which is one of the important pathophysiologic features of ARDS. Several investigators reported that damage to mitochondrial membrane by hydrogen peroxide leads to a loss of membrane potential, causing swelling, leakage of cytochrome C and finally to initiation of apoptosis [Newmeyer and Ferguson, (2003) and Zimmermann et al., (2001)].

2.2.3 Lipid peroxidation

Hydrogen peroxide and superoxide anions generate hydroxyl radical (HO’) by Fenton reaction (Fenton, 1894) and Haber Weiss reaction (Haber & Weiss, 1934) respectively.

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \quad \text{Fenton reaction} \]

\[ \text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{O}_2^+ + \text{OH}^- + \text{OH}^- \quad \text{Haber Weiss reaction} \]

Hydroxyl radical is the most reactive free radical produced in biological systems (Bielski and Shiue, 1979). Fridovich (1976) suggested that the hydroxyl radical is the only metabolite formed in biological systems that can abstract methylene hydrogen atoms from polyunsaturated fatty acids and further initiates lipid peroxidation.
Lipid peroxidation represents a classical free-radical reaction involving three stages: initiation, propagation and termination.

\[
\begin{align*}
\text{LH} + \text{HO}^- & \rightarrow \text{H}_2\text{O} + \text{L}^- & \text{Initiation} \\
\text{L}^- + \text{O}_2 & \rightarrow \text{LOO}^- & \text{Propagation} \\
\text{LOO}^- + \text{LH} & \rightarrow \text{LOOH} + \text{L}^- & \text{Propagation} \\
\text{L}^- + \text{L}^- & \rightarrow \text{L} - \text{L} & \text{Termination}
\end{align*}
\]

LH, L’, LOO’ and LOOH represent polyunsaturated lipid, lipid alkyl radical, lipid per hydroxyl radical and lipid hyderoperoxide respectively.

Lipid hydroperoxides interact with certain iron-containing compounds to break down to yield malondialdehyde. Several investigators have reported that peroxidation of lipids by hydroxyl radical leads to, enhanced rates of protein degradation (Yamguchi and Yamashita 1980), increased rates of mutagenesity (Yau and Mencl, 1981), changes in the membrane fluidity and permeability (Hicks and Gebicki, 1978), and lysis of target cells (Stocks and Dormandy, 1971).

Dillard et al., (1978) observed that intensive exercise induced lipid peroxidation in players. They used expired pentane level in breath as indicator of lipid peroxidation. Players were subjected to graded exercise on a cycle ergometer during three 20-minutes bouts at 25%, 50% and 75% of VO\(_2\) max. They observed a two fold increase in expired pentane levels in breath of players undergoing exercise at 75% VO\(_2\)max as compared to their resting levels. But exercise at 25% and 50% VO\(_2\)max did not induced any lipid peroxidation. Kanter et al., (1986) reported a 60% increase in plasma malondialdehyde levels following 80 km race as compared to pre exercise values. Similarly Lovlin et al., (1987) studied the effect of cycling ergometery on lipid peroxidation of participants. Thiobarbutric acid reactive species (TBARS) level in plasma was used as indicator of lipid peroxidation. They observed increased levels of thiobarbutric acid reactive species after exercising at 100 % VO\(_2\)max as compared to resting values but no difference while exercising at 40 % and 70% VO\(_2\)max. Several investigators had reported significantly
increased malondialdehyde levels in sports persons after half marathon race at 60% VO₂ max (Child et al., 1998), 95 km mountain bike race at a 70% VO₂ max (Ruiz et al., 2006), long distance running with 65% VO₂ max (Kostaropoulos et al., 2006) and 30 minute treadmill running at 60% and 90% VO₂ max (Kanter et al., 1993). Miyazki et al., (2004) reported that the rats undergoing training of running for 1.5 h/day, 5days a week for 8 weeks, had raised erythrocyte malondialdehyde level as compared to that in sedentary group of rats. Kinnunen et al., (2005) observed a high level of lipid peroxidation in trained horses after treadmill-exercise for 53 min at moderate intensity as compared to pre exercise levels.

The effect of exercise on lipid peroxidation under various pathological conditions has been reported by several investigators. Miyazaki et al., (2004) reported that plasma hydroperoxides level, a marker of lipid peroxidation, in rats having damaged liver with fibrosis and subjected to treadmill running was significantly higher than in the rats having damaged liver but no exercise. Jammes et al., (2008) observed higher levels of plasma TBARS in patients with chronic obstructive pulmonary disease and subjected to incremental cycling exercise until volitional exhaustion as compared to their pre-exercise levels. Earlier Heunks et al., (1999) also reported similar results in patients with chronic obstructive pulmonary disease.

