INTRODUCTION
EXERCISE:

Exercise is the process to maintain the physical fitness of the body. Physical exercise induces physiological changes in the tissues of the body (Baldvin et al., 2000). Exercise is a regular, vigorous, planned and a goal oriented program. In general, exercise refers to many types of physical exertions that may vary in its duration, intensity and type. Physical fitness promotes a sense of wholeness of the overall wellbeing and is an important element for the elderly. There are some evidences that physical fitness is a powerful predictor of the disease and that aerobic exercise is better preserved immune system in elderly humans (Ghizalkhan and Shahin, 1998; Brunsgaard and Pederson, 2000). Exercise is not only a leisure activity, but also an effective preventive and therapeutic tool in medicine. Regular physical exercise has well-documented health benefits and can prolong mean life span in animals. It reduces signs of aging and increases average life expectancy by approximately 10% in rats, however, maximum life span remains unchanged (McCarter, 2000; Leeuwenburgh and Heinecke, 2001). The well-documented benefits of regular physical exercise include reduced risk of cardiovascular disease (CVD), cancer, osteoporosis and diabetes (McCarter, 2000).

Regular aerobic exercise is associated with a reduced risk of atherosclerotic vascular disease and acute cardiovascular events, particularly in middle-aged and older adducts (Goldberg et al., 1996). Regularly performed activity induces adaptations that compensate for some of the functional declines associated with
aging, primarily because the habits of physically active older people may have prevented the development of the endocrine metabolic and cardiovascular risk factors that accelerate CVD and its complications (Kissebah and Krakower, 1994).

An understanding of the effects of regular exercise training on the physiological factors that influence the declines in cardiovascular and endocrine metabolic functions with aging may improve the health and functional well-being of the elderly (Goldberg et al., 1996). Population, in general, irrespective of age and gender, perform regular exercise to maintain good health. Exercise is known to evoke numerous physiological changes in vital organ systems of the body. Among those changes, the most important is the enhanced respiration and utilization of oxygen in the body. Increased oxygen influx during exhaustive exercise may be potentially harmful to the body. During the last 15 years, much evidence has accumulated implicating generation of reactive oxygen species (ROS) and other free radicals during exercise in the muscle and heart (Somani and Arroyo, 1995; Leeuwenburgh and Heinecke, 2001). Exposure to drugs and/or chemicals may also generate ROS. However, cells contain several antioxidant defense mechanisms to protect themselves from ROS injury. These include endogenous antioxidants (Glutathione, vitamin C, A, E, uric acid and iron binding protein) and antioxidant enzymes (AOE) superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), referred to as the antioxidant system. The activity of AOE, which are well distributed in all organs of the body, are dependent on oxygen consumption rate, metabolic rate and the amount of metal ions and fatty acids present. The AOE activity is prone to being
altered by changes in oxygen consumption (oxidative stress). Response of an antioxidant system are dependent on the type, mode, intensity, frequency and duration of exercise, animal species, tissue specificity and the extent of exposure to drugs and chemicals. The modulation of AOE activity primarily depends upon its substrate (ROS), co-substrate production, nature of catalytic center, affinity of the enzyme to the substrate (Km) and selectivity and specificity of the substrate.

The transition from rest to exercise is accompanied by substantial alterations in a number of body's function, which allow the body to successfully adapt to this additional stress. As the body is subjected to repeated bouts of exercise, long term adaptation in the bodily function occur. Exercise can be accomplished only through the series of complex interactions within the body involving all the body systems (Brites et al., 1999).

ETHANOL:

"I Was Going to Quit When Things Got Better,  
But Instead They Just Got Bitter"  ---- Confession of an Alcoholic

Alcoholic beverages have been used since the dawn of history. Alcoholism is defined as a complex illness, a chronic disease characterized by reported excessive drinking, which interferes with the individual's health, interpersonal relations or economic functioning. Alcoholism, if untreated becomes more severe and may be fatal. About 4 percent of the population drinks approximately 62 percent of all
alcoholic beverages consumed. This chronic heavy drinking is a significant factor in the development of alcohol dependence or alcoholism and is associated with serious adverse health consequences. Problems with alcohol appear to be a common curse afflicting almost all industrialized nations as well as developing countries. It is estimated that 2,05,000 individuals die prematurely each year from a variety of ethanol-induced factors including cirrhosis, cancer, heart disease, suicide, homicide, highway fatalities and other accidents. Chronic alcohol ingestion leads to impaired cardiac function, including depressed cardiac contraction, ventricular hypertrophy and electrophysiologic abnormalities (Nicholas and Jun, 2003). When alcohol is ingested it reaches the organs of highest blood flow and then presents itself either to effect the total organism or to be affected by the organisms detoxifying or removal apparatus. Chronic exposure to excessive ethanol consumption has adverse effects on virtually all organs and tissues in the body (Pastino et al., 2000). According to the report prepared by World Health Organization, about 20 years ago (1985), the production of alcoholic beverages had continued to rise in most parts of the world. Between 1965 and 1999 the world wide commercial production of alcohol beverages had risen by almost 300% (Ashok Sahni, 2000).

DiLuzio and Hartman (1967) from their experiments on liver suggested that ethanol and its metabolites can stress the balance toward auto-oxidation, either acting as prooxidants or reducing the antioxidant level. The protective action of antioxidants would then probably be due to an inhibition of free radical-induced chain reactions, with the resulting prevention of peroxidative deterioration of structural lipids in
membranous organelles (DiLuzio and Hartman, 1967). Many data show, however, that free radical mechanisms could be involved in the adverse ethanol effects even in tissues poorly metabolizing ethanol, such as the heart or the central nervous system (Nordmann et al., 1992). Production of ROS is a physiological process, but its increase and dysbalance between production of radicals and antioxidants could lead to oxidative stress with the affection of various biological functions and structural changes (Zima et al., 2001).

