E3 Journal of Biotechnology and Pharmaceutical Research Vol. 4(3), pp. 42-47, March 2013
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 © 2013 E3 Journals

Full Length Research Paper

The effect of glutathione, catalase and ß-carotene on free radicals using model diseases as reference

Ananya Sen[1], Vinaya B. Joseph[1], and Kavitha G. Singh[2*]

[1]Department of Life Science, Post Graduate Studies, Mount Carmel College, No. 58, Palace Road, Bangalore, India- 560052.
[2]Department of Chemistry, Mount Carmel College, No. 58, Palace Road, Bangalore, India- 560052.

Accepted 4 March, 2013

The study was designed to find out the correlation between lipid peroxide (LPO) levels and the severity and complication of stress related disorders with a focus on the potential benefit of antioxidants in the treatment of chronic diseases, as antioxidants play an important role in maintaining a balance between free radicals produced by metabolism or derived from environmental sources. The antioxidants used were glutathione, catalase enzyme and ß- carotene, all of which have been implicated in antioxidant reactions that occur in the body. The lipid peroxidation was assessed by measuring the concentration of plasma malondialdehyde (MDA). The TBA test was performed to measure the amount of MDA present in the sample. The MDA-TBA complex was measured by visible absorption spectrophotometry. The ability of these antioxidants to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was also measured by spectrophotometry. In the following study, it was seen how the antioxidants have the ability to slow, if not stop, the harmful degenerative effect of free radicals in oxidative stress related disorders by decreasing the lipid peroxide levels.

Key words: Lipid peroxides, MDA, DPPH, Antioxidants, Glutathione, Catalase, β-carotene.

INTRODUCTION

Oxidative Stress

The generation of free radicals can occur through natural mechanisms due to cellular metabolic reactions or through a range of environmental factors such as air pollutants, smoking cigarettes, inflammation, radiation (which includes UV rays from the sun), industrial chemicals, processed foods, as well as drugs both recreational and prescription. The generation of free radicals leads to oxidative stress. Oxidative stress (OS)has been implicated in the pathophysiology of many neurological, particularly neurodegenerative diseases. OS can cause cellular damage and subsequent cell death because the reactive oxygen species oxidize vital cellular components such as lipids, proteins, and DNA. Antioxidant defense mechanisms include removal of O_2, scavenging of reactive oxygen/nitrogen species or their precursors, inhibition of lipid proxide formation, and binding of metal ions needed for the catalysis of lipid peroxide generation and up-regulation of endogenous antioxidant defenses. Antioxidants of widely varying chemical structures have been investigated as potential therapeutic agents. Thus neurological disorders such as Alzheimer's are a potential target for the investigation of effect of exogenous antioxidants on oxidative stress.

Free radicals are generated by the following mechanisms.

*Correspondence author. Email: kavi182@yahoo.co.in. Tel: +91-80-22261759; Fax: +91-80-22286386.

Abbreviations: MDA: Malondialdehyde, **DPPH**: 1,1-diphenyl-2-picrylhydrazyl, **TBA**: Thiobarbituric acid, **ROS**: Reactive oxygen species, **OS:** Oxidative stress, **LPO:** Lipid peroxide, **BBB:** Blood brain barrier.

• By homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons.
$X: Y \rightarrow X^* + Y^*$

• By the loss of single electron from normal molecule.
$X: Y \rightarrow X^+ + Y^-$

• By addition of single electron to normal molecule.
$X + e^- \rightarrow X-$

Antioxidants act by preventing the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidants act as radical scavengers, and helps in converting the radicals to less reactive species (Kumar 2011)
.

Stress Related Disorders

Some of the major disorders which involve the formation of free radicals involve coronary diseases such as atherosclerosis and myocardial infarction. A crucial step in the pathogenesis of atherosclerosis is believed to be the oxidative modification of low density lipoprotein (LDL). The oxidation of LDL may be prevented by its endogenous antioxidant compounds, most prominent of which is α-tocopherol. Consequently, an improved antioxidant status may offer possibilities for the prevention of this major disease (Shao and Heinecke 2009).