2.3 Protection against oxidative stress

To protect cells from oxidative stress and associated oxidative damage, normal cells have antioxidant defense system. Gutteridge and Halliwell (1990) defined antioxidant as “Any substance that delays or inhibits oxidative damage to a target molecule”. Antioxidant enzymes together with antioxidant molecules provide the cell an ability to counteract the action of reactive oxygen species which are responsible for oxidative stress.
2.3.1 Enzymatic Defense system

The first line of defense against reactive oxygen species mediated injury is antioxidant enzymes: superoxide dismutase, catalase and glutathione peroxidase.

2.3.1.1 Superoxide Dismutase

McCord and Fridovich (1969), observed a unsuspected enzymatic activity in bovine erythrocytes which catalyzed the dismutation of superoxide anions. Fridovich (1976) reported that the protein orgotein, isolated initially on the basis of its anti inflammatory properties, was superoxide dismutase and form a primary line of defense against superoxide and hydroxyl ions mediated injury in the body. Superoxide dismutases are a family of metalloenzymes associated with different metal ions i.e. Cu-SOD, Cu-Zn-SOD, Mn-SOD and Fe-SOD. Fe-SOD and Mn-SOD are unequally distributed among living organisms and are located in different cellular compartments. In particular, Mn-SOD exclusively or together with Fe-SOD is found in facultative aerobes (Gardner and Fridovich, 1992), the liver of humans (Barra et al., 1984), chloroplasts of higher plants, and in mitochondria of higher plants, fungi and animals (O’Nell et al., 1988). More than 90% of the extracellular superoxide dismutase is present in the interstitial spaces of tissues and extracellular fluids and this account for the majority of the superoxide dismutase activity in the plasma, lymph and synovial fluid (Karlsson et al., 1993).

Neil and oliver (1985) reported that superoxide anions react with nitric oxide (NO) to form peroxynitrite (OO-NO).

\[ \text{NO} + \text{O}_2^- \rightarrow \text{OO-NO} \]

Buttery et al., (1996) reported increased concentration of peroxynitrite in atherosclerotic lesions of humans. Stralin et al., (1995) proposed that the concentration of extracellular superoxide dismutase within the arterial wall of normal persons was high
enough to suppress the pathological effects of superoxide anions i.e. the reaction of superoxide anions with nitric oxide.

Low superoxide dismutase activities has been reported in various pathological conditions involving oxidative stress. Lynch et al., (1997) observed increased inactivation of nitric oxide by superoxide anions which impaired *in vivo* endothelial dependent vasodilation in rats fed with copper deficient diet. Landmesser et al., (2000) reported a low extracellular superoxide dismutase activity in coronary artery disease patients. Superoxide dismutase activity was also depressed in subjects with coronary artery disease risk factors such as diabetes mellitus. Nischal et al., (1998) reported that superoxide dismutase activity in serum of patients suffering from non-insulin dependent diabetes mellitus was low as compared to that of healthy controls. Activity of this enzyme was highly reduced in diabetics with microvascular complications such as retinopathy and nephropathy as compared to those who were freshly diagnosed for diabetes and free from these complications. Kimura et al., (2003) observed low concentration of serum extracellular superoxide dismutase in patients with type II diabetes as compared to controls. There was a strong relationship between serum extracellular superoxide dismutase concentration and the severity of both micro- and macrovascular diabetic complications. They suggested that serum concentration of extracellular superoxide dismutase may act as marker of vascular injury in diabetics. Bhatia et al., (2003) reported low superoxide dismutase activity in erythrocytes of diabetic patients. Decreased activity was linked to the progressive glycation of superoxide dismutase in diabetics.

that overproduction of superoxide dismutase in such cases was to counteract the deleterious effects of raised levels of highly reactive oxygen species during various diseases.

