If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health. —Hippocrates (c. 460-377 BC)

Hippocrates espoused a rational approach to health without relying on oracles or divine intervention. His approach, based on inductive reasoning, created a science of medicine. Thus, Hippocrates, the Father of Medicine, occupies a special place in the field of Exercise Nutrition.
THE BEGINNING

Exercise physiology arose mainly in early Greece and Asia Minor, although the topics of exercise, sports, games, and health concerned even earlier civilizations. The greatest influence on western civilization, however, came from the Greek physicians of antiquity – Herodicus (5th century B.C.), Hippocrates (460-377 B.C.), and Claudius Galenus or Galen (A.D. 131-201).

The first female exercise physiology laboratory and associated Degree program in the United States was established in 1892 at Harvard University.

Much of the history of exercise physiology in the United States can be traced to the effort of a Kansas farm boy, David Bruce Dill, whose interest in physiology first led him to study the composition of crocodile blood. Fortunately for us, this young scientist redirected his research to humans when he became the first director of the Harvard Fatigue Laboratory, established in 1927.

This discussion regarding the overview of exercise physiology can be visualized with clippings of a passage from an American physiology and hygiene text book written over 130 years ago by J.C. Dalton, M.D., a professor of physiology in the College of Physicians and Surgeons in New York City. The comments are supposed to enlighten the modern views of nutritional impact on performances, which is the basis of antioxidant research.

“The natural force of the muscular system requires to be maintained by constant and regular exercise”.

“The muscular exercise of the body, in order to produce its proper effect, should be regular and moderate in degree. Exercise which is so-violent and long-continued as to produce exhaustion or unnatural fatigue is an injury instead of an advantage, and creates a waste and expenditure of the muscular force instead of its healthy increase” – this comment can attribute to the origin and perspective of oxidative damage by ROS.

“The exact quantity of exercise to be taken is not precisely the same for different persons, but should be measured by its effect. It is always beneficial when it has fully employed the muscular powers without producing any sense of excessive fatigue or exhaustion” – the comment vividly indicates the optimization of exercise stress.
Current literature of free radical research and antioxidant treatment in combating exercise-induced oxidative stress also indicates a similar theme.

In recent years, with ever improving levels of competition, athletes and coaches have developed considerable interest in nutrition. Unfortunately, this is an area in which the scientific efforts of trained nutritionists and biochemists have often been obscured by the misinformation and pseudo-wisdom of the so-called self-taught and ignorant quacks.

“Performance can be improved by modifying a basically sound diet” – is the novel idea of this century.

It is possible to obtain an adequate amount of every nutrient through food. It is not easy though, and requires both planning and taking a minimum number of calories from nutrient-rich sources, each day. For e.g. even if we are eating plenty of vitamin E rich vegetable oils and nuts, a diet of about 2,500 calories a day would provide, at most, 40 to 50 international units (IU) of vitamin E.

Vitamin E, vitamin C and β-carotene apparently seem to act as antioxidants. This role is, however, an important one. Vitamin E, the first vitamin discovered to act as an antioxidant, is considered to be the strongest antioxidant. So, if we want the antioxidant effect, we will have to take supplements.

In females, endurance exercise is likely to produce more oxidative stress if free radical (ROS) production is great enough to overcome the body’s normal antioxidant defense system (Alessio, 1993).

The research work undertaken here attempts to elucidate a lot of unknown factors like, the specificity of nutrient necessary for different conditions; the quantity of nutrients required; the efficacy of the need – permanent or temporary; the period of treatment required and so on. During the humble work, newer horizon of ideas developed; newer hopes with novel dimensions emerged. Sometimes those became the “turning points” of the project; sometimes attempts were futile. Still the search for the “mystery” was on and will be.

“… But I have promises to keep
And Miles to go before I sleep
And Miles to go before I sleep”
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I. EXERCISE INDUCED OXIDATIVE STRESS

Exercise represents a challenge to the body’s control system to maintain homeostasis. In general, the body’s main control systems are capable of maintaining a steady state during most types of submaximal exercise in a cool environment (Power and Howley, 1997).

The human body is made for physical work (Rodahl, 1989). Exercise physiology is concerned with the study of how the body adapts to acute stress of exercise or physical activity and the chronic stress of physical training (Fig. 1).

<table>
<thead>
<tr>
<th>NATURE OF WORK TO BE PERFORMED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type: Physical or mental</td>
</tr>
<tr>
<td>Load: (a) Light or heavy</td>
</tr>
<tr>
<td>(b) Small or large muscle</td>
</tr>
<tr>
<td>groups</td>
</tr>
<tr>
<td>Rhythm: (a) Continuous or</td>
</tr>
<tr>
<td>intermittent</td>
</tr>
<tr>
<td>(b) Static or dynamic</td>
</tr>
<tr>
<td>Duration: Brief or prolonged</td>
</tr>
<tr>
<td>Schedule: Day work or shift</td>
</tr>
<tr>
<td>work</td>
</tr>
<tr>
<td>Working position: Sitting or</td>
</tr>
<tr>
<td>standing</td>
</tr>
<tr>
<td>Working techniques</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SOMATIC FACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy or sick</td>
</tr>
<tr>
<td>Male or Female</td>
</tr>
<tr>
<td>Small or Large</td>
</tr>
<tr>
<td>Old or Young</td>
</tr>
<tr>
<td>Fed or Starved</td>
</tr>
<tr>
<td>Individual differences</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRAINING Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSYCHIC FACTORS</td>
</tr>
<tr>
<td>Attitude: positive or</td>
</tr>
<tr>
<td>negative</td>
</tr>
<tr>
<td>Motivation: reward</td>
</tr>
<tr>
<td>(selection)</td>
</tr>
<tr>
<td>Sleep deprivation</td>
</tr>
<tr>
<td>Stress: Positive or</td>
</tr>
<tr>
<td>negative</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SERVICE FUNCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. FUEL</td>
</tr>
<tr>
<td>(a) intake</td>
</tr>
<tr>
<td>(b) storage</td>
</tr>
<tr>
<td>2. OXYGEN UPTAKE</td>
</tr>
<tr>
<td>(a) Pulmonary ventilation</td>
</tr>
<tr>
<td>(b) Cardiac output</td>
</tr>
<tr>
<td>i. stroke volume</td>
</tr>
<tr>
<td>ii. heart rate</td>
</tr>
<tr>
<td>(c) Cardiac output</td>
</tr>
<tr>
<td>(a-v O₂ diff.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ENVIRONMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude</td>
</tr>
<tr>
<td>High gas pressure</td>
</tr>
<tr>
<td>(underwater operations)</td>
</tr>
<tr>
<td>Temperature: (a) heat</td>
</tr>
<tr>
<td>(b) cold</td>
</tr>
<tr>
<td>Humidity</td>
</tr>
<tr>
<td>Air velocity</td>
</tr>
<tr>
<td>Noise Vibration: (a) Local</td>
</tr>
<tr>
<td>(b) Whole body</td>
</tr>
<tr>
<td>Air pollution: (a) dust</td>
</tr>
<tr>
<td>(b) gases: O₂, O₃, CO, CO₂, SO₂, etc.</td>
</tr>
</tbody>
</table>

Energy yielding processes i.e., transformation of chemical energy into work

Physical performance

Fig.1: Factors affecting physical performance (modified from Åstrand and Rodahl, 1986).
Good exercise habits should be practiced over one's lifetime. Research indicates that individuals should exercise in order to decrease the risk of disease. Researchers professed that “Physical inactivity is a disease: exercise is a pediatric, primary care, general internal, rehabilitative, and geriatric medicine”.