Further, cancer is another disease that is intimately related to the generation of free radicals. The development of cancer in humans and animals is a multistep process. One type of endogenous damage is that arising from intermediates of oxygen (dioxygen) reduction—oxygen-free radicals (OFR), which attacks not only the bases but also the deoxyribosyl backbone of DNA. OFR are also known to attack other cellular components such as lipids, leaving behind reactive species that in turn can couple to DNA bases. In view of these findings, OFR are considered as an important class of carcinogens (Valko et al. 2004). Hence cancer is also an attractive target for the investigation of the action of antioxidants in retarding the progress of free radical generation. In view of these findings, the following study has taken into account the free radical levels of four diseases namely, breast cancer (2.79± 0.38 µmol/l) (Gönença et al. 2004), Alzheimer's (5.97± 2.37 µmol/l) (McGrath et al. 2001), atherosclerosis (2.54± 1.19 µmol/l) (Södergren 2000) and myocardial infarction (7.48± 1.5 µmol/l) (Levy et al. 1998). These disorders were taken as models during consideration of the average level of free radicals to be tested with the antioxidants.

In this study, three antioxidants have been investigated. They are- Glutathione, Catalase and ß -carotene.

Glutathione

Cells have a number of mechanisms for dealing with the toxic effects of oxygen. One of the most important mechanisms is connected with the widely distributed tripeptide thiol glutathione (L-y-glutamyl-L-cysteinyl-glycine; GSH). 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 (Meister 1994).The normal level of glutathione present in the serum is 480±50 µmol/L.

Catalase

Catalase is usually located in a cellular organelle called the peroxisome (Alberts et al. 2002). It is a very important enzyme in reproductive reactions. Catalase catalyzes the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. Hydrogen peroxide is a harmful by-product of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. The normal plasma level of catalase is 7.3 ± 0.8 U/ml (Leff et al. 1992).

ß-Carotene

β-Carotene is biosynthesized from geranylgeranyl pyrophosphate (Van Arnum 2000). The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals (Stahl and Seis 1996). Lycopene (eleven conjugated and two nonconjugated double bonds) is among the most efficient singlet oxygen quenchers of the natural carotenoids (Krinsky 1998). Carotenoid adduct radicals have been shown to be highly resonance stabilized and are predicted to be relatively unreactive. Beta-carotene has many benefits, such as, it is necessary for growth and repair of body tissue, it helps maintain smooth, soft disease-free skin, it helps protect the mucous membranes on the mouth, nose, throat and lungs, thereby reducing susceptibility to infections, it protects against air pollutants, it counteracts night-blindness and weak eyesight, and it aids in bona and teeth formation.

Malondialdehyde

Reactive oxygen species degrade polyunsaturated lipids,

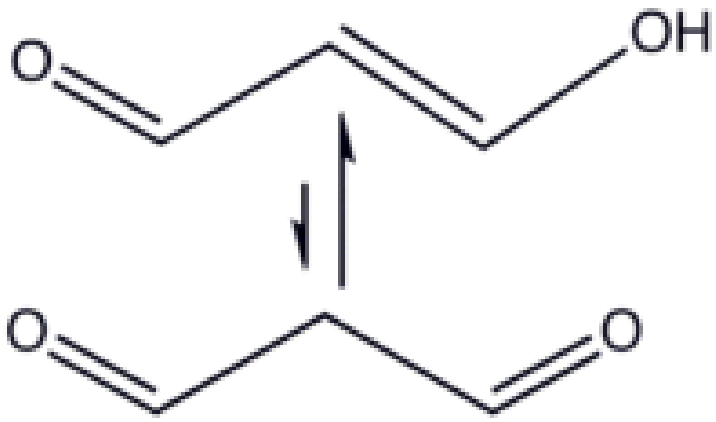

Figure 1. Structure of Malondialdehyde.