2.3.1.2 Effect of exercise on Superoxide Dismutase Activity:

Increase in superoxide anions production in players as a result of exercise has been reported by many researchers. To counteract the effect of these superoxide anions, superoxide dismutase activity may be enhanced in these players. Miyazaki et al., (2001) reported an increase in superoxide dismutase activity after completion of a training session of cycle ergometric running up to 80% VO$_2$max, 60 min/day, for 5 days/week for 12 weeks as compared to pre training levels of superoxide dismutase activity. An increase in erythrocyte superoxide dismutase activity after other type of exercises viz marathon running (Marzatico et al.,1997), strenuous jumping test consisting of six bouts of 30-s continuous jumping separated by 2 minutes of rest (Ortenblad et al., 1997) and 4 weeks of swimming exercise (Gonenc et al., 2000), has been reported as compared to pre exercise activity. Balakrishnan and Anuradha (1998) reported that even in players at rest, superoxide dismutase activity was higher as compared to that in sedentary controls. They reported that superoxide dismutase activity levels immediately before exercise in erythrocyte of players performing sports activity of football, hockey and long distance running, 6 days/week for 20 hr/week for a period of 3 years were raised as compared to the sedentary workers. Superoxide dismutase activity in erythrocyte has been reported to be higher in rats undergoing, treadmill running for 1.5h/day, 5 days/week for 8 weeks (Oztason et al., 2004 and Senturk et al., 2001), and treadmill exercise protocol for 6 weeks 15 meter·min$^{-1}$ for 60 min, 15 degree grade, 5 days/week (Asghar et al., 2007) as compared to superoxide dismutase activity in sedentary group of rats. Oztason et al., (2004) suggested that exercise induced increase in concentration of superoxide anion, (substrate of superoxide dismutase), and reactive oxygen species formation was responsible for the increased superoxide dismutase activity in exercised group immediately before exercise compare to sedentary workers or after exercise as compared to the pre-exercise values. However some other researchers reported unaltered
Erythrocyte superoxide dismutase activity after exercise. Erythrocyte superoxide dismutase activity was not affected in trained rats after exhaustive treadmill running at 2.1 km/hr, 60 min/day, 5 days/week for 4 weeks (Senturk et al., 2001), in trained male subjects after a duathlon competition when compared to pre competition superoxide dismutase values (Tauler et al., 1999) and in trained long distance runners after aerobic training at 70% VO$_2$max as compared to pre exercise values (Selamoglu et al., 2000). Sureda et al., (2007) reported a decrease in superoxide dismutase activity in cyclists after completion of 165 km cycling race as compared to pre exercise levels. Knez et al., (2007) reported higher superoxide dismutase activity in players (participating in half and a full Ironman triathlon competition, with 65% VO$_2$max, undertaking medium- to high-intensity training for 14 h/week) at resting condition as compared to sedentary group, but they observed a decrease in superoxide dismutase activity after completion of race in both half and full triathlon as compared to pre-exercise values. The decrease in antioxidant enzyme activity observed may reflect allosteric downregulation of the enzymes in addition to enzyme inactivation attributable to overwhelming oxidative stress.

2.3.1.3 Catalase

Catalase is another important antioxidant enzyme which catalyzes the decomposition of hydrogen peroxide to water.

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} \text{2H}_2\text{O} + \text{O}_2$$

Catalase is present in peroxisomes, cytosol and mitochondria. It consists of four protein subunits, each containing a heme Fe (III)-protoporphyrin group bound to its active site (Dixon and Webb, 1964). Xu et al., (1999) observed that increased catalase activity in beta cells of transgenic mice protected their islet cells from the toxic effects of hydrogen peroxide. Jingxiang and Arthur (2001) reported that overexpression of both cytosolic and mitochondrial catalase activity protected liver E47 cells from oxidant induced loss of mitochondrial membrane potential and permeability. In this way catalase plays an important role in the overall protection against oxidant-induced cytotoxicity.
2.3.1.4 **Effect of exercise on Catalase activity:**

Protection against exercise induced oxidative stress has been reported to be provided by enhanced catalase activity. Mine et al., (2001) observed that catalase activity was increased after 800 m swimming as compared to pre-swimming catalase activity. Kostaropoulos et al., (2006) compared catalase activity in serum of long distance and short distance runners. They observed a threefold higher catalase activity in long distance runners. They explained that high oxygen load imposed on long distance runners during their repeated prolonged exercise bouts, is the reason behind the increased activity of catalase. Knez et al., (2007) reported higher catalase activity in ultraendurance athletes in their resting condition as compared to that in control group of sedentary workers. Kyparos et al., (2007) also reported an increase in catalase activity in blood of males undergoing novel volitional fatigue test consisting of shuttle runs with a tennis racquet in the hand towards the left and right sidelines within the tennis singles court in an attempt to hit tennis balls until exhausting, at a frequency of 20 balls/ min, as compared to the pre-exercise activity of catalase. Teixeria et al., (2008) observed an increase in catalase activity after swimming exercise in rats as compared to the resting state. Urso and Clarkson (2003) attributed the increase in catalase activity during endurance exercise, to the high production of reactive oxygen species and insufficient scavenging of high hydrogen peroxide levels by glutathione peroxidase.

