UNIT 9 ANTIOXIDANTS

Structure

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9.1 INTRODUCTION

Antioxidants as you may be already aware are *compounds that scavenge free radicals*. Ample evidence exists that the antioxidants protect the body from the load of free radicals which are generated during the normal metabolic processes everyday. This unit gives an overview of physiological mechanisms available in the body to prevent free radical damage. What are free radicals? What are their ill effects? What is the importance of antioxidants in health and disease? These are a few issues discussed in this unit.

Objectives

After studying this unit, you will be able to:

- know how free radicals are formed,
- understand the significance of antioxidants, and
- explain the nutrient and non-nutrient antioxidants in health and disease.

9.2 ANTIOXIDANTS AND FREE RADICALS

What do vitamins A, E, C and beta-carotene, a precursor to Vitamin A, and the trace mineral, Selenium have in common? Well, they are all antioxidants.

What are antioxidants?

Any substance that prevents or impedes cell oxidation (destruction) by free radicals, etc. is an *antioxidant*. Antioxidants are the molecules that work to reduce damage done to cells and DNA by free radicals i.e. charged particles found in the environment and produced by processes in the body. Antioxidants are *components that combine with free radicals in the body and neutralize their damaging effects*. So, antioxidants work together in the body to maintain our health and vigor well into the late decades of life.

We have a fairly clear idea now about what is an antioxidant. Do you know what a free radical is? You may recall reading about antioxidants, free radicals in the Advance Nutrition Course. Well, now once again let us get to know about free radicals.

What is a free radical?

Free radicals are *atoms or molecules with an unpaired electron*. You will be able to understand this better after reading the discussion on free radicals given herewith:

Oxidation-reduction reactions are central to the supply of biological energy. In an oxidation-reduction reaction, electrons from one species are transferred to another. An *oxidizing agent* is a substance that gains electrons in an oxidation-reduction reaction and in the process gets reduced. A *reducing agent* is a substance that donates electrons and gets oxidized. Oxidation is always accompanied by reduction, hence there must be both an oxidizing agent and a reducing agent. Oxidation can take place in several ways:

- removal of hydrogen (dehydrogenation)
- addition of oxygen
- removal of electrons

Dehydrogenation is the most common form of biological oxidation. Most dehydrogenation occurs by C–H bond cleavage. Since covalent chemical bonds consists of pairs of electrons shared by two atoms, bonds can be cleaved in two ways – both electrons can stay with one atom or one electron can remain with each atom. In most reactions, both electrons stay with one atom. Cleavage of C–H bond almost always produces two ions –

1) if the carbon atom retains both electrons, the carbon containing compound becomes a *carbanion* (i.e. with a negative charge).

$$R\text{–}C\text{–}H \longrightarrow \underset{\text{Carbanion}}{R\text{–}C^{-}:} + \underset{\text{Proton}}{H^{+}}$$

2) if the carbon atom loses both electrons, the carbon containing compound becomes a cationic ion (i.e. with a positive charge) called *carbocation*.

$$R\text{–}C\text{–}H \longrightarrow \underset{\text{Carbocation}}{R\text{–}C^{+}:} + \underset{\text{Hydride ion}}{H^{-}}$$

However, if one electron remains with each product, then free radicals are said to be formed.

$$R_1O - OR_2 \longrightarrow R_1O' + 'OR_2$$

Thus, you can now understand better that a free radical is a molecule or atom with an unpaired electron.

In Unit 6, you may recall reading that the majority of intracellular oxidation of substrates results in transfer of two electrons to appropriate acceptors such as NAD^+ or FAD, which are then oxidized in the electron transport chain. In the last step, O_2 is reduced to water, catalyzed by the enzyme cytochrome C oxidase. The electronic structure of O_2 favours its reduction by addition of one electron at a time leading to generation of oxygen radicals. Thus the radical has a highly reactive unpaired electron in an outer orbital which can initiate chain reactions by removal of an electron from another molecule to complete its own orbital. The stepwise transfer of electrons to O_2 results in formation of various intermediates called *reactive oxygen species*, as illustrated herewith.