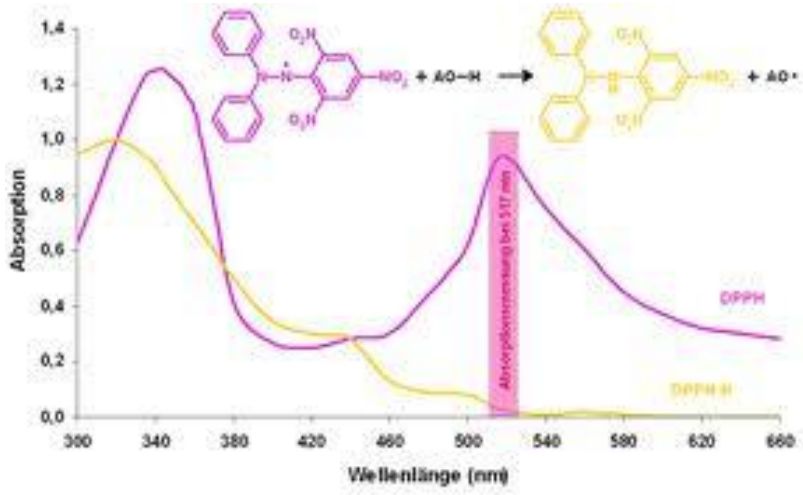

Figure 2. Structure and spectrophotometric characteristics of DPPH.

forming malondialdehyde (Pryor and Stanley 1975). This compound is a reactive aldehyde (Figure 1), and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Del Rio et al. 2005).

Malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M1G, which is mutagenic (Marnett 1999).The guanidine group of arginine residues condense with malondialdehyde to give 2-aminopyrimidines. Thus the measurement of malondialdehyde levels when exposed to antioxidants indicates the effectiveness of the antioxidants in decreasing the levels of reactive oxygen species.

1,1-Diphenyl-2-Picrylhydrazyl

The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH:1) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of colour (Figure 2). 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay is routinely practiced for the assessment of antiradical properties of different compounds. Scavenging of DPPH free radical is the basis of a common antioxidant assay. The basis of DPPH assay is if free radicals have been scavenged, DPPH will change its color to yellow. Due to

presence of odd electrons, it gives a strong absorption maximum at 520nm-540nm that can be measured by visible absorption spetrophotometery.

This study was designed to understand the potential benefit of antioxidants in the treatment of chronic diseases as antioxidants play an important role in maintaining a balance between free radicals produced by metabolism or derived from environmental source.

MATERIALS AND METHODS

Study of the Effect of Antioxidants on MDA

The basis of the TBA methods is the reaction of MDA with TBA at low pH and high temperature to form a coloured complex, the MDA-TBA complex, with an absorption maximum at 532-535 nm that can be measured by visible absorption spectrophotometry (Chirico 1994).The test works well in defined membrane systems (e.g. microsomes and liposomes), but its application to body fluids has produced a host of problems.

A major disadvantage of the TBA test is that it is non-specific and measures many parameters in addition to lipid peroxidation (Gutteridge 1986). A substantial amount of the MDA detected may be generated from decomposition of lipid hydroperoxides during the acid heating stage of the TBA assay as an artifact (Gutteridge 1981). The amount of MDA formed is dependent on the lipid content of the sample, the amount of metal ion contamination in the reagents and antioxidants present in the sample. The addition of antioxidants such as butylated hydroxytoluene (BHT) or EDTA to the sample before analysis is a way to suppress peroxidation during the test itself. Further, several other compounds (e.g. other aldehydes, carbohydrates, amino acids and bile pigments) react with TBA to form complexes that have absorption spectra overlapping that of the MDA-TBA complex (Kosugi et al. 1987). Thus a Standard graph was made taking different aliquots of MDA (1- 5 µmol/l).

Following this, the effects of glutathione, catalase and ß-carotene on MDA levels were measured. The standard concentration of MDA taken was 3 µmol/l as this was the average MDA level that was observed in several stress related disorders. In the case of ß-carotene and glutathione, 2 ml of these antioxidants were incubated with 2 ml of MDA in a water bath of 30°C for 20 min. A similar incubation of MDA with catalase was carried out at room temperature to avoid the degradation of the enzyme.