Beneficial effects of physical exercise on antioxidant defense in various pathological conditions have been observed by various researchers. Exercise is reported to enhance the activity of catalase, an antioxidant enzyme. Linke et al., (2005) reported an increase in catalase activity in chronic heart failure patients after treadmill exercise training, 10 min/day at 70% VO$_2$ max for the period of 6 months, as compared to the patients with sedentary life style. Free load bicycle exercise till exhaustion increased the catalase activity in ischemic heart disease patients as compared to their pre-exercise levels (Chursina et al., 2007 and Chursina and Shcherbatykh, 2006).

Catalase activity in the animals increased after completion of training as compared to their pre-training levels. Duncan et al., (1996) observed increased catalase
activity in rats having ethanol induced lung and liver cancers undergoing treadmill running, 60 min, 2 days/week for 7 weeks as compare to sedentary group of rats having ethanol induced cancers. Husain and Sumani (1997) studied the effect of ergometeric running for 60min/day at 10 degree of inclination for 12 weeks and 6.5 weeks respectively in chronic ethanol-induced hypertensive rats. They observed an increase in catalase activity in exercised group of chronic ethanol-induced hypertensive rats as compared to control group of sedentary hypertensive rats. Lawler et al., (2006) studied the effect of treadmill exercise training for 12 weeks, 5 days/weekk on catalase activity in Miniature Yucatan swine with occlusion of the proximal left circumflex artery. The enzyme activity increased in rats after exercise as compared to control group of rats without exercise. Contrary to above findings, Miyazaki et al., (2001) reported a decrease in catalase activity in runners undergoing 60min run/day for 12 weeks.

2.3.2 Non-Enzymatic Defense System

Second line of defense against reactive oxygen species mediated injury are small molecules that acts as antioxidants, particularly in the extracellular spaces, where antioxidative enzymes are either absent or present in very small quantities (Frei et al., 1988). Important non-enzymatic antioxidants are vitamin E, vitamin C and uric acid.

2.3.2.1 Vitamin E

Vitamin E is one of the most important lipid soluble non-enzymatic antioxidants, which occurs in plasma as α and γ- tocopherol. Reactive oxygen species particularly hydroxyl radical subtract hydrogen atom from poly-unsaturated fatty acids in the cell membrane. The fatty acid radicals formed, react with oxygen, which recycles to form more peroxy radicals in a chain reaction. The phenolic hydroxyl group of tocopherol reacts with this peroxy radical (LOO’) to form hydroperoxide (LOOH) and tocopheroxyl radical (Niki et al., 1988).

\[
\text{LOO'} + \text{Vitamin E-OH} \rightarrow \text{LOOH} + \text{Vitamin E-O'}
\]
Tocopheroxyl radicals are reduced to tocopherols by interaction with reductants like ascorbate serving as hydrogen donors (Chow, 1985).

Low levels of vitamin E have been reported in various pathological conditions involving free radical in their pathogenesis. Singh et al., (2005) in their case control study on Indian population, reported low levels of vitamin E in breast cancer patients as compared to healthy persons. Similarly the levels of vitamin E have been reported to be significantly lower in patients with prostate cancer compared to controls (Ozmen et al., 2006 and Surapaneni and Ramana, 2007). Sood et al., (2007) observed that injury to the myocardial tissue due to ischemia and reperfusion occurred because of imbalance between the formation of oxidants and available antioxidants in heart. The levels of vitamin E in two groups of patients with acute myocardial infarction, one reperfused group and another nonreperfused group were low as compared to vitamin E levels in control group of healthy persons. With in two groups of patients, reperfused group has even lower vitamin E level than nonreperfused group of patients. Krishna and Venkataramana (2007) reported that plasma vitamin E levels were low in patients with pregnancy-induced hypertension in preeclampsia as compared to control group with normal pregnancy. Surapaneni and Venkataramana (2007) observed low levels of vitamin E in osteoarthritis patients when compared to healthy controls. Vitamin E levels were also low in inactive inflammatory bowel disease patients as compare to the control group of healthy persons [Hengstermann et al., (2008)].