During exercise, bodily O2 consumption is greatly increased and it seems likely that more O2− and H2O2 form in vivo, since O2− can be a product of electron leakage from mitochondrial electron transport chains. Thus Davies et al., in the USA found that severe forced physical exercise in rats results in muscle damage, seen as a decrease in mitochondrial respiratory control, loss of structural integrity of sarcoplasmic reticulum, and increased levels of some markers of lipid peroxidation. Vitamin E-deficient rats have markedly lower endurance capacity for exercise. Protection against oxidative damage might be offered by careful endurance training. Quintanilha and Davies in the USA found that such training increased the activities of glutathione peroxidase, glutathione reductase, catalase, and SOD in rat heart and skeletal muscle, although it seemed to decrease the content of vitamin E in muscle mitochondria.

Do these observations have relevance in humans? Prolonged exercise, especially in untrained individuals, produces muscle damage, as demonstrated by microscopic studies or by release of muscle enzymes into the circulation. Some myoglobin can also leak into plasma. Exercise increases the number of circulating neutrophils and may produce some features of an acute-phase response, e.g., fall in plasma zinc and iron, and rise in C-reactive protein. Loss of iron and zinc in sweat may contribute to these changes. Scientists found that trained athletes have higher concentrations of caeruloplasmin in plasma than normal controls; caeruloplasmin is part of the extracellular antioxidant defenses. Dillard et al., 1978, in the USA found that some human subjects responded to exercise by increased exhalation of pentane (possibly arising from lipid peroxidation), an effect that could be diminished by pre-treating the subjects with excess oral vitamin E.
a. Free Radical Generation During Exercise

A free radical is a highly chemically reactive molecule or molecular fragment that contains at least one unpaired electron in its outer orbital or valence shell. Once formed, they can interact with other compounds to create new free radical molecules. Thus a free radical can be defined as “a chemical species possessing an unpaired electron”. Free radicals have important properties like:

(i) high reactivity with short life span (μs);
(ii) autocatalytic and diverse chemical reactivity;
(iii) generated both in vitro and in vivo;
(iv) low chemical specificity;

They can easily be formed when a covalent bond is broken, if one electron from each of the pair shared remains with each atom – a process known as homolytic fission.

Free radicals can be generated in 3 possible ways:

(a) Ion formation by heterolytic fission

\[ \begin{align*}
A_x^+ B & \longrightarrow A_x^- + B^+ \\
\end{align*} \]

(b) Radical formation by homolytic fission

\[ \begin{align*}
A_x^+ B & \longrightarrow A_x^- + B_x^- \\
\end{align*} \]

Where \( A_x^+ \) is an A -radical, often written as \( A^o \)

\( B_x^- \) is a B-radical (\( B^o \))

\( \times \) represents the electron pair

(c) Radical formation by electron transfer

\[ \begin{align*}
P + e^- & \longrightarrow P^- \\
\end{align*} \]

ROS and free radicals can be produced during exercise in a number of ways:
(i) Primarily via

**Mitochondrial Electron Transport Chain**

The majority of $O_2$ consumed by the eukaryotes is reduced in the mitochondria through the electron-transport chain (ETC). Both NADH-ubiquinone reductase and ubiquinone-cytochrome c reductase generate $O_2^-$ and $H_2O_2$. Because transition from two-electron carrier (NADH and $FADH_2$) to one-electron carrier (ubiquinone) involves the formation of semiubiquinone ($QH^+$), this segment of the ETC becomes a primary site for $O_2^-$ production (Davies et al., 1982). $O_2^-$ is readily reduced to $H_2O_2$ by mitochondrial superoxide dismutase (SOD; Mn containing). A metal-catalyzed Fenton reaction or Haber-Weiss reaction may give rise to $'OH$ (Halliwell and Gutteridge, 1989). Mitochondrial $H_2O_2$ production can be increased with increased $O_2$ tension, making it a viable source of ROS when metabolic rate and $O_2$ consumption are increased (Fig. 2).

Assuming that the percentage of $O_2$ to be converted to $O_2^-$ remains the same (i.e. ETC efficiency maintains the same), ROS production will increase roughly proportionally. It may be argued that $O_2$ production has been found to be lower in state 3 (ADP-stimulated) than state 4 (basal) respiration in isolated mitochondria. It is doubtful that a more efficient oxidative phosphorylation
can completely compensate for the electron leakage, however, due to a much greater \( \text{O}_2 \) flux during heavy exercise. Furthermore, there is evidence that heavy exercise may induce mitochondrial uncoupling due to inner membrane damage and hyperthermia (Davies et al., 1982). However, the actual rate of ROS production from mitochondrial source during exercise is unknown. The hypothesis that mitochondria comprise a primary site of ROS generation during exercise has been implicated in numerous studies.

(ii) The second source may be

Xanthine / Xanthine Oxidase Pathway

Xanthine oxidase (XO)-catalyzed reactions have been well established as one of the major sources of free radical generation in the ischaemic-reperfused (I-R) heart (Downey, 1990). During ischaemia, adenosine triphosphate (ATP) is degraded to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) because of the energy demand of contracting myocardium. It is reasonable to conclude that the XO hypothesis has merit at least under the conditions that skeletal muscle encounters an adenine nucleotide deficit and/or hypoxia followed by reoxygenation. This may occur during isometric contraction such as weightlifting and sprinting exercise.

(iii) Thirdly,

Respiratory burst and Neutrophils

Although there has been increasing research with respect to exercise and immune function, it became clear only recently that ROS may be involved in tissue inflammatory response to injury and that polymorphonuclear neutrophils (PMNs) play a key role in this process (Meydani and Evans, 1993).

Unaccustomed strenuous exercise has long been recognized to cause muscle injury accompanied by an inflammatory response, which is characterized by increased protease and lysozymal enzyme activities in working muscle (Salminen and Vihko, 1983). The response can last from several hours to several days after the cessation of exercise, depending on the intensity and duration of exercise. Furthermore, vitamin E
administration attenuated urinary markers of lipid peroxidation found during the post-
exercise period indicative of the oxidative nature of the injury (Meydani et al., 1992).