O_2
Oxygen
↓
O_2^-
Superoxide
↓
H_2O_2
Hydrogen Peroxide
↓
$H_2O + OH\cdot$
Hydroxyl radical
↓
H_2O
Water

The oxygen radicals readily react with a variety of cellular components causing cellular damage. In fact, the most potent oxygen species in biological species is probably the hydroxyl radical ($OH^{\cdot}$). Most free radicals are extremely short lived. However, they readily extract electrons from other molecules, converting them to free radicals and thereby initiating a chain reaction. Hydrogen peroxide itself is not a free radical but is converted to the hydroxyl radical in the presence of Fe^{2+} or Cu^{+} present in cells.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^{-}$$

You would be surprised to know that formation of free radicals is a normal oxidation process in foods and are formed during common food treatments such as toasting, frying, freeze drying, irradiation etc. Free radicals are generally very reactive, unstable structures that continuously react with substances to form stable products.

So we have seen that free radicals are highly reactive species that have an unpaired electron e.g. the hydroxyl and superoxide radical. The electron in an atom or molecule orbit the nucleus in shells or layers and the most stable configuration occurs when these electrons are in pairs that orbit in opposite directions. If an atom or molecule within the body loses or gains an electron, the resulting product is highly reactive and can react with and damage DNA, proteins, lipids or carbohydrates. Cellular damage caused by oxygen-derived free radical species has been implicated in the aetiology of a range of diseases, including cancer, atherosclerosis, cataract and retinopathy. It has been suggested that many of the degenerative changes associated with ageing may be due to the cumulative effects of free radical damage.

Now that we have an understanding about free radicals, surely you can now conceptualize what role these free radicals play. The next section focuses on this aspect.

9.3 ROLE OF OXYGEN FREE RADICALS

Evidence is accumulating that most of the degenerative diseases that afflict humanity have their origin in deleterious free radical reactions. These diseases include atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, degenerative eye disease etc. The process of biological ageing might also have a free radical basis.

Oxygen free radicals can react with DNA to cause breaks in the DNA chain and alteration of bases (mutation). This could initiate carcinogenesis. Free radicals can peroxidize polyunsaturated fatty acid (PUFA) residues in low density lipoprotein (LDL). This oxidized LDL is taken up by macrophages and generate foam cells and this ultimately leads to scarring and fibrosis of artery walls seen in atherosclerosis. Unoxidized LDL is considered relatively benign in its effects upon artery wall. The reactions of free radicals involve the loss or gain of an electron and this creates another free radical, which can initiate a damaging chain reaction unless the free radical is quenched by antioxidant systems and the chain halted. For example, peroxidation of a PUFA will generate another unstable compound (the lipid peroxyl radical) and this reacts with another fatty acid to produce stable lipid peroxide as shown in Figure 9.1. Susceptibility of PUFA to free radical damage is one of the concerns about recommending high levels of PUFA in the diet.