2.3.2.2 Vitamin C

Vitamin C also known as ascorbic acid is one of the important water soluble antioxidants in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. Mehlhorn et al., (1989) reported that ascorbate can donate either one or two electrons in redox reactions. Loss of the first electron results in the formation of ascorbate free radical. Mild oxidants such as ferricyanide can remove the second electron and convert the ascorbate free radical to dehydroascorbic acid. Dehydroascorbic acid is unstable at physiological pH and undergoes irreversible ring opening to form 2,3-diketo-1-gulonic acid.
Huang et al., (2001) reported that ascorbic acid was oxidized to form dehydroascorbic acid and protein disulfide isomerases reduced it back to ascorbic acid, by oxidizing their disulfide bonds. Schweinzer and Goldenberg (1992) reported that ascorbate can act as electron donor and cause reduction of α-tocopheroyl radical back to α-tocopherol.

Low levels of vitamin C have been reported in various pathological conditions in whose pathogenesis free radicals have been suspected to be involved. Ray and Husain (2001) reported low levels of vitamin C in breast cancer patients and explained that the reason behind this low vitamin C level was the high oxygen free radical induced oxidative stress. D'Odorico et al., (2001) collaborated increased free radical peripheral leukocyte DNA damage with decreased plasma vitamin C level in ulcerative colitis and crohn disease patients. Mecocci et al., (2002) reported that increased oxidative stress in
Alzheimer disease patient as indicated by high lymphocyte DNA 8-hydroxy-2'-deoxyguanosine (DNA 8-OHdG) content was related to low level of vitamin C in patients as compared to healthy workers. Krishna and Venkataramana (2007) reported low levels of vitamin C in patients with pregnancy induced hypertension: preeclampsia in Indian population as compared to its levels in healthy group. Surapaneni and Venkataramana (2007) reported significant low levels of vitamin C in osteoarthritis patients as compared to healthy persons. Esme et al., (2008), also reported low levels of vitamin C in patients with adenocarcinoma, squamous cell carcinoma and large cell carcinoma as compared to control group of healthy persons. With advancing stage of lung cancer, levels of this antioxidant molecule further decreased. Hengstermann et al., (2008) observed a low level of vitamin C in patients with inactive inflammatory bowel disease as compared to the control group of healthy persons.

2.3.2.3 Effect of exercise on Vitamin E and Vitamin C levels

During exercise oxidative stress increases in players. Vitamin E and C along with some other molecules and enzymes provide defense against this oxidative stress because of their antioxidant nature. Some investigators studied the level of vitamin E and C in players as compared with the levels in sedentary subjects. In cyclists undergoing long distance training, vitamin E and C levels were higher as compared to sedentary subjects (Robertson et al., 1991). Hubner et al., (1994) reported higher levels of vitamin E and C in long distance skiers as compared to control group of sedentary persons. Brites et al., (1999) observed high levels of vitamin E and C in soccer players undergoing 20 h of training/week and 6 soccer matches/week, as compared to the control group of sedentary persons. Aguilo et al., (2005), studied that in cyclists plasma vitamin E and C levels increased after completing 171 km cycling in 270 min as compared to their corresponding pre-exercise vitamin levels. Similarly, Cases et al., (2006) observed an increase in plasma vitamin E and vitamin C levels of cyclists after long distance cycling exercise as compared to their pre-exercise levels. Goldfarb et al., (2007) reported that exercise affected the levels of vitamin E and C to different extent in men and women. After running for 30 min at 80% VO₂max, women had higher plasma vitamin E and C
levels as compared to men. But in both men and women, after exercise, levels of vitamin E and C were higher as compared to their pre-exercise levels. In an another study, Sureda et al., (2007) reported an increase in vitamin E and vitamin C levels in male professional soccer players after playing a 60 minutes training match at medium and high intensity as compared to their pre-exercise values. In basketball players, significantly higher vitamin E and vitamin C levels were reported as compared to sedentary workers by Yilmaz et al., (2007).