(iv) Fourthly,

Due to alteration in blood flow and O₂ supply, underperfusion often occurs
during exercise and it is then followed by reperfusion in the recovery period.

Moreover, peroxisomes, liver microsomes, etc. generate free radicals primarily
through the cytochrome P₄₅₀ system. However the quantitative importance of
catecholamines as a source of ROS production during exercise has been hypothesized
but not investigated yet (Ji, 2000).

b. Prevention Cum Repair

Nature has created two levels of protection against oxidants in biological
systems:-

(i) By antioxidant enzymes.

(ii) By micro-nutrients and minerals.

Different Antioxidants

Human body has the ability to tackle the havoc producing free radicals by the
utilization of different types of antioxidants. This protection against free radicals is
mainly done by –

1. **Enzymes**: Antioxidant enzymes, able to remove the dreaded oxidants,
are mainly catalase, glutathione peroxidase, methionine sulfoxide
reductase, superoxide dismutase.

2. **Micronutrients (vitamins)**: Vitamins play an important role as
antioxidant. Lipidophilic vitamin, vitamin E, is involved in the protection
of lipid peroxidation process. The hydrophilic vitamin, vitamin C, acts in
phagocytic activity. Vitamin A also has some antioxidant activity.
3. **Minerals**: Certain trace elements, notably zinc, selenium and a few others are essential for working of the human antioxidant defence, especially the antioxidant enzymes.

**General Mechanism of Action of Antioxidant Enzymes**

The following figure shows the general pathway for ROS production and main steps of antioxidant defence mechanism.

![Diagram of ROS pathways and antioxidant defenses](image-url)

**Fig.-3**: Major ROS pathways and antioxidant defenses. Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase (Murray and Grauner, 1991).
Vitamins as Antioxidant

Except several antioxidant enzymatic protection in our body, there are also non-enzymatic protective elements which include the vitamins – Vitamin A, Vitamin E and Vitamin C. From a series of studies it has been concluded that a decrease in average levels of Vitamin A, Vitamin C and Vitamin E in blood indicates their lower antioxidant protection.

Vitamin A and β-Carotene

Vitamin A in excess is stored in liver, whereas its precursor, β-carotene is absorbed and is converted by the intestinal enzymes (carotene oxygenase) to retinol. β-carotene is consumed in LDL-C exposed to oxidizing conditions. β-carotene satisfies not only the O₂ molecule, but also acts as a radical carrier. This can inhibit lipid peroxidation at low physiological O₂ pressures. β-carotene acts an apparent anti-atherogenic agent.

Vitamin E (α-tocopherol)

Vitamin E is a lipid soluble vitamin which is an essential component of diet. Deficiency of Vitamin E may cause serious problems which mimics the damages due to oxidative stress. Also the damaging effects caused by Vitamin E deficiency can be completely alleviated by feeding of synthetic antioxidants. So, Vitamin E is considered to be a potential antioxidant. It is actually a protector against lipid peroxidation (Glessner and Vogel, 1973). Vitamin E is a sacrificial antioxidant that can donate hydrogen atoms. Vitamin E is located in membranes and lipoprotein, where it can interrupt the radical chain reaction of lipid peroxidation. Among eight different forms of Vitamin E, α-tocopherol is the most active form which is selectively retained in the body. α-tocopherol performs its antioxidant activity by the following way:

1. α-tocopherol both quenches and reacts with singlet O₂ and can therefore protect the membrane against this species.

2. α-tocopherol is also oxidized by superoxide generating system.
(3) Tocopherol, like most molecules, also react with \( \cdot \)OH at an almost diffusion-controlled rate.

(4) However, its major antioxidant action in biological membranes under most conditions is to react with lipid peroxy and alkoxy radicals, donating labile hydrogen to them and so terminating the chain reaction of peroxidation by scavenging chain propagating radicals.

\[
\begin{align*}
\text{RO}_2^* + \text{TH} &\rightarrow \text{RO}_2\text{H} + T^* \\
\text{RO}^* + \text{TH} &\rightarrow \text{ROH} + T^*
\end{align*}
\]

where, TH = Tocopherol, T* = Tocopheryl radical

Vitamin E may also protect against peroxidation by modifying membrane structure (Glesser and Vogel, 1973). Addition of \( \alpha \)-tocopherol into the phospholipid liposome during their preparation has a powerful protective effect.

So, low Vitamin E is indeed a significant risk factor in the etiology of heart disease and cancer at certain sites.

**Vitamin C (Ascorbic Acid)**

Vitamin C is a water soluble compound that exert a number of well defined biological activities. It is an essential cofactor but is often also regarded as an important chain breaking antioxidant in vivo, protecting against cancer by scavenging DNA-damaging ROS (Margaritis et al., 1977).

Ascorbate is a good reducing agent. Hence it can be oxidized by most of the ROS that are thought to contribute to tissue injury in several human diseases.

Ascorbic acid acts in concert with thiols, glutathione and dihydrolipoic acid in the regeneration of \( \alpha \)-tocopherol via the reduction of \( \alpha \)-chromanoxyl radical. Ascorbic acid has a sparing effect on cellular \( \alpha \)-tocopherol stores and \( \alpha \)-tocopherol and \( \beta \)-carotene within LDL-C.
II. ANTIOXIDANTS AND EXERCISE

a. Free Radicals and Antioxidants in Oxidative Stress

What is Free Radical?

A free radical is any species capable of independent existence that contains one or more unpaired electrons (Glesser and Vogel, 1973). Unpaired electron is the one that is alone in an orbital. This situation is very unstable making such species often highly reactive.

A radical might donate its unpaired electron to other molecule or it might steal an electron from another molecule in order to pair. However, if a molecule gives an electron to or takes an electron from another molecule, that another molecule itself becomes a radical (Fig. 4). Thus one radical begets another, and so on. In this way, the presence of a single radical initiates a chain sequence of electron transfer (redox) reaction.

![Diagram showing electron transfer](image)

Fig.-4: The reaction of a free radical with a non-radical.

Major Free Radicals

The major free radical entities affecting the human body are the :-

1) Superoxide radical (O$_2^-$)
2) Hydroxyl radical ('OH)
3) Nitric oxide radical (NO')

Among these, O$_2^-$ and 'OH are the two main entities that are produced in the body.
Superoxide radical is undoubtedly the most important that causes O₂ toxicity. Hydroxyl radical attacks and damages every type of living cell molecules. It has adverse effects on carbohydrate, amino acid, phospho-lipids and nucleic acid inside the cell.

Sources of Free Radicals

Oxidative stress in human biology comes from a variety of sources.