**Possible Sources of Alcohol-Induced ROS**

Chronic ethanol consumption has been demonstrated to increase the rate of hydrogen peroxide (H₂O₂) and hydroxyl radical (•OH) generation by isolated mitochondria in the presence of metal ion-chelate complexes (Kukielka et al., 1994). One of the most prevalent sources of ROS during chronic treatment with ethanol is cytochrome P-450 2E1 (CYP 2E1). This enzyme system induced by ethanol, is dependent on NADPH as a co-factor and is very reactive in producing superoxide anion (O₂⁻) and H₂O₂ during NADPH oxidation (Bondy and Naderi, 1994; Bailey and Cunningham, 1998). It is located on microsomes in most cells and catalyzes the production of acetaldehyde from ethanol. Because of its kinetic properties, cytochrome P-450 2E1 produces O₂⁻ at a high rate (Fig. 1). Although O₂⁻ is a principal product, H₂O₂ and •OH are also formed via Fenton Chemistry. Thus, the lipid peroxidation seen during chronic ethanol exposure is due, at least in part, to •OH-mediated attack on membranes. Not all ethanol-mediated peroxidation appears
Fig. 1 Diagram of enhanced mitochondrial ROS (i.e., $O_2^\cdot$ and $H_2O_2$) generation via the ubiquinone (Q) cycle after exposure to ethanol. During the Q cycle, ubiquinol ($QH_2$) is oxidized to ubisemiquinone ($Q^\cdot$), with a one-electron (e') transfer to the Rieske iron-sulfur (Fe/S) center. Consequently, $Q^\cdot$ is oxidized by cytochrome $b_{560}$, which itself is oxidized by cytochrome $b_{552}$. Finally, cytochrome $b_{560}$ transfers e' to Q and Q' to regenerate $QH_2$.

to be dependent on ROS. Under certain conditions, cytochrome P-450 2E1 can act as a peroxidase producing •OH-independent lipid peroxidation (Nordmann et al., 1992).

In addition, other peroxidase-like enzymes in the cytochrome P-450 family are altered by exposure to ethanol including cytochrome P-450 2B (Kukielka et al., 1994).

The direct action of ethanol on ROS production has been clearly implicated in ethanol toxicity; however, several studies have demonstrated a role for acetaldehyde in the oxidative damage. Ethanol metabolism by alcohol dehydrogenase to acetaldehyde results in the generation of NADH, potentially shifting the NAD+/NADH ratio (Nordmann et al., 1992). Changes in reducing equivalents have been proposed to favor an enhancement of the electron transport chain in mitochondria, thus increasing the “leak” of ROS from this mechanism (Nordmann, 1992). Alternately, Mira et al. (1995) have demonstrated that xanthine oxidase will metabolize acetaldehyde to acetate, resulting in the generation of O$_2$• as by-product of this reaction. Two conditions must be fulfilled for such an oxidation to occur efficiently: (1) Xanthine dehydrogenase (XDH), which represents the main form of the enzyme, must be largely converted into its oxidase form (XOD) and (2) acetaldehyde should be a substrate for the enzyme.

CYP 2E1-derived O$_2$• radicals increase the mobilization of iron from ferritin, which catalyzes lipid peroxidation. Ferritin was much more effective in stimulating lipid peroxidation of microsomes from rats chronically fed ethanol as compared to control animals, and this stimulation was decreased by anti-CYP2 E1 IgG (Kukielka
and Cederbaum, 1996). Since ferritin is the major storage form of iron within cells, increased mobilization of iron may play a role in the development of oxidative stress after ethanol treatment.

The activity of the citric acid cycle is depressed, partly because of a slowing of the reactions of the cycle that require NAD; the mitochondria will use the hydrogen equivalents originating from ethanol instead of those derived from the oxidation of fatty acids that normally serve as the main energy source of the liver.

**Implication of free radical mechanisms in ethanol-induced myocardial injury**

Tissues from patients who had died from acute ethanol consumption with a previous history of chronic alcohol misuse, show increased myocardial lipopigment (also called "age pigments" as their density is enhanced with age) (Jaatinen et al., 1993). Wannamethee and Shaper (1992) reported a strong correlation between alcohol intake and sudden cardiac death. An oxidative stress may represent a fundamental mechanism in the production of myocardial injury (Ames et al., 1995; Hagen et al., 2002). The increased conversion of XD into XO that has been detected in the heart after administration of a single ethanol dose, may contribute to this lipid peroxidation (Oei et al., 1986).
Since chronic ethanol treatment elicits an enhanced activity of peroxisomal acyl CoA-oxidase and catalase in rat myocardium, increased production of hydrogen peroxide resulting from the activation of acyl CoA-oxidase together with enhanced acetaldehyde generation through the catalase pathway may also play a role in the ethanol-induced enhancement in myocardial lipid peroxidation (El-Sokkary et al., 1999).

AGING:

Aging is a universal, intrinsic, progressive, irreversible and deleterious phenomenon (Strehler, 1962). Aging is a complex, biological process that leads to the gradual loss of the ability of an individual to maintain homeostasis, which is observed to occur in every individual of a given species although they may appear at different rates. It is accompanied by a general decline in the biochemical and physiological functions of most organs, a decrease in the ability of the individual to respond to challenges or stresses and an increase in the susceptibility to age-associated diseases (Wei et al., 1998a). Hence, aging is described as sum total of time-dependent progressive, deleterious and irreversible changes occurring in a cell, in an organ or in the total organism (Patel, 1981). Rockstein (1977) defines “aging as sum total of accumulated deleterious changes which appear during life span of an organism, resulting in failure to withstand to the stress of the environment”. As age advances, several enzymes show an increase, while some decrease and some do not show any change in their activities (Anasuya, 1979; Hussain and Mitra, 2004; Kakarla et al., 2005). These specific alterations in the enzymes must have inflicted a great impact on
the process of aging. Age-dependent changes in physiological capacity would be reflected in intracellular changes, particularly in enzyme systems concerned with effecting such physiological functions. Numerous theories on aging have been proposed on the basis of the experimental data. Some theories suggest that the body of any living organism is subjected to mechanical, thermal, chemical, pharmaceutical reactions, radiations and polluted environment leading to the production of free radical, affecting the cell organelles including nucleic acids (Gracy et al., 1999).