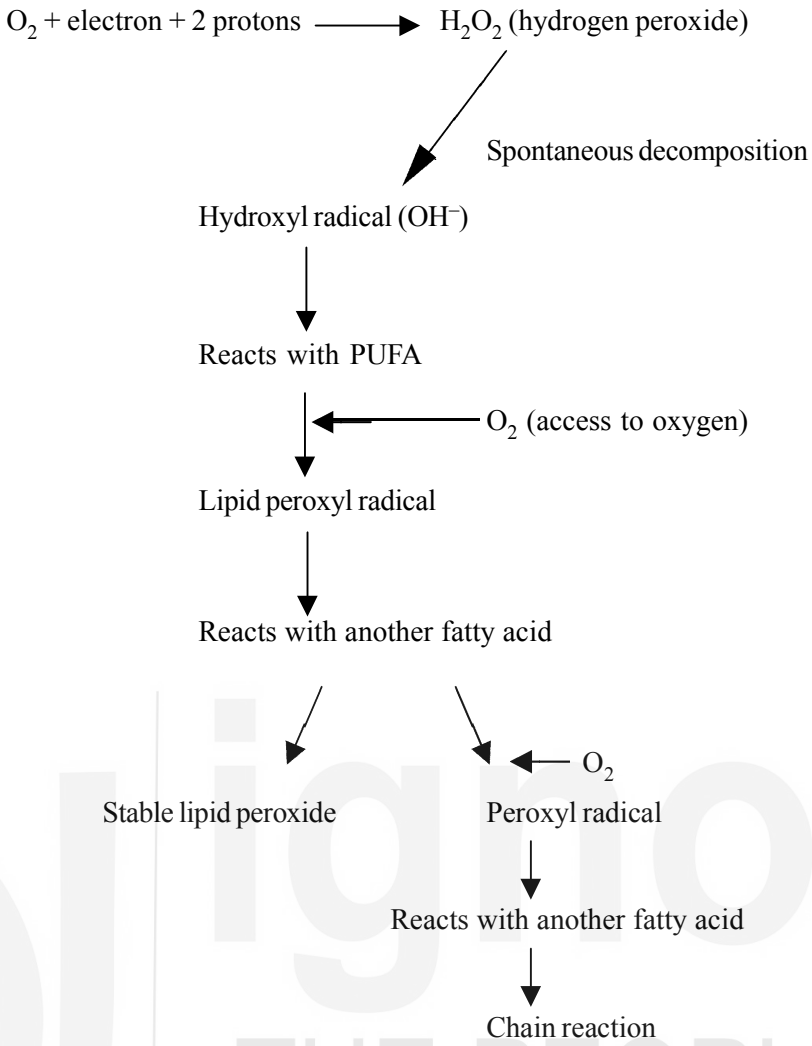

Figure 9.1 : Role of free radical in lipid peroxidation

From our discussion above, it is clear that free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed, these highly reactive radicals can start a chain reaction, like dominoes. To prevent free radical damage, the body has a defense system of *antioxidants* about which we shall learn in section 9.5. But, first let us get to know how are free radicals formed.

9.4 PRODUCTION OF OXYGEN FREE RADICALS

Oxygen free radicals are *a normal by-product of the oxidative processes of the cell*. Some free radicals arise normally during metabolism. Sometimes the body's immune system's cells purposefully create them to neutralize viruses and bacteria. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals. Some of the processes that generate free radicals include:

- Electron transport chain – Free radicals are a by-product of the electron transport chain.
- Dissociation of oxygen from haemoglobin generates superoxide radicals.
- Certain environmental factors increase the generation of free radicals e.g. cigarette smoke, exposure to high oxygen tension and ionizing radiation including sunlight.
- Phagocytic white cells generate oxygen free radicals to kill ingested bacteria and destroy other 'foreign bodies'. They can also secrete these reactive species into

surrounding tissues (e.g. to kill large parasites) and this can cause significant damage to surrounding tissues. Injured and diseased tissue thus has high levels of free radicals.

So we have seen that free radicals are formed as a result of certain environmental factors and as a by-product of our normal body metabolism. What can be done to prevent against free radical damage? Read the next section and find out. But first let us check what we have learnt so far.

Check Your Progress Exercise 1

1) What are antioxidants?

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2) What are free radicals? Briefly discuss their role in PUFA oxidation.

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3) Enumerate the processes that generate free radicals.

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9.5 PHYSIOLOGICAL MECHANISMS TO LIMIT FREE RADICAL DAMAGE

We now know that the free radicals are the normal by-products of the oxidative processes in cells and thus there are several physiological mechanisms whose specific role is to neutralize these free radicals and limit their tissue damaging effects. Figure 9.2 highlights the antioxidant defense system. The primary enzymatic defenses include superoxide dismutase, catalase, peroxidase etc. Non-enzymatic defenses include glutathione, alpha tocopherol, ascorbate, beta-carotene, hydroquinones, flavonoids and phenolic acids.