Table 1: Sources of Oxidative Stress in Human Pathophysiology (Glesser and Vogel, 1973)

<table>
<thead>
<tr>
<th>Source</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mitochondrial electron transport</td>
<td>• Leakage of superoxide due to inefficient reduction of oxygen.</td>
</tr>
<tr>
<td>• Transition metal ions</td>
<td>• Copper and iron facilitate hydroxyl radical formation.</td>
</tr>
<tr>
<td>• Inflammation</td>
<td>• Free radicals released by activated phagocytes.</td>
</tr>
<tr>
<td>• Enzymes, e.g., Xanthine oxidase.</td>
<td>• Release superoxide during reperfusion of ischemic tissue.</td>
</tr>
<tr>
<td>• Drug metabolism, e.g., paracetamol (acitaminophen), para quat.</td>
<td>• Free radical intermediates created during metabolism.</td>
</tr>
<tr>
<td>• Cigarette smoke</td>
<td>• Gas phase rich in free radicals.</td>
</tr>
<tr>
<td>• Radiation</td>
<td>• X-rays, ultraviolet light.</td>
</tr>
</tbody>
</table>

Table 2: Reactive Forms of Oxygen, and the Way in Which They are Formed

<table>
<thead>
<tr>
<th>Reactive oxygen species</th>
<th>Mode of formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singlet oxygen (O₂⁺)</td>
<td>Excitation of O₂</td>
</tr>
<tr>
<td>Superoxide anion radical (O₂²⁻)</td>
<td>One-electron reduction of O₂²⁻</td>
</tr>
<tr>
<td>Perhydroxyl radical (&quot;O-OH&quot;)</td>
<td>Protonation of superoxide anion radical</td>
</tr>
<tr>
<td>Peroxide anion (O₂²⁻)</td>
<td>One-electron reduction of O₂²⁻</td>
</tr>
<tr>
<td>Hydroxyl radical (&quot;OH&quot;)</td>
<td>From H₂O under the influence of ionizing radiation and from H₂O₂ through metal-catalyzed disintegration.</td>
</tr>
<tr>
<td>Ozone (O₃)</td>
<td>From O₂ through photolysis.</td>
</tr>
</tbody>
</table>

The existence of reactive forms of O₂ has its origin in the physicochemical properties of the oxygen molecule (in the ground state, i.e., as it occurs in the
atmosphere). Because of its electron configuration, oxygen can act as an electron acceptor:

The outer molecular orbitals of O$_2$ each contain one electron; the spins of these electrons are parallel. According to the Pauli exclusion principle, O$_2$ (in the ground state) can only undergo univalent reductions. As a consequence, the complete reduction of O$_2$ to water consists of four successive one-electron steps:

$$\begin{align*}
O_2 &\overset{e^-}{\longrightarrow} O_2^\bullet + \rightarrow O_2^{2\bullet} + \rightarrow "O^{3\bullet}" + "O^{3\bullet}" + \rightarrow 2" O^{3\bullet}" + \\
&\text{superoxide} \hspace{1cm} \text{anion radical} \hspace{1cm} 2H^+ \hspace{1cm} \text{+} \hspace{1cm} \text{+} \hspace{1cm} 4H^+ \\
&\text{hydrogen} \hspace{1cm} \text{peroxide} \hspace{1cm} \text{OH}^\bullet \hspace{1cm} \text{H}_2\text{O} \hspace{1cm} \text{radical} \hspace{1cm} 2\text{H}_2\text{O}
\end{align*}$$

*Fig.-5: Reduction of oxygen to water by addition of four electrons*

Well-known examples of reactive O$_2$ intermediates are the superoxide anion radical, hydrogen peroxide and the hydroxyl radical.

**b. The Antioxidant**

All the organisms have evolved a natural protecting mechanism within them. The components of the protecting phenomena are collectively called the antioxidants. So, antioxidant may be defined as *substances whose presence in relatively low concentration compared to those of an oxidizable substrate, significantly inhibits the rate of oxidation of proteins, lipids, carbohydrates, DNA and the cellular structures.*

**Defense Mechanisms Against the Injurious Effects of Reactive Oxygen Species**

Over the past 25-30 years it has become clear that the living cell disposes of a number of enzymatic and non-enzymatic defense mechanisms against ROS.
Enzymatic Defense Against Reactive Oxygen Species

There are three classes of enzymes known to provide protection against ROS: the catalases and peroxidases, that react specifically with hydrogen peroxide, and the superoxide dismutases.

General Mechanism for Antioxidant Defense

Antioxidants protect the biological system mainly by the following mechanism:

1. Enzyme antioxidants catalyze the breakdown of radical species usually in the intracellular environment.
2. Preventive antioxidants decrease the local $O_2$ concentration and bind transition metal ions such as iron and copper, preventing their interaction with hydrogen peroxide and superoxide to produce highly reactive hydroxyl radical.

3. Sacrificial (chain breaking) antioxidants are powerful electron donors and react preferentially with free radicals before more important target molecules are damaged. In doing so, the antioxidant is sacrificed (oxidised) and must be regenerated or replaced.

$$\text{Nucleic acids} \quad \rightarrow \quad \text{mutations, cancer}$$
$$\text{DNA damage} \quad \rightarrow \quad \text{cell injury}$$
$$\text{SH, Redox changes} \quad \rightarrow \quad \text{disturbances to SH-dependent enzymes}$$
$$\text{Covalent binding}$$
$$\text{Membrane damage} \quad \rightarrow \quad \text{ion transport, calcium influx}$$
$$\text{Lipid peroxidation} \quad \rightarrow \quad \text{toxic products}$$

*Fig. 7: Free radicals and cellular injury. Major routes are shown in which a free radical ($R^*$) can interact with neighboring components in cells to disturb their metabolic function(s) (Deshpande et al., 1996).*

**Non-Enzymatic Defense Against Reactive Oxygen Species**

The second line of defense largely consists of substances that eliminate radicals. The major radical scavengers of the cell are the vitamins E and C. Vitamin E ($\alpha$-tocopherol) is lipophilic and is incorporated in membranes. Vitamin C (ascorbic acid) is water-soluble and occurs in the cytosol. Ascorbic acid reacts quite rapidly with the superoxide anion radical and with hydrogen peroxide, but even faster with hydroxyl radicals. Furthermore, it can eliminate singlet $O_2$. This antioxidant exerts its protective effect, for example, in the lens of the eye (which does not contain any superoxide dismutases) and in the fluid surrounding the pulmonary alveoli, where it complements the action of SOD and catalase. The mechanisms of the detoxication of lipid radicals by vitamin C are schematically shown in Figure 8 below.

Fatty acid chains in biological membranes are mostly unsaturated. They are therefore highly sensitive to oxidation by singlet oxygen or hydroxyl radicals. The
oxidation consists of a chain of reactions which is known as lipid peroxidation. Lipid peroxidation causes serious membrane damage and may therefore lead to cell death. Membrane components with an antioxidant effect may block the injurious chain reaction. A well-known example is α-tocopherol radical and a harmless fatty acid. The α-tocopherol radical can then be reduced to α-tocopherol by glutathione (GSH). α-tocopherol is present in high concentrations in the membranes of the human eye and is probably the only lipid-soluble antioxidant occurring in human blood plasma. Figure schematically represents the reaction of α-tocopherol with lipid radicals.

