CHAPTER II

LITERATURE REVIEW

Exercise and Free Radicals

The beneficial effects of physical activity are well known, but the possibility for negative effects has only recently been addressed. Potentially negative effects may occur because elevated aerobic metabolism in exercise increases the production of free radicals. (Mc Ardle WD, 2000)

Exercise is postulated to generate free radical by other means, including

1) increases in epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated,
2) production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and
3) inflammatory responses to secondary muscle damage incurred with overexertion (Clarkson PM and Thompson HS ,2000, Alessio HM, 1993)

Some argued that the potential for free radical damage may also increase during trauma, stress, muscle damage, and from environmental pollutants including smog. With exercise, the risks depend on intensity and training state because exhaustive exercise by the untrained more likely produces oxidative damage in the active muscles. Gee DL and Tappel AL ,1981 suggested that acute exercise has been reported to result in elevated levels of lipid peroxidation by product as indicated by expired breath samples of ethane and pentane. Davies KJ ,1982 determined that the increases in lipid peroxidation levels during acute exercise might indicate that the body's defense system was unable to regulate lipid peroxidation formation during exercise since these periods of regulate lipid peroxidation may be limited.

Ohno H. ,1986 investigated that acute exercise resulted in increased glutathione reductase that appear to respond to exercise.

In recent year, there are rare researches for studying an acute exercise bout on the susceptibility of low density lipoprotein in human. In addition, it is difficult to detect the oxidation stress to increase the susceptibility of low density lipoprotein. Moreover there is no excepted gold standard.for the measurement of lipid peroxidation.

In 1998 , Wetzstein CJ.,et al. describes the effect of an acute exercise bout on the susceptibility of isolated low density lipoprotein (LDL) to in vitro oxidation. LDL was isolated from 23 exercisers and sedentary subjects immediately before and after a single bout of exercise (30 min of treadmill work at 55% & 70 % peak oxygen consumption (VO_2peak). There was statistically significant increase in plasma myeloperoxidase (MPO) levels following exercise compared to baseline values (1.58$\pm$0.91 mg/dl versus 2.08$\pm$12 mg/dl; n=12 ,p $\leq$0.03)

These results suggest that the 30 min exercise bout at a moderate intensity was a program that generate sufficiently oxidative stress to increase the susceptibility in vitro LDL oxidation. Additionally , the exercise bout appeared to activated neutrophils, subsequently releasing MPO protein.

Free radicals

A free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently.(Clark PM, 2000) The conventional radical dot (.) designates the presence of one or more of the unpaired electrons. (Aruroma OI,1994) Free radicals are highly reactive due to the presence of unpaired electron(s).Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be thousand of events long.(GoldFarb, 1999) The electron transport chain, which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen acts as the terminal electron acceptor within the electron transport chain. The literature

suggests that anywhere from 2 to 5 % of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron leak. (Sjodin et al 1990) During exercise oxygen consumption increases 10 to 20 fold to 35-70 ml/kg/min. Under normal conditions most molecular oxygen in biologic systems undergoes tetravalent reduction by efficient intracellular systems such as the cytochrome system. However, 1% to 2% leaks from this pathway to undergo univalent reduction as illustrated in Fig 2.1 (Bulkley GB., 1983). This chain reaction is thought to contribute to lipid peroxidation, DNA damage and protein degradation (Clarkson PM., 2000)