About five decades ago, Harman (1956) first reported that free radicals play a key role in the human aging process. He suggested that the accumulation of free radical-induced damage to vital molecules is an important cause of human aging, subsequently he re-shaped this free radical theory of aging and proposed that mitochondria are the major intracellular targets of free radical attack that leads to human aging (Harman, 1981). Over the years, this theory has received much attention and gained much support from the molecular and cellular biological research on aging. Wei (1998) proposed that during organism aging, the production of reactive oxygen species is increased as a result of the functional deterioration of mitochondria (Fig. 2). There is strong evidence that increased free radical generation may be the underlying reason for several age-related pathogenesis (Ji et al., 1992; Hagen et al., 2002).

It was first demonstrated in 1989 that the respiratory functions of mitochondria gradually decline in human liver (Yen et al., 1989) and skeletal muscle (Trounce et al., 1989). This phenomenon was confirmed in other human tissues
Fig. 2. Mitochondrial role in the determination of life and death of the cell.

Nohl and Kramer (1980) showed that the activity of adenine nucleotide translocase (ADP/ATP carrier) exhibited a 30% decrease in the efficiency of ATP synthesis but also increases the electron leak from the respiratory chain of mitochondria. Luo et al., (1997) showed that a defect in complex I (glutamate-malate supported) of the respiratory chain results in excess production of hydroxyl radicals and enhanced lipid peroxidation in human skin fibroblasts. This finding demonstrated that age-dependent decline of mitochondrial respiratory functions may elicit higher oxidative stress, which in turn may lead to a wide spectrum of oxidative damage.

The reason for the aged animals to increase ROS production is not entirely clear. Age-related defects in mitochondrial electron transport chain (ETC) are considered a major mechanism (Cadenas and Davies, 2000). Mitochondria from aged animals demonstrate much lower cytochrome C oxidase (Complex IV) activity than those from young ones, where as enzyme activities in complex I and II show lesser changes (Nohl et al., 1978). This alteration of ETC stoichiometry favors a greater electron "leakage" and formation of $\cdot OH$ found in the senescent organism. Peroxidative modification of membrane lipids has been proposed to be another major change in mitochondria at old age (Yu, 1994). Higher malondialdehyde (MDA) levels were noticed in the mitochondria from aged heart and skeletal muscle (Fiebig et al., 1996; Marzani et al., 2004). Biochemical and/or structural defects of membrane lipids may cause further ROS generation via enzymatic pathways involving cyclooxygenase, NADPH oxidase and xanthine oxidase (Sawada, 1992).
Under normal physiological conditions, approximately 1-5% of the oxygen consumed by mitochondria of human cells is converted to $O_2^{-}$, $H_2O_2$ and other ROS (Chance et al., 1979; Wei, 1998). Human cells can dispose off ROS by coordinated expression and functioning of an array of free radical scavenging enzymes (Fridovich, 1995; Wei, 1998). However, these antioxidant defense systems are not perfect and are subjected to alterations during aging. As a result, there is an age-dependent increase in the fraction of ROS and free radicals escaping these cellular defense systems and damage cellular constituents including lipids, proteins and nucleic acids (Richter et al., 1995). The rate of production of $O_2^{-}$ and $H_2O_2$ in mitochondria was found to increase with age in various mammalian tissues (Sohal et al., 1994). The enhanced production of ROS inevitably elevates the oxidative stress and oxidative damage of the cell. It is established that the activities and capacities of antioxidant systems of cells decline with age, which in turn leads to a gradual upset of the prooxidant/antioxidant balance and accumulation of oxidative damage, most notably in DNA, in the aging tissues (Wei et al., 1998b).

FREE RADICALS:

Oxygen is essential for life, but we can't live in the presence of oxygen indefinitely. So, oxygen is a dangerous friend. Oxygen toxicity appears to result from the formation of ROS, many of which are free radicals. Free radicals are any molecules with one or more unpaired electrons. These include oxygen-derived free radicals, such as $O_2^{-}$ and $'OH$. Endogenous biological oxidants formed during
metabolism or exogenous environmental sources of free radicals such as cigarette smoke, ozone, ultraviolet light and other free radical substances can damage biomolecules and alter normal functions, thus they could be involved in acute and chronic diseases.

Recent evidences suggest that there are two sources of \( \text{O}_2^{-}\) with in the electron transport chain in the mitochondria. The major source of \( \text{O}_2^{-}\) is ubisemiquinone free radical generated by the reduction of ubiquinone during electron transport. Ubisemiquinone interacts with \( \text{O}_2\) to form \( \text{O}_2^-\).

\[
\text{Ubiquinone} + \text{e}^- \rightarrow \text{Ubisemiquinone}^\cdot \\
\text{Ubisemiquinone}^\cdot + \text{O}_2 \rightarrow \text{Ubiquinone} + \text{O}_2^- 
\]

A second source of mitochondrial \( \text{O}_2^-\) formation has been reported to be NADH dehydrogenase. Quantitatively NADH dehydrogenase generates 50% of the \( \text{O}_2^-\) produced by the ubisemiquinone (Boveries and Cadenas, 1982). Ubisemiquinones are lipophilic and diffuse through the organelle and come in contact with oxygen to produce superoxide (Chance et al., 1979). The metabolism of certain drugs and chemicals in tissue microsomal complex may also result in the generation of ROS that interact with \( \text{O}_2\) to yield \( \text{O}_2^-\) and \( \text{H}_2\text{O}_2\) (Nordmann, 1994; Fernandez-Checa et al., 1997).
The mitochondrial production of $H_2O_2$ was first reported by Jensen in 1966. Further studies have shown that most, if not all, mitochondrial $H_2O_2$ is derived from dismutation of $O_2^-$ (Pollack and Leeuwenburgh, 1999). Superoxide radical generation by mitochondria is greatest when respiratory chain carriers located on the inner mitochondrial membrane are highly reduced. There are species-to-species and organ-to-organ differences in mitochondrial respiratory major sites and specific activities of $O_2^-$ and $H_2O_2$ production by mitochondria isolated from different sources (Turrens, 2003).