The radicals that evolve from vitamins E and C during detoxication reactions are less reactive, because they are stabilized by resonance. Moreover, they are transformed back into the original vitamins by glutathione-dependent systems.

**Vitamin C (ascorbic acid)**

**ascorbic acid radical**

![Diagram of vitamin C detoxication](image)

*Fig.-8: Detoxication of lipid radicals (L*) by vitamin C and subsequent regeneration of vitamin C (Halliwell and Gutteridge, 1989).*
c. The Antioxidant Vitamins in General

Vitamin E

Vitamin E is the principal component of the secondary defense mechanism against free-radical-mediated cellular injuries. In fact, it is the only natural physiological lipid-soluble antioxidant that can inhibit lipid peroxidation in cell membranes (Deshpande et al., 1996).

Chemistry and Properties. At least eight compounds exhibiting vitamin E activity and having a 6-chromanol ring structure and a side chain have been isolated from plant sources (Fig.-10)

![Chemical structures of tocopherol and tocotrienol series of compounds having vitamin E activity](image)

Fig.-10: Chemical structures of tocopherol and tocotrienol series of compounds having vitamin E activity (Deshpande et al., 1996).
The term “vitamin E” should be used as the generic description for all tocol and tocotrienol derivatives qualitatively exhibiting the biological activity of α-tocopherol. Thus, phrases such as “vitamin E activity”, “vitamin E deficiency”, and “vitamin E in the form of…” represent the preferred usage. The term “tocol” is the trivial designation of 2-methyl-2-(4'-8'-12'-trimethyltridecyl) chroman-6-ol.

The term “tocopherols” should be used as the generic description for all mono-, di-, and trimethyl tocols irrespective of biological activity. The term “tocopherols” is not synonymous with the term “vitamin E”.

The only naturally occurring stereoisomers of α-tocopherol, formerly known as d-a-tocopherol or α-tocopherol, should be designated as RRR-α-tocopherol. The totally synthetic α-tocopherol, formerly known as dl-α-tocopherol, should be designated all-<i>rac</i>-α-tocopherol. Esters of tocopherols should be designated as tocopheryl esters (e.g., α-tocopheryl acetate).

The adrenal and pituitary glands, testes, and platelets have the highest concentrations of the vitamin. It is most concentrated in cell fractions rich in membranes such as the mitochondria and microsomes.

**Physiological Functions**

*In vivo Antioxidant.* Vitamin E functions *in vivo* as a protector against lipid peroxidation (Deshpande et al., 1996). The evidence include such *in vitro* observations as its direct reactions with and quenching of superoxide and peroxyl radicals and singlet O₂ as well as its ability to prevent lipid peroxidation.
Tappel, 1981, first proposed that vitamin E functions as an *in vivo* antioxidant that protects tissue lipids from free-radical damage. It is now widely recognized that tocopherol is located primarily in the membrane portion of the cell and is a part of the cell’s defense against O₂-centered radicals. Vitamin E is unique in its more specific localization in membranes and the tenacity with which it remains in most tissues.

Vitamin E acts as a chain-breaking antioxidant in membranes. The autooxidation and antioxidant reactions involving vitamin E are summarized as follows:

1. **Initiation (formation of a free radical)**

   \[
   \text{LH} \xrightarrow{\text{Initiators}} \text{L}^*.
   \]

2. **Reaction of radical with oxygen**

   \[
   \text{L}^* + \text{O}_2 \rightarrow \text{LO}_2^*.
   \]

3. **Propagation**

   \[
   \text{LO}_2^* + \text{LH} \rightarrow \text{L}^* + \text{LOOH}.
   \]

4. **Antioxidant reaction**

   \[
   \text{LO}_2^* + \text{E} \rightarrow \text{E}^* + \text{LOOH}.
   \]

5. **Regeneration**

   \[
   \text{E}^* + \text{C} \rightarrow \text{E} + \text{C}^* \rightarrow \text{C} + \text{NADP} \quad \text{Semidehydro ascorbate reductase}
   \]

   \[
   \text{E}^* + 2\text{GSH} \rightarrow \text{E} + \text{GSSG} \quad \text{GSH reductase}
   \]

   \[
   \text{GSSG} + \text{NADPH} \rightarrow 2 \text{GSH} + \text{NADP} \quad \text{GSH reductase}
   \]

6. **Termination**

   \[
   \text{E}^* + \text{E}^* \rightarrow \text{E} - \text{E} \text{ (dimer)} \]

   \[
   \text{E}^* + \text{LO}_2^* \rightarrow \text{EOOL} \text{ (?)}
   \]

The abbreviations used in the above equations are \(\text{L}^*\) = fatty acid radical, \(\text{LO}_2^*\) = peroxy radical, \(\text{LH}\) = fatty acid, \(\text{E}\) = tocopherol, \(\text{LOOH}\) = hydroperoxide, \(\text{C}\) = ascorbic acid, \(\text{C}^*\) = ascorbyl radical, \(\text{GSH}\) = reduced glutathione, and \(\text{GSSG}\) = glutathione disulfide (oxidized form of GSH).
Vitamin C (Ascorbic Acid)

Vitamin C is chemically the simplest of vitamins and therefore was among the first to be isolated, characterized, and purified and to have its structure determined. Its ene-diol structure, however, provides it with a highly complex chemistry. Thus it has a very complicated redox chemistry involving comparatively stable radical intermediates and heavily modified by the acidic properties of the molecule.

Vitamin C was first isolated in crystalline form by the Hungarian scientist Albert Szent-Gyorgyi as a reducing factor from the adrenal glands with the empirical formula $C_6H_8O_6$, which he called "hexuronic acid".

Nearly all species of animals synthesize vitamin C and do not require it in their diets. However, humans, cannot synthesize the vitamin because of the loss of a liver enzyme, L-gulono-$\gamma$-lactone oxidase, during the course of evolution.

Chemistry, and Properties

L-Ascorbic acid is an $\alpha$-keto lactone with an almost planar five-membered ring.

![Stereoisomers of Vitamin C (L-ascorbic acid).](image)

The reversible oxidation-reduction with dehydro-L-ascorbic acid is L-ascorbic acid's most important chemical property and the basis for its known physiological activities and stabilities.

Physiological Functions

Most physiological functions of ascorbic acid are related to its ability to act as an electron donor. In addition to its antiscorbutic properties, it can exert a significant
influence on biological activities directly via its characteristic physicochemical properties of ionized state, oxidation/reduction, its capacity to its lower interfacial tension, and its involvement in hydrogen bonding.

**Oxidation and Hydroxylation**

Ascorbic acid is an essential factor in several hydroxylation reactions of the type RH + O → ROH, primarily because of its ability to act as a redox couple, ascorbic acid/dehydroascorbic acid (H₂A/A), which undergoes cycling similar to that of cytochromes. It is also involved in the metabolism of several amino acids, leading to the formation of hydroxyproline, hydroxylysine, noradrenaline (norepinephrine), serotonin, homogentisic acid, and carnitine.