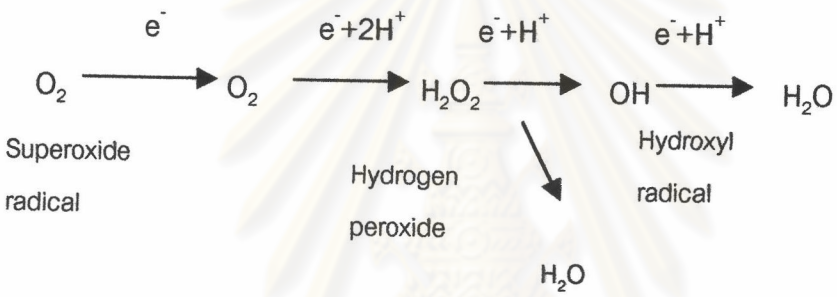


Figure 2.1 Univalent pathway for the reduction of molecular oxygen. By a series of single- electron transfers molecular oxygen is reduced first to the superoxide radical (O_2•) and from superoxide, with the addition of two electrons, to hydrogen peroxide (H_2O_2). Hydrogen peroxide is itself then univalently reduced, with the addition of another proton, to water and the hydroxyl radical (OH'). A final univalent reduction and the addition of another proton convert the hydroxyl radical to water. (Adapted from Bulkley GB 1983)

Lipid peroxidation

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoprotein (LDL) (Dekker J.C., 1996) The PUFAs allow for fluidity of cellular membranes. Lipid peroxidation occurs when free radicals are generated adjacent to polyunsaturated fatty acids (PUFAs) (such as arachidonic acid and linolenic acid) in

membrane lipids. The reactive radical will steal a hydrogen atom from one of the =CH- groups in the fatty acid to generate a carbon-center radicals within the membrane. This process is particularly easy with the polyunsaturated fatty acid (PUFAs). Next, the carbon radical tends to be stabilized by a molecular rearragement to produce a conjugate diene, which rapidly reacts with O_2 to give a hydroperoxy radical. Hydroperoxy radicals abstract hydrogen atoms from other lipid molecules and so continue the chain reaction of lipid peroxidation. (Halliwell and Gutteridge 1984, Jialal and Devarj 1996) Fig. 2.2 and Fig. 2.3. These lipid hydroperoxides tend to migrate away from the hydrophobic interior of the membrane to the surface ,thus disrupting membrane organization. Peroxidation of biological membranes increases their leakiness to ions and causes damage to transmembrane proteins such as receptors and enzymes.

Lipid hydroperoxides decompose in the presence of iron and copper ions to form a wide range of cytotoxic aldehydes, such as malondialdehyde and hydroxynonenal, which themselves are capable of chemically modifying proteins and DNA.

Transition metal ions can thus be involved in lipid peroxidation in two ways. Firstly, they can participate in first- chain reactions which involve attack by any species that is capable of abstracting a hydrogen atom. The highly reactive hydroxyl radical has properties. Several iron ion- oxygen complexes such as perferryl ($FeO_2^{\cdot 2+}$) may also initiate peroxidation. However, since lipid systems will always contain traces of peroxides, the second and principal action of iron will be to decompose these peroxides into peroxyl and alkoxyl radicals and thus perpetuate the chain reaction(Table 2.1). The term reactive oxygen species is used to describe not only oxygen-centered radicals but also some non-radical derivatives of oxygen, e.g. hydrogen peroxide. Hypochlorous acid (HOCl), another reactive oxygen species, produced by the reaction of hydrogen peroxide with chloride ions, plays an important role in the leukocyte respiratory burst which is involved in the host defense system. Both superoxide and hydrogen peroxide have limited chemical reactivity. They can both cross membranes to attack cellular targets, but can only participate in the formation of hydroxyl radicals if

trace amounts of iron or copper are presented. However, in normal circumstances in the absence of such transition metals, they react only slowly.

Some of the more important reactive oxygen species are listed in Table 2.2. It should be noted that free radicals may be positively (H^+) or negatively ($O_2^{\cdot-}$, $^{\cdot}OH^-$) charged, although most are neutral.