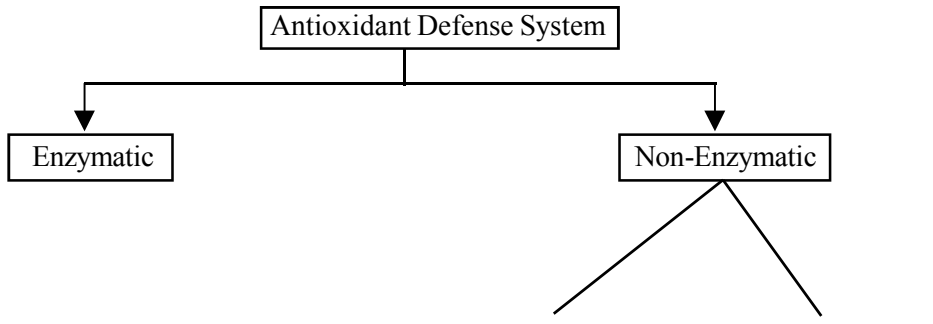

Figure 9.2 : Antioxidant defense system

There are a number of metal containing enzymes whose function is to scavenge and dispose of free radicals. Several essential nutrients are components of or cofactors for enzymes that are involved in free radical disposal. Some examples are listed below:

- *Zinc* and *Copper* are components of the enzyme *superoxide dismutase*, which disposes of the superoxide radical by converting two superoxide radicals to hydrogen peroxide and oxygen.
- *Selenium* is a component of the enzyme *glutathione peroxidase*, which neutralizes hydrogen peroxide and converts it to water and oxygen. It also converts peroxidized lipids into stable and harmless products thus breaking the chain reaction of free radical production.
- *Iron* is a component of the enzyme *catalase* which converts hydrogen peroxide to water and oxygen.
- The enzyme *glutathione reductase* regenerates glutathione, which is oxidized by the glutathione peroxidase reaction mentioned above. This enzyme is a flavoprotein and utilizes a *riboflavin derivative* as a prosthetic group.
- In addition to these enzyme systems, vitamins and other plant pigments (flavonoids) have antioxidant properties and so have the capacity to scavenge free radicals e.g. *vitamin E* in the lipid phase and *vitamin C* in the aqueous phase.
- Some of the substances in food that are known to have or probably have an antioxidant effect are:
  a) the essential minerals– selenium, zinc, copper and iron
  b) vitamins C and E
  c) β-carotene and several other carotenoids, including lycopene (abundant in tomatoes), lutein (found in green vegetables), α-carotene, zeaxanthin and cryptoxanthin, and
  d) other plant pigments, such as polyphenols found in some fruits, tea, olive oil and red-wine and the flavonoids found in grapes, nuts, oranges and strawberries.

Some of the mechanisms involved in the disposal of free radicals are summarized in Box 1.

Box 1: Mechanisms for the disposal of free radicals

Superoxide dismutase (*SOD*) (Zn containing) converts superoxide radicals to H_2O_2

$$\dot{O}_2^- + \dot{O}_2^- + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Glutathione peroxidase (Se containing) converts H_2O_2 to water

$$H_2O_2 + \text{reduced glutathione} \xrightarrow{\textit{Glutathione reductase}} H_2O + \text{oxidized glutathione}$$

Glutathione peroxidase (B_2 containing) regenerates reduced glutathione

$$\text{Oxidized glutathione} \xrightarrow{\textit{Glutathione reductase}} \text{reduced glutathione}$$

The enzyme *catalase* (Fe containing) converts H_2O_2 to water and O_2

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

Vitamin E can quench free radical when it is oxidized.

Free radical + Vitamin E ⟶ Water + oxidized Vitamin E

Lipid peroxyl radical + Vitamin E ⟶ stable hydroperoxide + oxidized Vitamin E

Vitamin E can be *regenerated* by a mechanism that involves Vitamin C

$$\text{Oxidized Vitamin E} \xrightarrow{\text{Vitamin C}} \text{Vitamin E}$$

Some important enzymatic and non-enzymatic physiological antioxidants, their location and properties are summarized in Table 9.1.