**Antioxidant Properties**

In recent years, the antioxidant properties of vitamin C have received considerable attention. Unlike the oxidation-reduction reactions in which ascorbate donates two electrons, the antioxidant reactions use its ability to donate a single electron to free-radical species. The products of such reactions are the quenched reactive species and the less reactive ascorbyl free radical. The ascorbyl radical then can be either reduced back to ascorbic acid or oxidized to form dehydro-ascorbic acid.

Ascorbate reacts rapidly with both superoxide and peroxyl radicals and even more rapidly with hydroxyl radicals (Deshpande et al., 1996). It also scavenges singlet O₂, reduces thyl radicals, and combines quickly with hypochlorous acid, a powerful oxidant generated at sites of inflammation. These free-radical-scavenging reactions are especially important in the eye and in the extracellular fluid of the lung, where they provide protection against other oxidizing agents such as ozone.

Ascorbic acid also plays a vital role in maintaining the balance between oxidative products and the various cellular antioxidant defense mechanisms. The interdependency of such reactions involve ascorbic acid, vitamin E, selenium, catalase, and GSH.

**Carotenoids**

Carotenoids are a group of fat-soluble pigments that contribute to the yellow, orange, and/or red colouration of fruits and vegetables. The biological activity is defined
in terms of the carotenoids containing at least one \(\beta\)-ionone ring that is not hydroxylated in order to show the vitamin A activity.

The recent renewed interest in carotenoids can be partly attributed to the role of \(\beta\)-carotene in reducing the risks of certain cancers through a mechanism that does not require its conversion to vitamin A.

**Chemistry and Properties**

The conversion of biologically active carotenoids that contain at least one \(\beta\)-ionone ring that is not hydroxylated to vitamin A occurs by two primary oxidative reactions. Among the carotenoids, even though \(\beta\)-carotene contains the highest provitamin A activity, its inefficient conversion to retinal, susceptibility to oxidative reactions leading to the formation first of retinoic acid and then to inactive products, and poor absorption compared to that of preformed vitamin A in the diet result only in about one-sixth the overall utilization of retinal. Therefore, in calculating the vitamin A activity, six units of \(\beta\)-carotene by weight is considered to be equivalent to one unit of retinal.

![Structure of \(\beta\)-carotene.](image)

**Dietary Sources**

Animals, including humans, are not capable of de novo synthesis of \(\beta\)-carotene. The micronutrient must therefore be supplied in the diet. Fresh fruits and vegetables are excellent sources of carotenoids in the human diet (Fig.13).

**Physiological Functions**

The biological functions may be defined as a preventive role of carotenoids in whose absence physiological capability may be impaired.
Antioxidant Functions: Carotenoids are known to scavenge and deactivate free radicals both in vitro and in vivo. β-carotene especially has been shown to protect isolated lipid membranes from peroxidation, LDL-containing lipids from oxidation, and liver lipids from oxidation induced by trichloromethyl radical, although theoretically all carotenoids with a conjugated double bond system should act similarly.

\[
\text{β-Carotene} + \text{ROO}^* \rightarrow \text{β-carotene}^* 
\]

The carotenoid radical is resonance-stabilized to such an extent that its subsequent reaction with molecular \(O_2\) to form a peroxy-β-carotene radical is reversible.

\[
\text{β-carotene}^* + O_2 \rightarrow \text{β-carotene-OO}^* 
\]
Under conditions of low O₂ pressure, the shifting of the equilibrium to the left greatly reduces the concentration of the highly reactive peroxy-β-carotene radicals.

Singlet Oxygen Quencher: Carotenoids are very effective quenchers of singlet O₂ (Deshpande et al., 1996). Due to its instability and high energy level, singlet O₂ can potentially transfer this energy to other molecules, generating free radicals in the process. Singlet O₂ is therefore involved in oxidative reactions that may impair or destroy important cellular components such as membrane lipids, enzymes, and nucleic acids.

The antioxidant, antimutagenic, chemopreventive, and immunoenhancing functions of carotenoids are summarized in Fig. 14.

Fig. 14: Schematic representation of the biological functions of carotenoids (Deshpande et al., 1996).
III. OXIDATIVE STRESS AND ITS INDICATORS

a. Lipid Profile and Exercise

Regarding bio-energetics of exercise, blood lipid fractions and their derivatives do contribute a substantial portion which varies from anaerobic to aerobic type of work and obviously with the intensity of the work.

Specified physical labor imposes an influence on the total cholesterol and LDL-C level. The study also attempts to highlight the influences of exercise on plasma triglyceride (TG) level as a corollary biochemical study. TG is a fundamental derivative of serum lipid fractions.

Generally, females possess a bit higher serum lipid derivative concentration at specific regions like buttock, hip regions and so on. This study specifies the female subjects with a view to find out whether this excess fat in female subjects would otherwise contribute in the process of bio-energetics following exercise; and if so upto what extent.

In this study, vitamin E and the mixture of vitamins E, C and β-carotene supplementation for a specified time period has been done to find out whether the major dietary antioxidant vitamins do possess any particular effects or any influence on blood haemoglobin concentration and on blood lipid profile.

b. Lipid Peroxidation and Exercise Stress

In addition to disturbances in Ca\(^{2+}\) homeostasis, oxidative stress followed by depletion of reducing equivalents may also lead to another cause of membrane damage, namely lipid peroxidation. Lipid peroxidation and cell death often go hand in hand. Some of the processes through which the intermediates produced in the body may cause damage to the cell are as follows:

i) abstraction of a hydrogen atom from a polyunsaturated fatty acid chain by singlet O\(_2\) and hydroxyl radicals (radical formation, initiation).

ii) reaction of the resulting fatty acid radical with molecular O\(_2\) (oxygenation or, more specifically, peroxidation).
iii) These events may be followed by a detoxication process, in which the reaction chain is stopped. This process, which may proceed in several steps, is sometimes referred to as termination.

Endoperoxides disintegrate to yield malondialdehyde and other products. If the processes described above involve biological membranes (cell membrane, endoplasmic reticulum, mitochondrial membrane, lysosomal membrane etc.), this will ultimately lead to the destruction of these membranes, with all its consequences.

In cellular systems, lipid peroxidation can occur mainly in biomembranes, where the content of unsaturated fatty acids is relatively high. Lipid peroxidation is a very complicated chemical and biochemical reaction process involving free radicals, $O_2$, metal ions, and, in biological systems, a number of other factors.

c. **Uric Acid and Responses to Oxidative Stress**

A wide range of natural antioxidants have been shown to occur in plants and animals. Among these chemicals, Vitamin E, Vitamin C, $\beta$-carotene and uric acid are very interesting as synthetic products of natural origin capable of participating in the in vivo radical defense mechanism (Halliwell and Gutteridge, 1989; Tappel and Dillard, 1981; Ames et al., 1983).