Hydroxyl radical production by mitochondria has been reported in aerobic organisms (Cadenas and Davies, 2000). Formation of $\cdot OH$ and $H_2O_2$ accounts for many of the effects of $O_2^-$ generating systems, since $\cdot OH$ radical scavengers, in addition to SOD and Catalase, can protect free radical targets in many *in vitro* and *in vivo* test systems. The generation of the potent oxidant $\cdot OH$ seems to require not only $O_2^-$ or $H_2O_2$ but a transition metal such as iron. These substances can react by what has been termed an iron-catalyzed Haber-Weiss reaction:

$$Fe(III) + O_2^- \rightarrow Fe(II) + O_2$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + \cdot OH$$

Thus, $O_2^-$ reduces iron, which in turn reduces $H_2O_2$ to form $\cdot OH$. Reductants such as ascorbate can also reduce $Fe(III)$, implying that a source of peroxides in the
presence of transition metals can generate -OH in the absence of $O_2^-$. (Winterbourn, 1979).

The plasma membrane is a critical site of free radical reactions for several reasons. Extracellularly generated free radicals must cross the plasma membrane before reacting with other cell components and may initiate toxic reactions at the membrane. The unsaturated fatty acids present in membranes and the trans-membrane proteins containing oxidizable amino acids are susceptible to free radical damage. Also, increased membrane permeability caused by lipid peroxidation or oxidation of structurally important proteins can cause a breakdown of transmembrane ion gradients, loss of secretary functions and inhibition of integrated cellular metabolic processes (Freeman and Crapo, 1982).

Hydrogen peroxide can cross membranes almost as readily as can water. The charged $O_2^-$ molecules can cross membranes and enter cells via trans-membrane anion channels (Kellogg and Fridovich, 1977). Perhydroxyl radical ($H_2O_2^-$) is a stronger oxidant than $O_2^-$ and would be expected to better partition into lipid and the hydrophobic core of proteins and exert toxic effects. Thus, cell surfaces are capable of serving as both targets of reactive free radicals and as a gating mechanism that provides a barrier to charged species and can modify other radical species to a more permeable and reactive form.
LIPID PEROXIDATION:

The aerobic environment involves a potential threat for polyunsaturated lipids. One of the most common actions of ROS in cellular systems is the extraction of hydrogen from susceptible membrane lipids. This reaction can be divided into three steps: the initial production of alkoxyl or peroxyl radicals by $\cdot$OH, the propagation of these reactions by the formation of secondary lipid radicals and the eventual termination of the radical chain reaction by the donation of $H^+$ from antioxidants such as vitamin E (Buettner, 1993). The process is termed lipid peroxidation and is associated with the oxidative degradation of the membrane lipids with polyunsaturated fatty acids (PUFAs) are particularly susceptible targets and molecules with multiple bonds are often lost during lipid peroxidation. These oxidative changes can result in changes in membrane fluidity, in membrane permeability and in the function of membrane-bound transport systems (Halliwell and Gutteridge, 1989).

It has been noted that the extreme reactivity of the hydroxyl radical may result in random attack on organic macromolecules in the vicinity of its origin. These may include enzymes and other proteins and in particular the polyunsaturated fatty acid moieties of membrane phospholipids. The initial carbon-centered radical formed by $\cdot$OH radical attack interacts with molecular oxygen to produce a peroxyl radical, which in turn gives rise to a lipid hydroperoxide. The formation of the hydroperoxide
depends on the abstraction of hydrogen, either from a lipid antioxidant such as vitamin E or in the absence of vitamin E, from other polyunsaturated fatty acid molecules in the vicinity, giving rise to further radical species and the initiation of a chain reaction.

\[
\begin{align*}
  \text{LH} + \cdot \text{OH} &\rightarrow \text{L} + \text{H}_2\text{O} \\
  \text{L} + \text{O}_2 &\rightarrow \text{LOO} \\
\end{align*}
\]

Either \( \text{LOO} + \text{E-H} \rightarrow \text{LOOH} + \text{E} \),

Or \( \text{LOO} + \text{LH} \rightarrow \text{LOOH} + \text{L} \).

Lipid peroxidation can be indicated by a variety of means. Loss of cell membrane unsaturated fatty acids, formation of lipid peroxides and oxygen uptake by lipid preparations all indicate peroxidation. Peroxidation of fatty acids containing three or more double bonds will produce malondialdehyde (MDA). The presence of this oxidation by-product can be measured with thiobarbituric acid (TBA) which, although not a specific or quantitative indicator of fatty acid oxidation, correlates with the extent of lipid peroxidation.

Age pigments, termed lipofuscin, probably, result from lysosomal accumulation of insoluble conjugated Schiff's bases formed from reaction of MDA with lipid and protein during lipid peroxidation. Plasma membrane and organelle
lipid peroxidation can be stimulated by all of the previously mentioned sources of free radicals and is potentiated by the presence of metals. These metals can serve as redox catalysts and also catalyze the conversion of $O_2^-$ and $H_2O_2$ to more potent oxidants (Svingen et al., 1979). Lipid peroxides and lipid peroxy radicals can exert their toxicity by reacting with many of the same cellular components as $O_2$-derived free radicals. Because of the hydrophobic nature of the lipid radicals, most of the reactions will take place with membrane associated molecules. After peroxidation of membrane fatty acids, the presence of shortened-chain fatty acids containing R-OOH, R-COOH, R-CHO and R-OH groups may seriously affect membrane permeability and micro viscosity (Kovacic et al., 2002).

