Free radicals and oxidative stress

1. Introduction

Free radicals are normally present in the body in relative small amounts. An excess can be produced by different factors such as exposure to radiation (sun rays or medical x-rays), exposure to environmental pollutants such as vehicle exhaust fumes and tobacco smoke, exposure to medicines, toxins, chemicals and foods high in fat and unhealthy oils.

A free radical can be defined as an individual or group of atoms that posses at least one unpaired electron thus making it highly reactive and unstable. It interacts with key cell components causing irreversible damage to a cell resulting in the increased development of diseases such as cancer, cardiovascular disease and other age related diseases. It is believed that anti-oxidants aid in the fight off of free radicals (Wiendow, 2009).

2. Why are free radicals so harmful?

Free radicals are compounds such as oxygen, hydrogen peroxide, or hydroxyl groups that have lost an electron. These unstable molecules latch onto another molecule, "stealing" its electrons, which in turn try to steal an electron from another molecule. This process happens usually at a nearby cell membrane, setting off a chain reaction of free-radical formations called lipid peroxidation, which ultimately leads to damage.

Free radicals, also known as oxidants, are increasingly recognised as being responsible for tissue and organ damage, which could lead to the functional disturbances associated with chronic degenerative diseases (Serfontein, 2001). Free radicals can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Mathews and van Holde, 1990).

Accumulation of free radicals over an extended period of time can lead to oxidative stress. According to Heilbronn and co-workers (2006), oxidative stress refers to oxidative damage caused by reactive oxygen species (ROS) resulting in cancer, premature aging and ultimately death.

ROS is produced by various cell types and includes immune and endothelial cells, and inner cellular organelles like the mitochondria (Carter *et al*., 2007). ROS plays a maladaptive role in the body by attacking lipids, protein and DNA and in the process generates a variety of products such as harmful oxidized lipids, less functional proteins, carbohydrates and nucleic acids that negatively affect normal cellular function (Heilbronn *et al*., 2006).

Free radicals increase in the body during stress and exercise (van der Merwe, 2004). They cause oxidative stress to the body and may contribute to more than sixty other health conditions, including:

- atherosclerosis and heart disease
- increased aging of bones, organs, brain and skin
- interference with cell replication
- malignant tissue formation
- enzyme malfunction

3. How does the body cope?

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Mathews and van Holde, 1990; Serfontein, 2001).

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases.

Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either

prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases.

4. Oxidative stress in disease

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. In many of these cases, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage. One case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. Here, low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally cardiovascular disease (Serfontein, 2001).

The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids, the target of lipid peroxidation. Consequently, antioxidants are commonly used as medications to treat various forms of brain injury. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and as a way to prevent noise-induced hearing loss.

Antioxidants can cancel out the cell-damaging effects of free radicals. Furthermore, people who eat fruits and vegetables, which happen to be good sources of antioxidants, have a lower risk of heart disease and some neurological diseases, and there is evidence that some types of vegetables, and fruits in general, protect against a number of cancers (Serfontein, 2001). There is also evidence that antioxidants might help prevent other diseases such as suppressed immunity due to poor nutrition, neurodegeneration, and macular degeneration.

5. Classification of antioxidants

Antioxidants are generally classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). Hydrophilic antioxidants react with oxidants (free radicals) in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet.

The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors.

The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes.

5.1 Ascorbic acid

Ascorbic acid is a monosaccharide antioxidant found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation, it must be obtained from the diet and is a vitamin. Most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In addition

to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants.

5.2 Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Mathews and van Holde, 1990).

5.3 Melatonin

Melatonin is a powerful antioxidant that can easily cross cell membranes and the blood-brain barrier. Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant.

5.4 Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form.

It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Mathews and van Holde, 1990).

6. Pro-oxidant activities

Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide, however, it will also reduce metal ions that generate free radicals through the Fenton reaction.

$2\ Fe^{3+} + \text{ascorbate} \rightarrow 2\ Fe^{2+} + \text{dehydroascorbate}$
$2\ Fe^{2+} + 2\ H_2O_2 \rightarrow 2\ Fe^{3+} + 2\ OH\cdot + 2\ OH^-$

The relative importance of the antioxidant and pro-oxidant activities of antioxidants are an area of current research, but vitamin C, for example, appears to have a mostly antioxidant action in the body.

7. Enzyme systems

As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide (Mathews and van Holde, 1990).

7.1 Superoxide dismutase, catalase and peroxiredoxins

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the

mitochondrion. There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites.

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. This protein is localized to peroxisomes in most eukaryotic cells.

Peroxiredoxins are peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides, as well as peroxynitrite. These enzymes share the same basic catalytic mechanism, in which a redox-active cysteine (the peroxidatic cysteine) in the active site is oxidized to a sulfenic acid by the peroxide substrate.

In its active state, thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining other proteins in their reduced state. After being oxidized, the active thioredoxin is regenerated by the action of thioredoxin reductase, using NADPH as an electron donor.

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione S-transferases. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. In addition, the glutathione S-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism (Mathews and van Holde, 1990).

8. Physical exercise

During exercise, oxygen consumption can increase by a factor 10 or more. Energy production involves reduction of molecular oxygen (O_2); the reduction is not always complete, and about 2-5% of the molecular oxygen turns into the superoxide radical, O_2^-. This increase in free radicals may result in damage that contributes to muscular fatigue during and after exercise (Burke and Deakin, 2010).

The inflammatory response that occurs after strenuous exercise is also associated with oxidative stress, especially in the 24 hours after an exercise session. The immune system response to the damage done by exercise peaks 2 to 7 days after exercise, which is the period during which most of the adaptation that leads to greater fitness, occurs. During this process, free radicals are also produced by neutrophils to remove damaged tissue.

References

Burke, L. and Deakin, C. 2010. Clinical sports nutrition. 4th ed. McGraw-Hill Australia.

Carter, C.S., Hofer, T., Seo, A.Y. and Leeiwenburgh, C. 2007. Molecular mechanisms of life and health-span extension: Role of calorie restriction and exercise intervention. *Nutr. Metab.* **32**: 954-966.

Heilbronn, L.K., de Jonge, L. and Frisard, M.I. 2006. Effects of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals. A*merican Medical Association.* **295** (13):1539-1547.

Mathews, C.K. and van Holde, K.E. 1990. Biochemistry. The Benjamin/Cummings Publishing Company Inc.

Serfontein, W. 2001. New nutrition. Transform your life. Tafelberg Publishers, Cape Town.

Van der Merwe, A. 2004. Stress solutions. Understand and manage your stress for a balanced, energised life. Tafelberg Publishers, Cape Town.

Wiendow, R.A. 2009. Biological and physiological ageing. *Research Starters*. 1-5.