Study of the Effect of Antioxidants on DPPH

A stock solution of ascorbic acid was prepared by dissolving 0.1 g of the salt in 100ml distilled water (1000 µg/ml). The stock was diluted suitably in order to obtain different concentration of ascorbic acid, ranging from 10 µg-100 µg. Clean dry test tubes were taken and labeled appropriately. 0.1 ml of the above each concentration of ascorbic acid was taken in respective tubes. To this 3 ml of DPPH solution was added. Tubes were incubated for 10mins at room temperature after which absorbance was measured at 540 nm. A blank (distilled water) and control (DPPH) was also prepared.

DPPH Scavenged (%) = A (control) - A (test) ÷A (control) ×100

RESULTS AND DISCUSSION

The aim of the study is to investigate the effect of stress related disorders with the antioxidant treatment on lipid peroxidation levels. The antioxidants used were glutathione, catalase, ß carotene all of which have been implicated in antioxidant reactions that occur in the body. We assessed lipid peroxidation by measuring the concentration of plasma malondialdehyde (MDA) as well as the amount of DPPH scavenged by these antioxidants.

MDA is lipid peroxide which is involved in several cardiovascular disorders. The mechanism involves generation of oxidative stress which in turn results in endothelial dysfunction, generation of kinases and inflammation, this causes altered gene responses and modified cellular responses, this results in apoptosis and myocardial cell injury and dysfunction (Lakshmi 2009). The TBA test is performed to measure the amount of MDA present in the sample. MDA is generated as a degradation product from peroxidised lipids[22] and as a side product of enzymatic metabolism of thromboxanes and prostaglandins (Janero 1990). It has been shown to be formed from PUFAs containing at least two double bonds (Shimizu et al. 1981), but has also been proposed to be derived mainly from fatty acids with three or more double bonds (Esterbauer and Cheeseman 1990).

1, 1-diphenyl-2-picrylhydrazyl is a stable free radical with red color (absorbed at 540nm). If free radicals have been scavenged, DPPH will generate its color to yellow. This assay uses the character to show free radical scavenging activity. Due to presence of odd electrons it gives a strong absorption maximum at 540nm. As these electrons becomes paired off in the presence of a hydrogen donor that is a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured.

We have investigated three antioxidants in this study which are Glutathione, Catalase and ß-carotene. 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. 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 (Meister and Anderson 1983).

Catalase is an enzyme found in nearly all living organisms exposed to oxygen.It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani 2004). The reaction is believed to occur in two stages:

$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+)$

$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2$

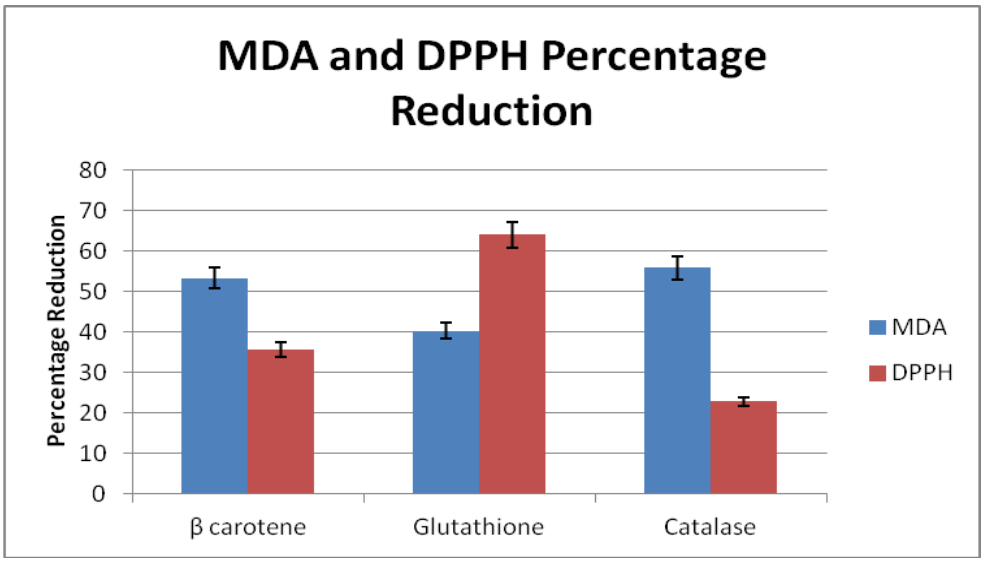

Figure 3. Percentage reduction of MDA and DPPH on incubation with β carotene, Glutathione and Catalase. The error bars indicate the standard deviation whose values have been mentioned in the discussion.

Here Fe()-E represents the iron center of the heme group attached to the enzyme. Fe(IV)-E(.+) is a mesomeric form of Fe(V)-E, meaning that iron is not completely oxidized to +V but receives some "supporting electron" from the heme ligand. This heme has to be drawn then as a radical cation. As hydrogen peroxide enters the active site, it interacts with the amino acids Asn147 (asparagine at position 147) and His74, causing a proton (hydrogen ion) to transfer between the oxygen atoms. The free oxygen atom coordinates, freeing the newly-formed water molecule and Fe(IV)=O. Fe(IV)=O reacts with a second hydrogen peroxide molecule to reform Fe(III)-E and produce water and oxygen.

Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani 1996).

β-Carotene: The prevention of lipid peroxidation by carotenoids has been suggested to be mainly via singlet oxygen quenching (Krinsky 1998). β-Carotene is also a scavenger of peroxyl radicals, especially at low oxygen tension (Burton and Ingold 1984). The interactions of carotenoids with peroxyl radicals may proceed via an unstable β-carotene radical adduct (Burton and Ingold 1984). They may further undergo decay to generate non-radical products and may terminate radical reactions by binding to the attacking free radicals (Rice-Evans et al. 1998). Carotenoids act as antioxidants by reacting more rapidly with peroxyl radicals than do unsaturated acyl chains. In this process, carotenoids are destroyed (Woodall et al. 1997).

The facts outlined in our study support the view that antioxidants play an important role in decreasing the lipid peroxide levels. The MDA levels were considerably lowered when acted upon by each antioxidant. In the case of glutathione, the MDA levels decreased from 3 µmol/l to 1.83±0.28, in n=6 trials. Therefore the level of reduction in MDA levels was 40%. When MDA was treated with ß carotene, it was seen that the MDA levels decreased to 1.5±0, n=6, indicating that the level of reduction was 53%. Incubation with catalase reduced the MDA levels to 1.33±0.28, n=6, indicating that the level of reduction of the MDA levels was 55%. Thus it was observed that the maximum reduction in MDA levels was caused by catalase followed by ß-carotene and glutathione respectively (Figure 3).

In the case of glutathione, the level of DPPH scavenged was 63.99±2.50 %, n=6. When DPPH was treated with ß carotene it was seen that the level of DPPH scavenged was 35.795±5.22 %, n=6. The level of DPPH scavenged in Catalase was 22.85±1.27 %, n=6. Thus it was observed that the maximum level of DPPH scavenged was caused by glutathione followed by ß carotene and catalase respectively (Figure 3).

ACKNOWLEDGEMENT

Acknowledgement

We thank Myrene D'Souza, Shasidhar, and Mount

Carmel College for their invaluable help and support.

Statement of Authorship

Kavitha Singh participated in design of the experiment and provision of significant advice. AS participated in collection of data, analysis of data, writing of the manuscript. VJ participated in collection of data, analysis of data, writing of the manuscript.

Sources of Funding

The experiment was carried out utilizing the chemicals and equipments present in the college laboratory.

Conflict of Interest

None of the authors have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

REFERENCES

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002). Molecular Biology of the Cell, 4th Ed., Garland Science: New York, p. 401.

Burton GW, Ingold KU (1984). β-Carotene: an unusual type of lipid antioxidant. Science, 224: 569-573.