Malondialdehydes produced by peroxidation can cause cross-linking and polymerization of membrane components (Hochstein and Jain, 1981). This can alter intrinsic membrane properties such as deformability, ion transport, enzyme activity and the aggregation state of cell surface determinants. Because MDA is diffusible, it will also react with nitrogenous bases of DNA (Donato, 1981).

**ANTIOXIDANT DEFENSE SYSTEM:**

About a decade ago, scientists from various countries signed in Saas Fee (Switzerland), a declaration on the significance of antioxidants in preventive medicine. This declaration stated that antioxidant nutrients may have major significance in the prevention of a number of diseases. These include cardiovascular
and cerebro-vascular diseases, some forms of cancer and several other disorders, many of which may be age-related (Nordmann, 1994).

Although oxygen free radicals are generated as natural byproducts of various biological pathways, serious cell and tissue damage does not usually occur under physiological conditions. Aging might be an exception, during which the slow but the deleterious effect of ROS leads organisms to functional deterioration and death (Behzad and Jawahar, 2004). Heavy physical exercise rarely causes large scale oxidative damage in healthy individuals. This is because higher organisms have developed a remarkably efficient antioxidant system during the course of evolution (Halliwell and Gutteridge, 1989). Thus, the extent of oxidative damage during physical exercise is determined not only by the level of free radical generation, but also by the defense capacity of antioxidants. The system consists of antioxidant vitamins (water-soluble ascorbic acid and fat-soluble α-tocopherol and β-carotene), thiol-containing, low molecular weight compounds, mainly glutathione (GSH) content and antioxidant enzymes, such as SOD, CAT and GSH-Px (Fig. 3). Each of these antioxidants plays a unique role in the cell and components one another geographically and functionally (Chance et al., 1979). Furthermore, there is evidence that certain antioxidants, such as GSH may be involved in inter organ transport (Ji and Leeuwenburgh, 1996). These antioxidant defense system preserve homeostasis for normal cell function at rest and perhaps during mild oxidative stress. However, the protective margin of ROS production is excessive, or when the antioxidant
Fig. 3: A summary of the major antioxidant enzymes which play a role in controlling free radical damage.

defense is severely compromised due to nutritional deficiency or biochemical inhibition, extensive cell and tissue damage may occur, leading to various pathogenic conditions and/or aging (Ames et al., 1995). The resultant oxidative damage can induce further ROS production thereby forming a vicious cycle.

**Pro-oxidants and Antioxidants**

It has been shown that \( \mathrm{O}_2^{•−} \) may be generated by monoelectronic reduction of dioxygen through various enzymatic mechanisms. Furthermore, \( \mathrm{O}_2^{•−} \) is able to generate the highly potent and aggressive pro-oxidant \( \cdot\mathrm{OH} \) through the iron-catalyzed Haber-Weiss reaction.

In order to avoid such cellular disorders, efficient cellular antioxidant mechanisms are present. They include enzymes such as SOD, which enhances the generation of \( \mathrm{H}_2\mathrm{O}_2 \) from \( \mathrm{O}_2^{•−} \); and catalase and glutathione peroxidase, which destroy hydrogen peroxide and thus hinder the generation of \( \cdot\mathrm{OH} \).

Another line of antioxidant defense is represented by substrates acting as chain-breaking antioxidants against destructive process linked to prooxidants. The main membranous chain breaking antioxidant is \( \alpha \)-tocopherol (vitamin E) (Niki, 1993) which is able to quench lipid peroxyl radicals resulting from peroxidative attacks on membranous polyunsaturated fatty acids. During this reaction, \( \alpha \)-tocopherol is converted into its free radical form. Since the concentration of vitamin E is extremely low inside the membranes, \( \alpha \)-tocopherol would be rapidly lost if it was not regulated from its radical form by processes preventing the irreversible
formation of α-tocopherol quinine as well as of further degradation products. The regeneration of α-tocopherol from its radical form is mainly achieved by reduction through ascorbate (Packer et al., 1979). Owing to its localization close to the interface of the membranes, α-tocopherol is able to react with the water-soluble ascorbate, which therefore efficiently contributes to the regeneration of vitamin E.

Glutathione represents one of these auxiliary systems contributing to the regeneration of α-tocopherol from its radical form (Meister, 1994). Glutathione has many other antioxidant properties and is the substrate of the already mentioned glutathione peroxidase as well as of glutathione-S-transferase, a family of enzymes well represented in the cell and playing a prominent role in antioxidant defense (Boyer, 1989).

Endogenous Non Enzymatic Antioxidants

This category of antioxidants refers to antioxidant vitamins (vitamin E, vitamin C and β-carotene), GSH and other thiols. Several biological compounds such as α-lipoic acid, uric acid and ubiquinone also demonstrate antioxidant functions (Yu, 1994; Packer et al., 1995).

Vitamin E (α-tocopherol)

Vitamin E is chemically referred to as α-tocopherol (Burton et al., 1982) and is the most widely distributed antioxidant. Because of the lipophilic property of the α-tocopherol molecule, it is the major free radical chain termination in plasma.
lipoproteins. Intracellularly, vitamin E is associated with lipid rich membranes such as mitochondria and endoplasmic reticulum.

**Vitamin C (Ascorbic Acid)**

Vitamin C is a water soluble antioxidant existing in the cytosol and extracellular fluid. Its chemical properties allow it to interact directly with O₂ and •OH, thereby functions as an antioxidant (Beyer, 1994). It can also regenerate oxidized vitamin E, wherein ascorbate is oxidized to dihydroascorbate (DHA). DHA may be reduced by a GSH and/or dihydrolipoic acid redox cycle (Halliwell and Gutteridge, 1989). Vitamin C is especially efficient in scavenging free radicals formed in the aqueous phase such as plasma, thereby preventing damage to erythrocyte membrane (Beyer, 1994).