Aguilo et al., (2003), studied the effect of exercise intensity and training on vitamin E and vitamin C levels in both amateur and professional cyclists. The amateur cyclists exercised 14±1 h per week, and their VO$_{2\text{max}}$ was 62.5±1.8 ml/Kg x min while the professional cyclists exercised 24 ±1 h per week, and their VO$_{2\text{max}}$ was 80.2±1.6 ml/Kg x min. Amateur cyclists were subjected to the maximal and submaximal prolonged exercise tests and professional cyclists were subjected to a mountain stage (170 km) of cycling exercise (which is an endurance exercise). Before exercise, no significant difference was observed between vitamin levels (vitamin E and C) in players of both the groups. Plasma levels of vitamin E and C increased in well-trained professional cyclists after cycling at mountain stage but not in amateur cyclists. Shing et al., (2007) examined the influence of high-intensity cycling on blood vitamin E and C levels. Highly-trained male cyclists (VO$_{2\text{max}}$ 76 ± 4 ml.kg$^{-1}$.min$^{-1}$) completed a session of 9 exercise bouts lasting 30 s each, at 150% peak power output) on day 1, followed by 2 laboratory-simulated 30 km time trials on days 2 and 3. They observed a decrease in vitamin E and C levels after exercise as compared to basal levels. On the contrary, Viguie et al., (1993) observed no change in plasma vitamin E and vitamin C levels due to exercise on either day 1 or 3 in trained males (24.3±1.1 yr) exercised 90 min at 65% peak O$_2$ uptake on a cycle ergometer for 3 consecutive days. Bachur et al., (2007) in their animal study, divided rats into three groups, group A (swimming for 50 min) and group B (swimming for 100 min), which were further subdivided into 3 different subgroups based on their exercise intensities, i.e non-weight bearing (subgroup I), 3% weight load (subgroup II), and 5% weighted load (subgroup III), as well as a control-rested group (C). They observed that vitamin E and vitamin C levels were significantly higher only in groups BII
and BIII. When groups were compared by intensity at each swimming time there were no differences between I, II, and III sub groups at 50 min for vitamin E and vitamin C levels. They further explained that the contradictory results on effect of exercise on vitamin E and C levels in different studies were due to factors like different exercise intensities and different exercise duration used in different studies.

### 2.3.2.4 Uric Acid

Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen. Ames et al., (1981) observed that uric acid protected erythrocyte membrane from peroxidation and acted as singlet oxygen scavenger. Davies et al., (1986) reported that urate acted as antioxidant in unilamellar liposomes and rat liver by inhibiting the oxidation of dihydroascorbate to the ascorbyl radical (monohydroascorbate). Increased oxidative stress due to exercise is reported to cause DNA degradation and further catabolism of purines which leads to higher production of uric acid (Aruoma et al., 1989).

### 2.3.2.5 Effect of exercise on Uric Acid Concentration:

Exercise leads to oxidative stress and uric acid acts as one of the antioxidants against this stress. Several investigators studied the effect of exercise on uric acid concentration. Klapcinska et al., (2001), estimated blood uric acid of sports persons before the warm-up and 5 min, 2 hrs and 20 hrs post sprint. They observed increased uric acid levels after the race as compared to their pre-exercise levels. Evans et al., (2002) studied the effect of endurance race of 1760 m and 2160 m on blood uric acid concentrations of horses 30-60 min before and 8 and 30 min after the race. They observed higher uric acid concentration in horses after endurance race as compared to before race. Castejon et al., (2006) studied the relationship between uric acid and endurance exercise in horses; the day before, and 5-10 mins after, successfully finishing a 121 km and 164 km endurance race. They reported that the fastest horses showed significantly higher plasma uric acid levels compared with the slowest and medium speed horses. Stathis et al., (2006) studied uric acid concentration in cyclists after a 30 s sprint,
before and after 7 days of sprint training. The training consisted of 15 sprints, each lasting 10s, on an air-braked cycle ergometer performed twice a day. The sprint training attenuated the exercise-induced increases in plasma uric acid during the first 120 min of recovery but uric acid level was reduced in the 24 h recovery period following intense exercise.