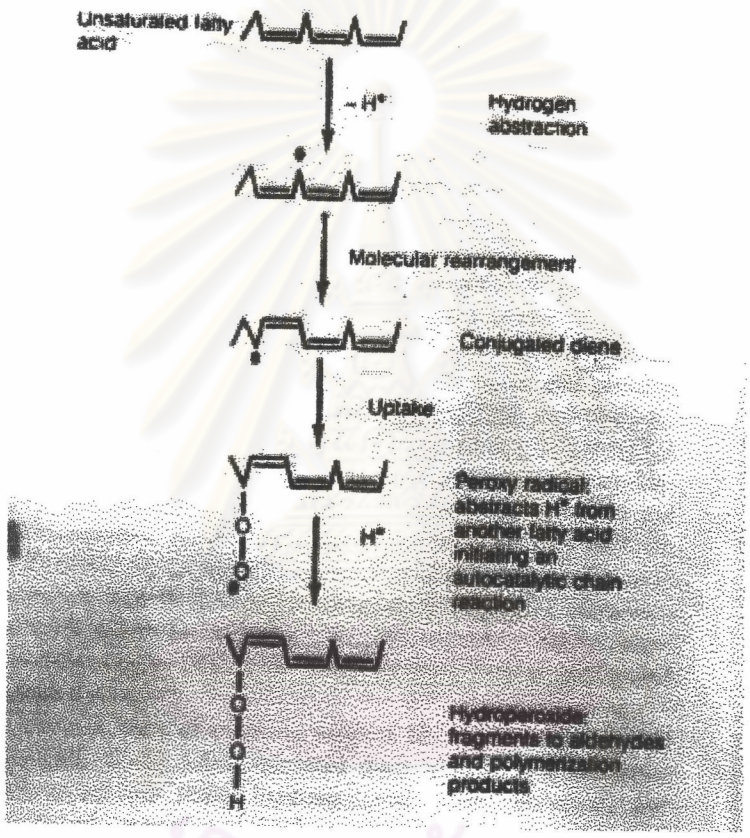


Fig.2.2 Peroxidation of a PUFA adapted from Jialal and Devaraj 1996

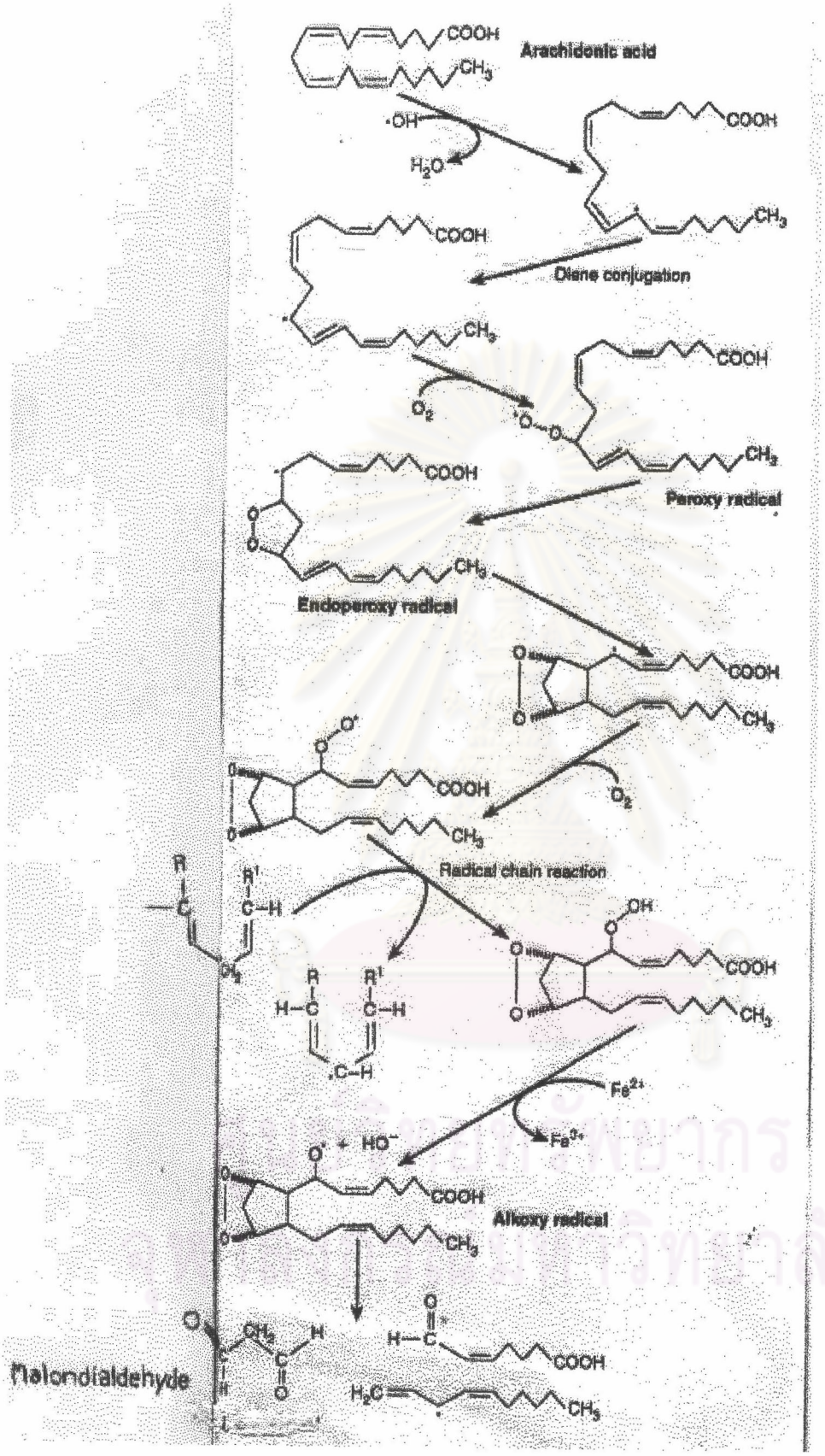


Figure 2.3 Initiation and propagation of lipid peroxidation of arachidonic acid resulting in the formation of malondialdehyde.

Table 2.1 Involvement of iron in lipid peroxidation

(a)	Lipid-OOH + Fe^{2+}	⟶	Lipid-O· + Fe^{3+} OH^-
	Lipid hydroperoxide		Alkoxyl radical
(b)	Lipid-OOH + Fe^{3+}	⟶	Lipid-OO· + Fe^{2+} + H^+
	Lipid hydroperoxide		Peroxyl radical
(c)	Lipid-O· + Lipid-H	⟶	Lipid-OH + Lipid
(d)	Lipid-OO· + Lipid-H	⟶	Lipid-OOH + Lipid
(e)	Lipid· + O_2	⟶	Lipid-OO·

Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting further hydrogen atoms.

Lipid-OO· + Lipid-H ⟶ Lipid-OOH + Lipid

Table 2.2 Free radical species of importance in pathology

Species	Molecular formula
Superoxide ion	O_2^-
Hydroxyl	HO·
Nitric oxide	NO·
Ferryl ion	FeO^{2+}
Perferryl ion	FeO_2^{2+}
Allyl	R·
Alkoxyl	RO·
Peroxyl	ROO·

(R is a polymer of $-CH_2-$ and $-CH=$ groups)

(adapted form Marshall WJ. and Bangest SK. 1995)

Generation of reactive oxygen species during normal aerobic metabolism respiratory burst (Marshall WJ. and Bangest SK. 1995)

The host defence system against harmful organisms includes neutrophils, monocytes, eosinophils and macrophages , which accomplish their protective role by a metabolic event known as the respiratory burst, in which a group of powerful oxidizing agents , including hydrogen peroxide, hypochlorous acid and a number of oxygen radicals, are injected into the phagocytic vacuole (Fig. 2.4) . Radical production is thus important in allowing phagocytes to kill some of the internalized bacteria. This can be

illustrated by patients with chronic granulomatous disease,a group of genetic disorders in which the plasma membrane bound NADPH oxidase system is defective. As a result, although the phagocytes of these patients will engulf bacteria normally, the defect in the respiratory burst is such that several bacterial strains are not killed. Patients with chronic infections with such organisma as *Staphylococcus aureus.*

Eicosanoid metabolism

During the formation of the endoperoxide 9,11-endoperoxy-15-hydroperoxyprostaglandin (PGG_2)from arachidonic acid, a trace of hydroperoxide is required to react with the Fe (III) haem at the active site of cyclo- oxygenase enzyme to form a peroxy radical. This reactive oxygen species can stereospecifically abstract a hydrogen atom from arachidonic acid to commence the process of PGG_2 formation. Excess of lipid peroxides can inactivate cyclo-oxygenase activity.