**Glutathione**

Reduced glutathione (GSH), a tripeptide (γ-glutamyl cysteinyl glycine), is the most abundant thiol present in all mammalian cells. GSH concentration in the cell is in the millimolar range for most tissues, but there is variability in different organs depending on their functions of oxidative capacity. GSH serves multiple functions in protecting tissues from oxidative damage and in keeping the intracellular environment in the reduced state (Meister and Anderson, 1983). GSH reduces hydrogen and organic peroxides via a reaction catalyzed by GPx; it serves as a scavenger of •OH and singlet oxygen (O₂⁻) (Halliwell and Gutteridge, 1989); and GSH is believed to reduce tocopherol radicals, either directly or indirectly by
reducing DHA radical, thereby prevent lipid peroxidation (Niki et al., 1985). By donating its proton, GSH is oxidized to GSSG, which can be reduced back to GSH by glutathione reductase (GR), a flavon-containing enzyme, using NADPH as the reducing power.

GSH can be synthesized from endogenous or dietary amino acids, but only the liver contributes to significant de novo GSH synthesis, supplying 90% of the circulating GSH (Meister and Anderson, 1983).

**Uric acid**

Uric acid is the end product of purine metabolism in mammals. However, its antioxidant action was first reported by Howell and Wyngarden (1960). Its antioxidant properties were confirmed by its protection against oxidative damage (Ames et al., 1981). Besides being an excellent scavenger of •OH, uric acid may preserve plasma ascorbic acid under oxidative stress (Sevanian et al., 1985).

**Endogenous Enzymatic Antioxidants**

Cells are equipped with a host of enzymes that are directly or indirectly involved in the antioxidant defense against ROS. Enzymes that provide primary defenses include SOD, CAT, and GSH-Px. GR and enzymes producing NADPH, such as G-6-PDH, malic enzyme and isocitrate dehydrogenase (ICDH) are important in reducing GSSG to GSH, such that an adequate substrate level is maintained for
GSH-Px. GST is an important enzyme in metabolizing pro-oxidant xenobiotics in the liver. Secondary defense includes a group of loosely defined enzymes, which either repair cellular damage caused by ROS or remove the damaged molecules, such as phospholipase A₂ or specific proteases (Yu, 1994).

**Superoxide Dismutase**

The catalytic function of SOD was discovered by McCord and Fridovich (1969). It exists in virtually all O₂-respiring organisms and its major function is to catalyze the dismutative reactions.

\[
O_2^- + O_2^- \xrightarrow{\text{SOD}} H_2O_2 + O_2
\]

Superoxide dismutases are classified into three distinct classes depending on the metal ion content. They are Cu/Zn-SOD, Mn-SOD and Fe-SOD. In mammalian cells, there are two isozymes located in different sub-cellular compartments. The cytoplasm contains Cu/Zn-SOD (dimeric proteins) and mitochondria contain Mn-SOD (tetrameric protein). Mammalian cells also have the third SOD-isozyme, extracellular SOD, which is Cu/Zn-SOD (tetrameric protein) (Fridovich, 1995). The activity of the SOD varies among the tissues.

Most SOD assays involve an *in vitro* O₂ generating system making it impossible to evaluate the influence of O₂⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻긩
indefinitely with the increased substrate concentration and no apparent Km or V max (Chance et al., 1979; Halliwell and Gutteridge, 1989). Superoxide anion can generate H$_2$O$_2$ independent of SOD in the cell by attacking the iron-sulfur protein (4 Fe-4S) cluster, thereby releasing Fe (II), which sets the stage for •OH production via the Fenton reaction or via Haber-Weiss reaction (Halliwell and Gutteridge, 1989). This "cooperativity" between O$_2$ and H$_2$O$_2$ is an important mechanism for cellular damage, particularly DNA damage (Fridovich, 1995).

**Catalase**

Catalase is a major component of the antioxidant system and catalyzes the decomposition of H$_2$O$_2$. It has also a peroxide role in which the peroxide is utilized to oxidize H donors (AH$_2$).

\[
\begin{align*}
(i) \quad 2\text{H}_2\text{O}_2 & \quad \text{CAT} \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \\
\text{and} \quad (ii) \quad \text{AH}_2 + \text{H}_2\text{O}_2 & \quad \text{CAT} \rightarrow \text{A} + 2\text{H}_2\text{O}
\end{align*}
\]

Although the tissue distribution of catalase is widespread, the level of activity varies not only between tissues but within the cell itself. Catalase is present predominantly in the peroxisomes (microbodies) in liver and kidney and also in the micro peroxisomes of other tissues. It is important to recognize that although GPx and catalase have an overlap of substrate H$_2$O$_2$, GSH-Px (at least in mammals) has a much greater affinity for H$_2$O$_2$ at low concentrations (Km = 1µM) (Sies, 1985). Thus,
the threshold for activation for catalase may be higher than for GSH-Px. These kinetic properties probably explain why most studies find no significant alteration in catalase activity in several tissues that show prominent training adaptation of GSH-Px (Ji, 1995).

**Glutathione Peroxidase**

Glutathione peroxidase (GSH-Px) catalyzes the reduction of \( \text{H}_2\text{O}_2 \) and organic hydroperoxide and peroxides as follows.

\[
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GSH-Px}} \text{GSSG} + 2\text{H}_2\text{O}
\]

or

\[
2\text{GSH} + \text{ROOH} \xrightarrow{\text{GSH-Px}} \text{GSSG} + \text{H}_2\text{O} + \text{ROH}
\]

Both types of GSH-Px enzymes, selenium-dependent and selenium-independent, have been shown to catalyze these reactions and thus protect against radical damage by reducing peroxides. However, they possess different substrate specificities. Glutathione peroxidase is mostly present in the cytosol. The cytosolic and membrane-bound monomeric GSH-Px and the tetramere plasma GSH-Px are able to reduce phospholipids and hydroperoxides. This enzyme has an absolute specificity for \( \text{GSH} \) as its electron-donating substrate: however, its specificity for peroxide is much less selective. Within the cell, the distribution of GSH-Px is almost complimentary to that of catalase.
GSH-Px is susceptible to inactivation by O₂ and hydroperoxides in vitro due to the oxidation of the selenocysteine residue at the enzyme's active site (Blum and Fridovich, 1985). A high concentration of GSH is required to keep this active site in the reduced state.