2.4 Exercise induced oxidative stress and atherosclerosis

Exercise induces oxidative stress. Oxidative stress plays a major role in the pathogenesis of coronary artery disease. Three hypothesis has been proposed to understand the complex events associated with the atherosclerosis. Ross and Glomset (1973) proposed “response to injury hypothesis” and explained that endothelial injury caused by free radicals, blood pressure fluctuations, bacterial and viral insult etc is the initial step in atherogenesis. Williams and Tabas in 1995 proposed another hypothesis called “response to retention hypothesis” and emphasized that the inciting event for atherosclerosis was retention of the atherogenic lipoproteins such as low density lipoproteins. Hessler et al., (1979) reported that the oxidation of LDL was cytotoxic to the artery wall cells. They suggested that LDL oxidation might be important in the process of atherogenesis. In the same year, Goldstein et al reported that the scavenger receptors present on macrophages take up cholesterol from the acetylated LDL and not from the native LDL. Fogelman et al., (1980) reported that malondialdehyde, a product of the oxidation of fatty acids formed schiff-base with the epsilon amino groups of the lysine residues in apoB of LDL. This altered lipoprotein was recognized by the scavenger receptors resulting in uptake and accumulation of cholesterol, thereby forming foam cells. Based on these observations, Steinberg et al., (1989) proposed the “oxidative modification hypothesis” to explain the mechanism of initiation of atherosclerosis. According to their hypothesis, oxidized LDL facilitates the recruitment of circulating monocytes into the intimal space, inhibits the ability of resident macrophages to leave the intima, enhances the rate of LDL uptake by macrophages to form foam cells and finally leads to loss of endothelial integrity as oxidized LDL is cytotoxic. Thus oxidative stress is known to play a major role in coronary artery disease (CAD).
Lipid profile is used as a diagnostic tool to evaluate the risk the coronary artery disease. Number of workers has studied the effect of exercise which is known to induce oxidative stress, on lipid profile. Hartung (1980) reported that athletes had higher serum HDL-C concentration as compared to sedentary counterparts and concluded that regular exercise brings a favorable change in cholesterol and lipoprotein metabolism. Enger et al., (1980) observed a decrease in serum total cholesterol and triglycerides concentrations after a single bout of 70 km cross country ski race as compared to before and immediately after 1, 2 and 4 days of race. On the contrary, Durstine et al., (1983) reported that single bout of walking exercise had no effect on serum triglyceride and LDL-C levels but increased total cholesterol levels as compared to levels before exercise. Hughes et al., (1990) studied that different exercise duration had specific effects on lipoprotein cholesterol levels in players. They observed an increase in HDL-C level in sports persons, running on treadmill at 20% VO$_2$max for 15, 30 or 45 min, after exercise as compared to pre exercise values. HDL-C levels were higher in players who ran for 45 min as compared to those who ran for either 15 min or 30 min. A single bout of moderate exercise duration had no effect on post exercise levels of total cholesterol, LDL-C and triglycerides. In response to an acute single bout of aerobic cycling exercise an increase in HDL-C level as compare to baseline value was observed by Gordon et al., (1998). Park et al., (2003) reported a significant increase in total cholesterol and HDL-C levels in players exercising for 15 min after achieving VO$_2$max of 68% on motor driven treadmill as compared to their levels prior to exercise. Degoutte et al., (2003) reported an increase in total cholesterol and triglycerides concentration in judokas after a judo match of 7.18 min as compared to pre exercise concentration.

Mena et al., (1991) observed a significant increase in HDL-C levels but decrease in total cholesterol, triglycerides, LDL-C and TC/HDL-C ratio after completion of two cycle races of 800 and 900 km in 6 days in professional cyclists as compared to pre exercise levels. Sady et al., (1988) explained that low levels of triglyceride in physically active persons as compared to sedentary workers was because of an improved ability of physically active persons to clear circulating fat rapidly. They documented an enhanced triglyceride clearance rate in male athletes. Later on, similar results were reported in
female endurance athletes by Podl et al., (1994). The prolonged exercise alters the hepatic lipid metabolism in a way that results in reduced fatty acid synthesis, increased fatty acid oxidation and accumulation of triglycerides in liver and finally allowing decreased VLDL-TG secretion into the plasma (Gorski et al., 1990). Earlier Lopez (1974) reported a decrease in LDL-C and VLDL-C levels of cyclists who were trained for 4 weeks, cycling 140 km/week, as compared to lipoprotein cholesterol levels before training and in sedentary workers.

Longer the duration of an exercise session of a specific intensity, higher is the energy expenditure. The energy expenditure during exercise is further related to the effect of exercise on lipid profile of the sports persons. Low concentrations of triglycerides, cholesterol and VLDL-C after 1 hr ergometric cycling at 65% VO2max in boys and girls of younger age group of 12-17 years (Gillum,1987). Stein et al., (1990) reported that 12 weeks of cycle ergometric training at intensities more than 80% VO2max increased the concentration of HDL-C, compared to low intensities of aerobic exercise. Study of Romijin (1993) revealed that 2 hr of treadmill running at high intensity, more than 70% VO2max, resulted in a greater intramuscular triglyceride utilization compared with that of low intensity exercise. Ferguson et al., (1998), also observed that treadmill running at 70% VO2max for 1 hr resulting in energy expenditure of 800 kcal did not cause an increase in HDL-C level, whereas energy expenditure of 1100 kcal increased the HDL-C levels. Zhang et al., (1998) related the effect of average maximal oxygen uptake of 56.2 ml/kg/min and energy expenditure during treadmill running at 60% VO2max for 1 hr to the positive effects on lipid and lipoprotein metabolism. They observed that individuals whose VO2max was on an average 48 ml/kg/min decreased their triglycerides levels with an exercise energy expenditure of between 600 and 700 kcal , while HDL-C levels increased in same individuals. Moderate exercise intensities between 50% and 80% VO2max favorably changed HDL-C, total cholesterol and triglyceride levels, if exercise duration and caloric expenditure is sufficient (Crouse et al., 1997, Ferguson et al., 1998 and Bounds et al., 2000). Gill and hardman, (2000) reported that 90 min brisk walking at 60% VO2max on treadmill decreased triglyceride, LDL-C and cholesterol levels where as
HDL-C levels increased in women of post menopausal age. Several investigators have reported that to have beneficial effects of exercise on the lipid and lipoprotein metabolism, total energy expenditure during exercise should be at least 1000 kcal (Malkova et al., 1999 and Herd et al., 2001, Katsanos et al., 2004).