Table 9.1 : Important enzymatic and non enzymatic physiological antioxidants

Enzymatic antioxidants	**Location**	**Properties**
Superoxide dismutase (SOD)	Mitochondria and cytosol	Destroys superoxide radicals
Glutathione peroxidase (GSH)	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxide
Catalase (CAT)	Mitochondria and cytosol	Removes hydrogen peroxide
Nonenzymatic antioxidants	**Location / Nature**	**Properties**
Vitamin C	Aqueous phase of cell	Acts as free radical scavenger and recycles vitamin E
Vitamin E	Cell membrane	Major chain-breaking antioxidant in cell membrane
Uric acid	Product of purine metabolism	Scavenger of OH radicals
Glutathione	Nonprotein thiol in cell	Serves multiple roles in cellular antioxidant defense
Lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
Carotenoids	Lipid soluble antioxidants, located in membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
Bilirubin	Product of heme metabolism in blood	Extracellular antioxidant
Ubiquinones	Mitochondria	Reduced forms are efficient antioxidants
Metals ions sequestration: transferrin, ferritin, lactoferrin,		Chelating of metals ions, responsible for Fenton reactions
Nitric oxide		Free radical scavenger, inhibitor of LP

In Table 9.1 you have read about ubiquinones, uric acid as non-enzymatic antioxidants providing protection against free radicals. What are these compounds? Let's learn about them.

Non-enzymatic small molecular weight antioxidants

Despite low levels of enzymatic defenses against free radical, human blood plasma possesses highly efficient small molecular weight compounds which act as antioxidants.

Some such as glutathione, ubiquinone and uric acid are produced by normal metabolism. Other examples of small molecular weight antioxidants are peptides such as carnosine, anserine etc. The role of these small molecular weight antioxidants is highlighted next.

- *Uric acid* has been shown to inhibit lipid peroxidation and scavenge free radicals. Urate is very efficient scavenger of highly reactive and harmful oxygen species – namely hydroxyl radicals, superoxide anion, singlet oxygen and oxygenated heme intermediates in high iron valence states.
- *Ubiquinone* is the only known fat soluble antioxidant synthesized by animal cells. It is believed to play a role in cellular defense against oxidative damage.
- *Carnosine* and *anserine* are dipeptides found in skeletal muscles. They have been shown to decrease membrane lipid peroxidation. The antioxidant mechanism has been postulated as being caused by metal chelation and/or free radical scavenging.

We have studied about the antioxidant defence mechanism to prevent free radical damage above. However, there might be situations that might increase damage by free radicals. What are these situations? Read and find out.

Situations that increase damage by free radicals

The following circumstances may increase the risk of disease due to free radical damage of cellular components:

- increased generation of free radicals beyond the capacity of the mechanisms for their safe disposal and repair of the damage that they induce, and
- impaired capacity of the disposal mechanisms to handle any free radicals that are generated, for example due to dietary deficiency of a key antioxidant nutrient.

So what are the outcomes of the increased generation of free radicals? The next section summarizes the relationship between free radical and diseases. Before we move on to study this aspect, let us recapitulate what we have learnt so far.

Check Your Progress Exercise 2

1) Name the enzymatic antioxidant defense system.

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2) Which minerals are associated with the enzymatic antioxidant defense system?

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3) Name the vitamins which function as antioxidants.

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4) Which vitamin regenerates oxidized tocopherol?

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5) Name the non-enzymatic small molecular weight antioxidants.

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9.6 FREE RADICAL IN HUMAN PATHOLOGY AND DISEASE

Overproduction of free radicals, as you may recall reading earlier, have been implicated in the etiology of a host of degenerative diseases including cardiovascular diseases, diabetes, cancer, Alzheimer's disease, retinal degeneration, ischemic dementia and other neurodegenerative disorders and aging. In addition, they also play a role not only in acute conditions, such as trauma, stroke and infection, but also in physical exercise and stress. Let us learn about the role of free radical, in the context of cardiovascular disease, carcinogenesis and in physiological condition such as aging.