**Glutathione Reductase**

Although glutathione reductase (GR) is not directly involved in removing ROS, it serves an important role in converting GSSG to GSH, thereby maintaining GSH-Px catalytic function and a reduced intracellular redox status (Halliwell and Gutteridge, 1989). Glutathione reductase is an ancillary enzyme to limit the amounts of ROS via its reduction of GSSG in the presence of an adequate supply of NADPH. Thus, the ratio of GSH/GSSG is maintained at a high level so that the cell maintains the capacity to combat oxidative stress.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADPH}
\]

**Glutathione-S-transferase**

Glutathione-s-transferase (GST) catalyzes the conjugation of GSH with a wide variety of organic compounds, including certain species of hydroperoxides, thereby shares peroxidase activity with GSH-Px (Habig et al., 1984). Unlike GSH-Px, GST activity is not affected by selenium deficiency; however, adequate GSH concentration is critical for the enzyme's catalytic function.
EXERCISE AND AGING:

The interplay of exercise in the aging process is the subject of interest for scientists in the field of exercise on old age and the role of training adaptation and metabolic events leads to improvement of health and physical strength of elderly. The performance of physical exercise involves the integrated activity and coordination of many different organ systems. The primary requirement involves the delivery of an increased supply of oxygen to tissue and removal of waste products. However, the maximal oxygen uptake significantly diminishes with advancement of age. It is observed that the trained animals have more oxygen intake than untrained individuals. Numerous studies have shown that middle aged and older men who engage in regular exercise are able to develop higher respiratory capacity during exercise than the sedentary people (Evans et al., 1995).

Physical training can readily produce a profound improvement of functions essential for physical fitness in old age and thus effectively postpone physical deterioration for some individual up to 10-20 years (Astrand, 1992). Both healthy individuals and those with chronic illness and functional handicaps can improve their performance and therefore, their quality of life can be increased by physical activity (Astrand, 1988).

Fiatarone (1992) suggested that exercise training and life-long physical activity may contribute to a slower decline of age associated functional systems and may prevent or delay the onset of degenerative diseases. Exercise clearly has
beneficial effects on several aging processes as well as on certain age-related disorders like cardiovascular disease (Brites et al., 1999; Gunduz et al., 2004). In rodents and humans weight loss that accompanies exercise is beneficial. Fitness programs improve physical performance and also cognition memory and stress reaction (Vinod Kumar and Prasad, 1990).

EXERCISE AND OXIDATIVE METABOLISM:

Physical exercise training brings about specific metabolic and physiological adaptations that involve subtle cellular as well as gross physiological changes. Anaerobic training increases resting levels of anaerobic substrates and key glycolytic enzymes (Shaver, 1982). Aerobic changes include increase in mitochondrial size and number as well as the activity of aerobic enzymes, increased myoglobin and enhanced oxidation of fats and carbohydrates. Physical training develops strength, endurance and speed performance in various physical and physiological works (Westerterp, 2000; Indira Sriram and Jhansi Lakshmi, 2001).

Exercise influences oxidative metabolism and produces ROS which appear to play a key role in changing the membrane fatty acid composition, permeability and leakage of enzymes and chemotactive factors. All of which elicit sets of metabolic events that lead to the repair process (Yu, 1994). Meites (1993) reported that regular moderate exercise in elderly humans decreases incidence of heart disease, improves lung function and reduces bone loss. During exercise, oxygen consumption by the
body can increase by as much as 20 folds; ROS are generated during this time (Sjodin et al., 1990; Somani et al., 1995). ROS not only interact with membrane lipids causing lipid peroxidation but they are capable of reacting with enzyme proteins and DNA. Lipid peroxidation resulting from oxidative stress produces MDA and cause cytotoxic effects (Chaudhury et al., 1994).

Gurumurthy (2001) reported improved glycolytic pathway and increase in the activities of Krebs cycle enzymes to produce more energy to meet energy demands. Lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities are elevated during exercise training. Exercise shows a significant effect on lipid metabolism. Several studies demonstrated the effects of exercise in reducing cardiovascular diseases by reducing cholesterol and or by raising HDL cholesterol (Jhansi Lakshmi, 1998; DeSouza et al., 2000).

Exercise is known to evoke numerous physiological changes in vital organ systems of the body. Among those changes the most important is the enhanced respiration and utilization of oxygen in the body. Physical training increases the muscle respiratory capacity due to increased concentrations of mitochondrial, myoglobin and enzymes of TCA cycle and cytochrome system suggesting that the metabolic transducer gets adapted to increase energy requirements by physical training. Bigard et al. (1991) observed that training increased the activity levels of enzymes involved in glucose phosphorylation, the TCA cycle and β-oxidation of fatty acids.
EXERCISE AND ANTIOXIDANT SYSTEM:

During physical exercise large amount of oxygen is inhaled into the body and earlier studies provided evidence that body is subjected to oxidative stress during exercise (Schroder et al., 2001; Sen, 2001; Vijay Kumar and Naidu, 2002; Chevion et al., 2003). Biological antioxidants play a vital role in coping with an exercise induced oxidative stress. Deficiency or depletion of various antioxidant system enzymes has been shown to increase the extent of oxidative tissue injury in exercise (Leeuwenburgh and Heinecke, 2001).

Since exercise increases metabolic rate which is reflected by a greater amount of oxygen uptake, ROS production is also expected to increase during physical exertion, while this seem to be detrimental to the elderly who are physically active, exercise is also known to cause adaptive responses such as improvement in antioxidant defense capacity (Ji, 1995).