2.5 Effects of Vitamin E and C supplementation on exercise induced oxidative stress.

To combat the deleterious effects of exercise induced oxidative stress, one of the strategy is to supplement the diet of sportspersons with antioxidants. Several researchers investigated the effect of antioxidant supplementation in diet of players on antioxidant levels in blood and oxidative stress. Rokitzki et al., (1994), observed that supplementation of 400 I.U/ day alpha-tocopherol and 200 mg/ day ascorbic acid for 4.5 weeks in diet of athletes prior to a marathon race increased their ascorbic acid, alpha-tocopherol serum concentrations and decreased serum malondialdehyde level. Tauler et al., (2002) reported that plasma vitamin E and C levels of amateur trained male athletes whose diet was supplemented with antioxidants (500 mg/day vitamin E and 30 mg/day beta-carotene for 90 days and 1 g/day vitamin C ) for 15 days were significantly higher as compare to control group without supplementation. Robson et al., (2003) also reported that supplementation of 900 mg/day vitamin C and 90 mg/day of vitamin E for 7 days prior to 2 hr treadmill run at 65% VO₂ max decreased malondialdehyde concentration in blood of healthy endurance athletes. Zoppi et al., (2006) studied the effect of vitamin E and C supplementation on oxidative stress in elite soccer players. Players whose diet was supplemented with vitamin E and C for 3 months prior to session training, had lower lipid peroxidation and catalase activity as compare to control group with placebo receiving a pill containing maltodextrin. Bloomer et al., (2006) supplemented the diet of aerobically trained men and women with 400 IU of vitamin E and 1 g of vitamin C for 2 weeks. Plasma concentration of vitamin E and C increased in players of supplemented group after 30 min run at 80% VO₂ max as compared to that in control placebo group. Malondialdehyde concentration in such players was low after the run. Furthermore, Tauler et al., (2006) studied the effect of antioxidant supplementation for 90 days on sportsmen's basal neutrophil antioxidant defenses. They observed an increase in
neutrophil superoxide dismutase and catalase activities and vitamin E and C levels in supplemented group as compared to placebo group. Vitamin E and C supplementation also resulted in a decrease in serum malondialdehyde level. Goldfarb et al., (2007) studied the effects of 400 IU/day vitamin E and 1 g/day vitamin C supplementation on oxidative stress in endurance trained players who ran for 30 min at 80% VO₂ max, once before and once after 2 weeks of supplementation, and again after a 1-week wash-out period. They reported that plasma vitamin E and C levels were increased and malondialdehyde (MDA) level was reduced in antioxidant supplemented group as compared to the placebo group. Further, antioxidant supplementation attenuated exercise-induced oxidative stress equally in both genders. Machefer et al., (2007) investigated the effect of multivitamin and mineral supplementation [β-carotene (4.8 mg/day) along with vitamin C (150.0 mg/day) and vitamin E (24.0 mg/day)] for 3 weeks prior to an extreme running competition that consisted of six long races in the desert on oxidative stress in players. In supplemented group, plasma alpha-tocopherol and vitamin C levels increased, while malondialdehyde level decreased.

You et al., (2005), studied the effect of vitamin E and C on oxidative stress in rats induced by running for 90 min on a rodent treadmill at a speed of 16 m/min at 16 degrees grade. Male rats were pretreated with a normal rat diet supplemented with antioxidants (2,000 mg vitamin C and 1,000 IU vitamin E/kg diet) for 2 weeks. Malondialdehyde concentrations were lower in blood of rats taking vitamins E and C supplemented diet as compared to rats having normal non supplemented diet. Deaton et al., (2002) studied the effect of dietary supplement containing a mixture of natural antioxidants together with vitamins E and vitamin C for 4 weeks, on oxidative stress resulting from 2 min run at 70, 80 and 90% of the horses' individual maximum oxygen uptake. After run there was an increase in vitamin E and C levels in blood and a decrease in malondialdehyde concentration.