Cardiovascular Diseases

Heart diseases we know are one of the main causes of mortality worldwide. Understanding and potentially controlling oxidative events as they affect cardiovascular disease (CVD) therefore, has the potential to provide enormous benefits to our population in health and lifespan.

A high saturated fat diet tends to raise the LDL cholesterol concentration and further cigarette smoking increases free radical production. If the diet does not provide adequate antioxidants then scavenging free radicals becomes a problem. An unquenched free radical can react with LDL and can get converted to atherogenic oxidized form. With a continuated high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque, the symptom of atherosclerosis. Oxidized LDL is atherogenic, and is thought to be important in the formation of atherosclerotic plaques. Furthermore oxidized LDL is cytotoxic and can directly damage endothelial cells. Figure 9.3 presents a schematic representation of how free radicals might contribute to the risk of cardiovascular disease.

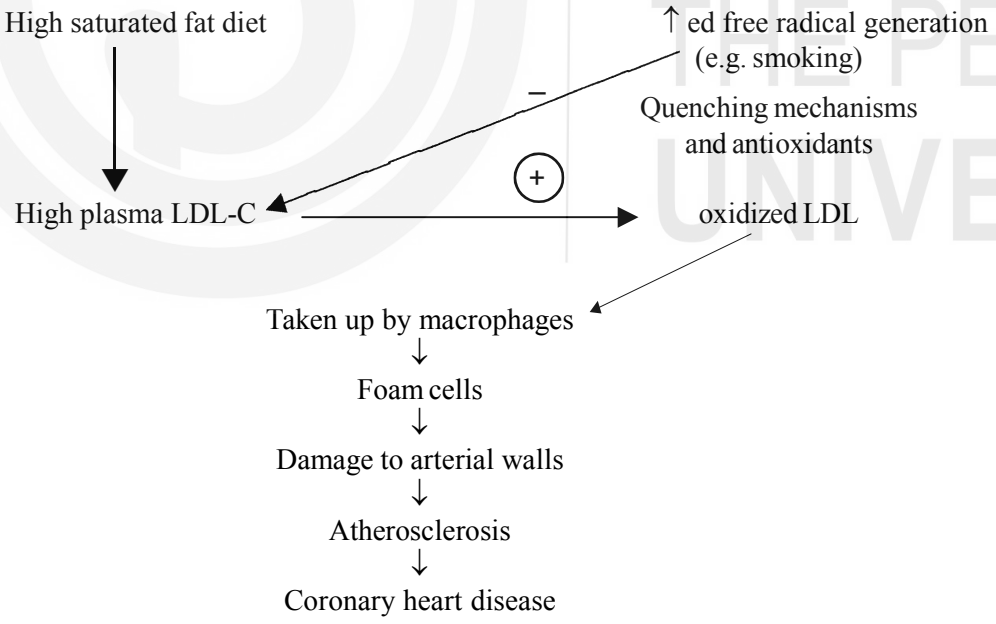

Figure 9.3 : A hypothetical scheme showing how free radicals might contribute to the risk of cardiovascular disease

Carcinogenesis

Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation and transformation, particularly in the past 10 years. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystem could lead to mutation, transformation and ultimately cancer.

Free Radicals and Aging

Strong experimental evidence supports the free radical theory of aging. An increasing number of diseases and disorders, as well as, aging process itself, demonstrate link either directly or indirectly to these reactive and potentially destructive molecules. Not much is known about the mechanism of aging and what determines lifespan. Leading theories attribute these to programs written in DNA and/or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

So what can be done to prevent the onslaught of the free radicals? It is simple. The body relies on the antioxidants, natural or diet-derived to deal with the onslaught. The last section in this unit highlights this aspect.