Physical activity is recognized as an important component of healthy life style and recommended throughout life by scientists and clinicians (Donaldson, 2000). Exercise training is recommended for improving physiological and functional capacity in the elderly (Rhodes et al., 2000). However, exercise like aging, is one of the physiological conditions characterized by increased production of free radicals (Clarkson, 1995). The production of free radicals increases in parallel with the increase in oxygen consumption during exercise, and this increase is directly related
to the intensity and/or the duration of exercise (Ji, 1996). On the other hand the antioxidant enzymes which constitute a defense mechanism against free radicals produced during exercise are also affected by the exercise (Clarkson, 1995).

ANTIOXIDANT DEFENSE AND AGING:

With aging due to increased free radical production the antioxidant capacity decreases. Ji (1993) reported decreased heart cytosolic superoxide dismutase activity. Ji et al., (1991) reported weakened myocardial antioxidant capacity during aging. Decreasing activity or constitutive levels of oxidant repair enzymes may contribute to progressive accumulation of oxidant damage with aging (Pacifici and Davies, 1991). Antioxidant defense provides the function of detoxification and it prevents the formation of ROS and lipoperoxides with SOD, CAT, GSH-Px, GSH, α-tocopherol and ascorbate. The reason for the detoxification disturbance during aging probably may be due to insufficiency of antioxidant defense (Logenov and Matyushin, 1997).

INTERACTION OF EXERCISE AND ETHANOL WITH ANTIOXIDANT SYSTEM:

The biotransformation of chemicals can be both harmful and helpful for the body under certain conditions; a chemical can be safely biotransformed and excreted from the body. However, several metabolic pathways could form electrophilic
intermediate metabolites and generate $O_2^{-}$ and free radicals. These metabolites can covalently bind to macromolecules and produce tissue-specific toxicity. Two specific pathways that produce free radicals or superoxide anions include NADPH-cytochrome P-450 reductase (Ortiz de Montellano, 1986) and one electron reduction under low oxygen tension (De Groot and Noll, 1983). Once these reactive metabolites are generated interaction with the ferrous ion could produce the hydroxyl radical, which could lead to toxic effects. Because exercise will increase oxygen consumption and the status of AOE, it may be a means of decreasing the toxicity that several chemicals produce. Both exercise as well as ethanol is known to exert oxidative stress to vital organs and tissues of the body (Bailey et al., 1999; DiMeo and Venditti, 2001; Leeuwenburgh and Heinecke, 2001). However, the interactive effects of the combination of both on the tissues were not known. Kendrick et al. (1993) reported that ingestion of ethanol adversely influenced treadmill exercise performance in human. Exercise training was shown to prevent the toxicity of Adriamycin, known to cause cardiac toxicity, by enhancing the activity of the AOE (Kanter et al., 1985). These toxicities may result from the free radical and oxidative stress that they generate. The question to be answered is whether exercise will decrease the free radicals and oxidative stress that these agents may generate. This is an area that has not been investigated extensively and an attempt has been made in this present investigation to understand the interaction of exercise and ethanol on aging subjects.
OBJECTIVES OF THE PRESENT STUDY:

Both aging and chronic ethanol consumption have been found to produce changes in lipid composition and antioxidant defense. Severity of intoxication, withdrawal and release of gamma-amino butyric acid following chronic ethanol consumption has been shown to be associated with age. It is logical, therefore, that differences in the cardiovascular response to ethanol consumption, when comparing younger with older individuals, may exist. Elderly people may be more susceptible than younger people to the oxidative stress, induced by alcohol drinking. As the number of elderly drinkers in the population is quite large, who are also doing regular exercise; the effects of regular exercise on aging and alcohol-induced oxidative injury deserve thorough investigation. An understanding of the effects of regular exercise training on the physiological factors that influence the decline in cardiovascular and endocrine metabolic functions with age and ethanol toxicity may improve the health and functional well-being of the elderly.

The main objectives of the present investigation are

1. To examine whether treadmill exercise training could alter the effects of ethanol-induced oxidative stress in the myocardium of two different age groups of rats, i.e., young and old age groups.

2. To investigate the interactive effects of exercise training and ethanol on the age associated free radical production and antioxidant defense mechanism.

3. To evaluate the oxidative metabolic responses of myocardium due to the interactive effects of exercise and ethanol in young and old age groups of rats.
PROGRAM OF THE PRESENT STUDY:

The following aspects of energy metabolism and antioxidant defense system in myocardial tissue of sedentary control and experimental groups i.e., exercise trained, ethanol treated and exercise plus ethanol treated animals of younger and older age groups of male albino rats.

(i) The effect of ethanol and aging on the anaerobic and aerobic energy pathways and the beneficial impact of exercise training have been assessed by quantifying the enzyme systems such as LDH, ICDH, SDH, MDH and G-6-PDH.

(ii) The lipid peroxides formed were measured as malondialdehyde (MDA) content to examine the fate of free radical production through lipid peroxidation during ethanol toxicity in aging rats with reference to exercise training.

(iii) Xanthine oxidase (XOD) activity levels were measured to assess the production of free radicals and also uric acid formation in response to exercise training and ethanol toxicity in two different age groups of rats.

(iv) Effect of the combination of exercise training and ethanol on the contents of antioxidants such as glutathione, ascorbic acid and uric acid was evaluated in younger and older rats.
The activities of GSH-Px, GR and GST were measured to analyze the role of glutathione mediated antioxidant system in combating oxidative stress induced by ethanol and aging in exercised rats.

To assess the extent of dismutation and hydroperoxide detoxification by SOD and CAT enzymes respectively were measured in control and experimental rats of both the age groups.

The results obtained in the present study have been discussed in the light of available recent reports to understand the combined action of exercise and ethanol on the oxidative metabolism and antioxidant defense system in the myocardial tissue of two different age groups of rats.