The potential source of uric acid is phenolic acids and it is a by-product of purine metabolism. Uric acid (Fig.15) is produced as a by-product of xanthine dehydrogenase, an enzyme that oxidizes hypoxanthine to xanthine and then to uric acid while reducing $\text{NAD}^+$ to $\text{NADH}$ (Halliwell and Gutteridge, 1989).

![Chemical structure of uric acid (8-hypoxanthine).](image-url)
Uric acid is a powerful scavenger of singlet $O_2$ and peroxyl and hydroxyl radicals and may function as an antioxidant *in vivo*. The reactions of HO$^\cdot$ with uric acid produces a range of carbon-centered radicals that mostly react with oxygen to give urate peroxyl radicals.

Although much less reactive than $-\cdot$H $+\cdot$HO$^\cdot$ $\rightarrow$ $\cdot$R $+\cdot$H$+\cdot$O,$\cdot$C $+\cdot$O$\rightarrow$ $\cdot$R $+\cdot$COO$^\cdot$, urate-derived radicals are not completely harmless. They can inactivate the enzyme alcohol dehydrogenase from yeast and the human $\alpha$-antiproteinase. Thus, like GSH, uric acid, is not always a "perfect antioxidant".

d. Glutathione Status and Exercise Profile

Glutathione (Glu-Cys-Gly) and reduced glutathione (GSH) are the only significant electron donor substrates for the metabolic reactions involving glutathione peroxidase (GSH-Px), although the latter can use a variety of hydroperoxide acceptor substrates (*Deshpande et al.*, 1996). In addition, GSH is also a scavenger of HO$^\cdot$ radicals and singlet $O_2$. Since GSH is present at high concentrations in many cells, it may help to protect against these radical species. GSH can reactivate some enzymes inhibited by exposure to high $O_2$ concentration, presumably...
by reducing of the disulfide bonds of the enzyme.

**Halliwell and Gutteridge, 1989**, reviewed other deleterious aspects of GSH biochemistry. Its rapid reaction with HO' \((k_2>109 \text{ M}^{-1}\text{s}^{-1})\) and oxidation by peroxides or by \(O_2\) in the presence of transition metal ions such as Cu\(^{2+}\) or Fe\(^{2+}\) yield thyl radicals. The reactions of GSH with \(O_2^-\) can also lead to the formation of singlet oxygen \((^1O_2)\) as follows:

\[
\begin{align*}
\text{GSH} + \text{O}_2^- + \text{H}^+ & \rightarrow \text{GS}^- + \text{H}_2\text{O}_2 \\
\text{or GSH} + \text{HO}_2^- & \rightarrow \text{GS}^- + \text{H}_2\text{O}_2 \\
\text{GS}^- + \text{O}_2 & \rightarrow \text{GSO}_2^- \\
\text{GSO}_2^- + \text{O}_2^- + \text{H}^+ & \rightarrow \text{O}_2 + \text{GSOOH} \\
\text{GSO}_2^- + \text{GSO}_2^- & \rightarrow \text{GSSG} + 2\text{O}_2 \\
\text{GSO}_2^- + \text{GS}^- & \rightarrow \text{GSSG} + \text{O}_2
\end{align*}
\]

A schematic representation of the GSH cycle in biological systems is shown in Fig. 17.

---

e. **Glucose-6-Phosphate Dehydrogenase Assay – a Novel Perspective of Exercise.**

G-6-PD, the key regulatory enzyme in the hexose monophosphate (HMP) shunt, catalyzes the oxidation of glucose-6-phosphate to 6-phospho-gluconolactone and the production of reducing equivalents in the form of NADPH to meet the cellular needs for reductive biosynthesis and maintenance of the cellular redox status. The importance of G-6-PD is illustrated by clinical manifestations of its deficiency.

As G-6-PD is indispensable to maintenance of the cellular redox balance and detoxification of ROS (Pandolfi et al., 1995), it is likely that the G-6-PD activity...
regulates cell growth and any change related to it alters the course of cellular senescence and thus performance at large.

Oxidative stress is often induced by strenuous physical exercise. In that state production of ROS within the body transcends the antioxidant defense from within. Earlier reports proved that even submaximal exercise may elevate the stress indices like plasma lipid peroxides and higher blood glutathione oxidation (Bagchi et al., 2001).

Moreover, the mixture of vitamin E, vitamin C and β-carotene are being supplemented here as the major non-enzymatic antioxidants available, at clinical dose, for the purpose of stress management. A new oxidative stress index is being attempted to be developed, i.e., G-6-PD level, which is probably involved with strenuous physical exercise. Therefore, the study aims in revealing the effects of antioxidant vitamin supplementation on exhaustive exercise-induced oxidative stress in females.

The final mission is to evaluate the exercise-mediated changes in G-6-PD level considered as a probable marker of oxidative stress management programme.

IV. EXERCISING FEMALE

Exercise is multi-dimensional. It is beneficial as well as destructive. The war of antioxidant is on against ROS. But female subjects are unique in exercising conditions due to the presence of unique antioxidant steroid oestrogen. Nutrition possesses a special role in augmenting it.

Nutrition and Performance including Body Composition

Table 3: New Approach to Improved. Balanced Nutrition to Promote Health and Prevent Disease (Modified from Deshpande et al., 1996)

<table>
<thead>
<tr>
<th>Old concept</th>
<th>New concept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended dietary allowance (RDA)</td>
<td>Optimal nutrition</td>
</tr>
<tr>
<td>Avoidance of deficiency diseases</td>
<td>Avoidance of chronic diseases and against</td>
</tr>
<tr>
<td></td>
<td>environmental toxicants</td>
</tr>
<tr>
<td></td>
<td>Optimal levels of macronutrients</td>
</tr>
<tr>
<td></td>
<td>Optimal levels of micronutrients</td>
</tr>
</tbody>
</table>
Under the above circumstances, one can easily apprehend the importance and relevance of proper nutrition enriched with the so-called antioxidant vitamins and minerals, in view of better performance, cumulatively correlated with reduced exercise-induced oxidative damage.

Body composition - by definition means "the quantification of the major structural components of the body - muscle, bone and fat".