9.7 NATURAL AND DIET-DERIVED ANTIOXIDANTS

Our daily foods contain a wide variety of free radicals scavenging molecules. Vegetables, fruit, tea, wine are the products rich in natural antioxidant compounds. Fruits and vegetables are rich sources of antioxidants, flavonoids and vitamins. One can get sufficient quantities of these by consuming at least 4-5 servings of fruits and vegetables daily. The recommendation has been based on various epidemiological studies wherein it has been demonstrated that there is a diminished risk of chronic diseases with diets rich in fruits and vegetables. It has been hypothesized that antioxidants, found in fruits and vegetables, may be responsible for this protective effect.

Dietary antioxidants may contribute to the decrease of cardiovascular disease by reduction of free radical formation, as well as, oxidative stress in general, by protection of LDL oxidation and platelet aggregation and by inhibiting synthesis of pro-inflammatory cytokines. Epidemiological studies have shown that a higher intake of these compounds is associated with a lower risk of mortality from cancer and coronary heart disease.

Check Your Progress Exercise 3

1) What happens if oxidized LDL is circulating freely in the system?

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2) Our foods contain a wide variety of free radicals scavenging molecule. Name a few molecules and briefly explain their role.

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9.8 LET US SUM UP

In this unit we learnt about antioxidants and free radicals. We studied that free radicals are highly reactive species that are normal by-products of metabolic processes and immune defense mechanism. These free radicals can react with DNA, membrane phospholipids, proteins and other cellular components. Oxidative damage caused by the reaction of free radicals with cellular components has been implicated in the aetiology of several chronic diseases and perhaps even in ageing.

To prevent free radical damage, the body has a defense system of *antioxidants*. Antioxidants we learnt are substances/molecules that work to reduce damage done to cells and DNA by free radicals. Several cellular enzymes quench free radicals and these enzymes have riboflavin or a dietary mineral as an essential cofactor. Vitamins E and C and some plant pigments such as the carotenoids have inherent antioxidant and free radical disposal property.

9.9 GLOSSARY

Antioxidant	:	components which combine with free radicals in the body and neutralize their damaging effect.
Free radicals	:	atoms or molecules with an unpaired electron.
Alzheimer's disease	:	a specific disease associated with the breakdown of nervous tissue in the brain, giving rise to a dementia in the patient.
Cytokines	:	powerful chemical substances secreted by cells.
Oxidized LDL	:	modification of LDL molecule.

9.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) Antioxidants are components that combine with free radicals in the body and neutralize their damaging effects.

2) Free radicals are highly reactive species that have an unpaired electron. Free radicals can peroxidize polyunsaturated fatty acid (PUFA) residues in low density lipoprotein (LDL) and this ultimately leads to scarring and fibrosis of artery walls.

3) Some of the processes that generate free radicals include electron transport chain, dissociation of oxygen from haemoglobin, certain environmental factors such as cigarette smoke, exposure to high oxygen tension, exposure to ionizing radiation including sunlight and phagocytic white cells .

Check Your Progress Exercise 2

1) The enzymatic antioxidant defense system includes glutathione peroxidase, superoxide dismutase, catalase, glutathione reductase and glutathione transferase.

2) The minerals associated with the enzymatic antioxidant defense system are Zn and Cu as components of SOD, Se as a component of glutathione peroxidase and iron as a component of catalase.

3) β-carotene, α-tocopherol and ascorbic acid are the vitamins which function as antioxidants.

4) Ascorbic acid is the vitamin which regenerates oxidized tocopherol.

5) Uric acid, ubiquinol, carnosine and anserine are the non-enzymatic small molecular weight antioxidants.

Check Your Progress Exercise 3

1) The freely circulating oxidized LDL is taken up by the macrophages which become foam cells. These foam cells cause atheroma, arterial lesions, finally developing into atherosclerosis.

2) Foods such as vegetables, fruit, tea, wine contain a wide variety of free radical scavenging molecules such as antioxidants, flavonoids and vitamins. Their role can be discussed as diminished risk of chronic diseases and contribution to the decrease of cardiovascular disease by reduction of free radical formation as well as oxidative stress in general, by protection of LDL oxidation and platelet aggregation and by inhibiting synthesis of pro-inflammatory cytokines. A higher intake of these compounds is associated with a lower risk of mortality from cancer and coronary heart disease.

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