Chelikani P, Fita I, Loewen PC (2004). Diversity of structures and properties among catalases. Cell. Mol. Life Sci., 61: 192-208.

Chirico S (1994). High-performance liquid chromatography-based thiobarbituric acid tests. Methods Enzymol. 233: 314-318.

Del Rio D, Stewart AJ, Pellegrini N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metab. Cardiovasc. Dis., 15: 316-28.

Esterbauer H, Cheeseman KH (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol., 186: 407-421.

Gaetani G, Ferraris A, Rolfo M, Mangerini R, Arena S, Kirkman H (1996). Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood, 87: 1595-9.

Gönença A, Ertena D, Aslanb S, Akıncıb M, Şimşeka B, Toruna M (2006). Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. Cell Biol. Int., 30: 376-380.

Gutteridge JM (1986). Aspects to consider when detecting and measuring lipid peroxidation. Free Radic. Res. Commun., 1: 173-184.

Gutteridge JM (1981). Thiobarbituric acid-reactivity following iron-dependent free radical damage to amino acids and carbohydrates. FEBS Lett., 128: 343-346.

Janero DR (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med., 9: 515-540.

Kosugi H, Kato T, Kikugawa K (1987). Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. Anal. Biochem., 165: 456-464.

Krinsky NI (1998). Overview of lycopene, carotenoids, and disease prevention. Proc. Soc. Exp. Biol. Med., 218: 95-97.

Kumar S (2011). Free Radicals and Antioxidants: Human and Food System. Advances in Applied Science Research, 2: 129-135.

Lakshmi SVV (2009). Oxidative Stress in Cardiovascular Disease. Indian J. Biochem. Biophysics, 46: 421-440.

Leff JA, Oppegard MA, Curiel TJ, Brown KS, Schooley RT, Repine JE (1992). Progressive increases in serum catalase activity in advancing human immunodeficiency virus infection. Free Radical Biol. Med., 13: 143-149.

Levy Y, Bartha P, Ben-Amotz A, Brook JG, Dankner G, Lin S, Hammerman H (1998). Plasma Antioxidants and Lipid Peroxidation in Acute Myocardial Infarction and Thrombolysis. J. Am. Coll. Nutr., 17: 337-341.

Marnett LJ (1999). Lipid peroxidation-DNA damage by malondialdehyde. Mutat. Res., 424: 83-95.

McGrath LT, McGleenon BM, Brennan S, McColl D, McILroy S, Passmore AP (2001). Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. Q. J. Med., 94: 485-490.

Meister A, Anderson ME (1983). Glutathione. Ann. Rev. Biochem., 52: 711-60

Meister A (1994). Glutathione-Ascorbic Acid Antioxidant System in Animals. J. Biol. Chem., 269: 9397-400.

Pryor WA, Stanley JP (1975). Letter: A suggested mechanism for the production of malondialdehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation. J. Org. Chem., 40: 3615-3617.

Rice-Evans CA, Sampson J, Bramley PM, Holloway DE (1997). Why do we expect carotenoids to be antioxidants in vivo? Free Radic. Res., 26: 381-398.

Shao B, Heinecke JW (2009). HDL, lipid peroxidation and atherosclerosis. J. Lipid Res., 50: 599-601.

Shimizu T, Kondo K, Hayaishi O (1981). Role of prostaglandin endoperoxides in the serum thiobarbituric acid reaction. Arch. Biochem. Biophys., 206: 271-276.

Södergren E (2000). Lipid Peroxidation in vivo. Ph.D. thesis, Uppsala University.

Stahl W, Sies H (1996). Lycopene: a biologically important carotenoid for humans? Arch. Biochem. Biophys., 336*:* 1-9.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004). Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cellular Biochem., 266: 37-56.

Van Arnum SD (2000). Kirk-Othmer Encyclopedia of Chemical Technology, New York. pp. 34-42.

Woodall AA, Britton G, Jackson MJ (1997). Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: Relationship between carotenoid structure and protective ability. Biochim. Biophys. Acta., 1336: 575-586.