It includes the following parameters:

a) **Body Surface Area**: which is used by researchers to evaluate the approximate proportionality of one’s body weight and stature (height).

b) **Body Mass Index**: A somewhat Better Alternative - The body mass index (BMI), derived from body mass and stature, is used frequently by clinicians and researchers to evaluate the "normalcy" of one’s body weight. The BMI has a somewhat higher association with body fat than do estimates based simply on stature and mass. The importance of this easy-to-obtain index lies in its curvilinear relationship to the all-cause mortality ratio: as the BMI becomes larger, so also does the risk of a variety of diseases, such as cardiovascular complications (including hypertension), diabetes, and renal disease. The lowest health-risk category is that of individuals whose BMIs range from 20 to 25, and the highest risk category is that of individuals whose BMIs exceed 40. Within this context, the suggested desirable BMI range for women is 21.3 to 22.1. The surgeon general has defined being overweight as having a BMI between 25 and 30, with obesity defined as a BMI in excess of 30; this value corresponds to a moderate category of risk.

c) **Body Density**.

d) **Total weight of fat**.

e) **Percentage of body fat**
Body fat exists in two storage sites, or depots. The first depot, termed essential fat, is the fat stored in the marrow of bones and in the heart, lungs, liver, spleen, kidneys, intestines, muscles, and lipid-rich tissues of the nervous system. This fat is required for normal physiologic functioning. In the heart, for example, the quantity of dissectable fat determined from cadaver studies represents about 22.7 g, or 8.6%, for an average heart weight of 256 g in females. Essential fat in females also includes sex-specific, or sex-characteristic fat. It is not at all clear whether this fat depot is expendable or serves as reserve storage for metabolic fuel.

The other major fat depot, storage fat, consists of fat that accumulates in adipose tissue. This nutritional reserve includes the visceral fatty tissues that protect the various internal organs from trauma and the larger subcutaneous fat volume deposited beneath the skin's surface. Although the proportional distribution of storage fat in men and women is similar (12% in men, 15% in women), the total percentage of essential fat in women, including the sex-specific fat, is four times higher than in men. More than likely, the additional essential fat is biologically important for child-bearing and other hormone-related functions. Considering the reference body's total quantity of storage fat (about 8.25 kg), this depot, at least theoretically, represents approximately 74,250 kcal of stored energy, or the energy equivalent of running nonstop at a 9-minute per mile pace for 133 hours.

f) Lean Body weight: The terms fat-free body mass and lean body mass are often considered interchangeable when they should not be. The lean body mass contains a small percentage of essential fat stores (perhaps as much as 3%), chiefly within the central nervous system, marrow of bones, and internal organs. In contrast, use of the term "fat-free" mass refers to the body mass devoid of all extractable fat. Behnke points out that fat-free mass is an in vitro entity and is the appropriate term for carcass analysis. Behnke views lean body mass, on the other hand, as an in vivo entity that remains relatively constant throughout the active adult life span with regard to compositional components such as water, organic matter, and minerals. In normally hydrated, healthy adults, the only difference
between fat-free body mass and lean body mass is the "essential" lipid-rich stores in bone marrow, brain, spinal cord, and internal organs. Thus, in calculating lean body mass (LBM), the small quantity of essential fat is still present, whereas in computing fat-free body mass "total" body fat is subtracted (FFM=Body mass – Fat mass). Lean body mass in men and minimal body mass in women are composed chiefly of essential fat (plus sex-specific fat for females), muscle, water, and bone.

V. INTER-RELATIONSHIP BETWEEN THESE INDICATORS IN THE PERSPECTIVE OF EXERCISE

a. Interrelationship of Vitamin E, Selenium, Vitamin E, and GSH in Membrane Protection:

Although Tappel's theory (1981) regarding the in vivo function of vitamin E as an antioxidant continues to constitute the cornerstone of most explanations of the biological activity of \( \alpha \)-tocopherol in mammalian tissues, the inconsistencies in the hypothesis as pointed out by Green and Bunyan led Diplock and Lucy to propose an alternative hypothesis for the action of vitamin E (Deshpande et al., 1996). It was founded primarily on the physicochemical interaction between the phytol side chain of vitamin E and certain PUFAs. With the discovery of the selenium-containing GSH-Px, a lipid peroxide-destroying enzyme, the biochemical rationale for the close metabolic relationship between selenium and vitamin E was clearly demonstrated. Moreover, in biological systems, vitamin E is regenerated from the tocopheroxy radical by vitamin C and GSH. The interdependency and cooperativity of these various nutrients is chemically shown in Fig. 17. Vitamin E (ascorbic acid) reduces the tocopheroxy radical with concurrent formation of an ascorbate radical, which in turn can be enzymatically reduced back to ascorbate by an NADH-dependent system. Presumably, these reactions with ascorbate radical occur at the lipid/water interface of the membranes, because vitamin C is found primarily in the cytosol whereas vitamin E is located in the cell membranes. GSH can also reduce tocopheroxy radical, perhaps by an enzymatically mediated process.

Although the physiological functions are described above primarily from the viewpoint of preventing free-radical-mediated cell injuries, vitamin E also plays an important role in nucleic acid, protein, and lipid metabolism. It may also protect the
sulphhydryl groups of dehydrogenases in the electron transport chain in mitochondria from oxidation or from reaction with metal ions.

b. **Functional Interaction of Vitamin E with Other Nutrients**

The antioxidant function of vitamin E is interrelated with the following dietary nutrients (Fig. 18):

![Diagram](attachment:image.png)

Fig. 18: Interaction and synergism between antioxidant systems operating in the lipid phase (membranes) of the cell and the aqueous phase (cytosol). $R^*$, free radical; (PUFA-OO•), peroxyl free radical of polyunsaturated fatty acid in membrane phospholipid; PUFA-OOH, hydroperoxy polyunsaturated fatty acid in membrane phospholipid released as hydroperoxy free fatty acid into cytosol by the action of phospholipase $A_2$; PUFA-OH, hydroxy polyunsaturated fatty acid; TocOH, vitamin E (a-tocopherol; TocO•, free radical of a-tocopherol; Se, selenium; GSH, reduced glutathione; GS-SG, oxidized glutathione, which is returned to the reduced state after reaction with NADPH catalyzed by glutathione reductase; PUFA-H, polyunsaturated fatty acid. (Murray RK, Granner DK, Mayes PA and Rodwell VW, ed. 25, 2000: 650.)
Therefore, the purpose of the present research work is to reveal the effects of antioxidant vitamin supplementation (vitamin E, vitamin C, β-carotene) at clinical dose and to highlight its effectiveness in controlling exercise induced oxidative stress in female.

**Objectives**

Under the above circumstances, the present study has been performed to meet the tentative status of the following objectives:

1) To investigate the importance of body composition of an individual in predicting the performance ability of an individual.

2) To integrate the relationship between exercise, lipid profile and lipid peroxidation status.

3) To highlight the ambiguous character of exercise itself, as it produces pro-oxidant and antioxidant environment *in vitro*.

4) To investigate whether the antioxidant role of uric acid hold good, or at least beneficial, on exercise induced oxidative damage condition.

5) To evaluate the exercise-mediated changes in G-6-PD level, considered as a probable marker of the blood glutathione status, which in turn serves as an important index of oxidative stress management programme.

6) To reveal the fate of oral administration of antioxidant vitamins, either individually or in an integrated manner (i.e. vitamin E alone or antioxidant vitamin mixture) at clinical dose and its effectiveness in controlling exercise-induced oxidative stress in trained and untrained females.
VI. REFERENCES


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