Endothelium-derived relaxing factor

Endothelium-derived relaxing factor(EDRF) is produced by vascular endothelium and is an important mediator of vasodilator responses induced by several pharmacological agents, including acetylcholine and bradykinin. EDRF has now been identified as nitric oxide. The endothelium seems to produce continuously small amounts of superoxide which can react with nitric oxide (both are free radicals) to form nitrate ions, a non – radical product.

$NO + O_2^{\cdot -} \longrightarrow$ Unknown intermediates $\longrightarrow NO^{3-}$ nitrate

Thus variations in the production of nitric oxide and superoxide by the endothelium may provide one mechanism for regulation of vascular tone. It is possible that the impaired endothelium-mediated vasodilatation in diabetic patients could be related to increased radical formation *in vivo.* Nitric oxide is also produced by macrophages. In brain tissue, nitric oxide synthetase has been localized within neuronal cells with highest activity in neurons of the cerebellum and olfactory bulb. It is suggested that nitric oxide may modulate multiple messenger pathways in the developing and adult human brain.

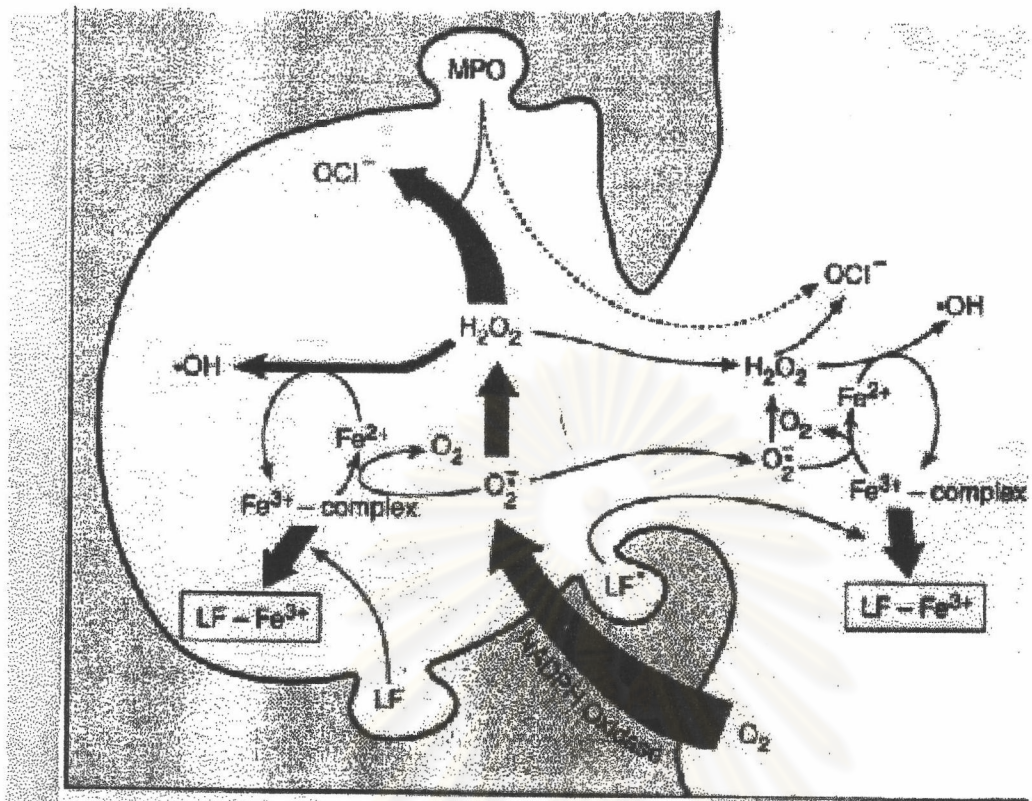


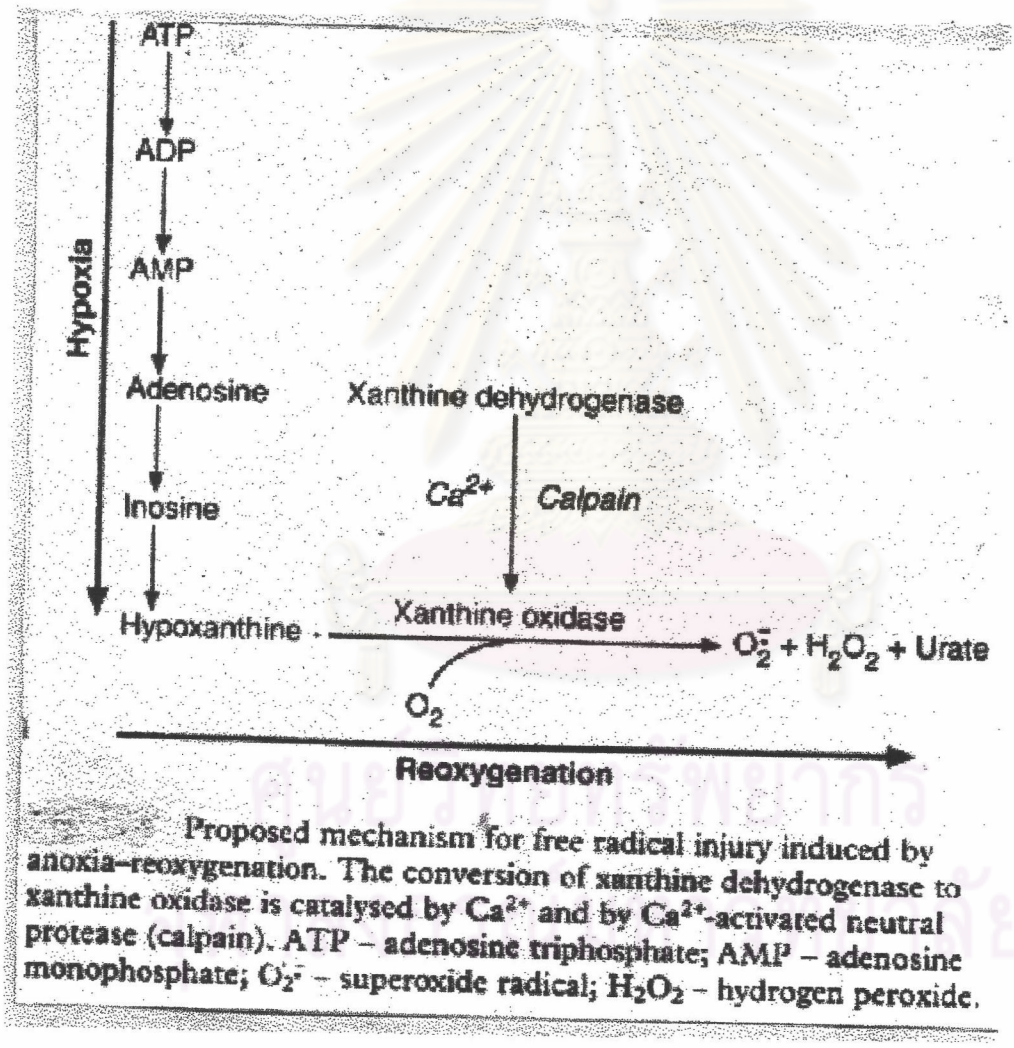
Fig. 2.4 Schematic diagram showing oxygen reduction reactions after neutrophil stimulation leading to hydroxyl (•OH) radical production and the impact of the release of neutrophil granule myeloperoxidase (MPO) and lactoferrin (LF) on this process. Activation of the neutrophil NADPH-dependent oxidase leads to the formation of superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). These compounds react in the presence of an appropriate iron catalyst (Fe^{3+} complex) to yield hydroxyl radicals (•OH). Since neutrophils do not appear to possess such a catalyst, it would have to be supplied by the target and/or microenvironment. MPO (released primarily into the phagosome) would inhibit •OH production by removing H_2O_2 from the system and generating hypochlorite ions (OCl^-). LF release inhibits •OH formation by binding Fe^{3+} in a form incapable of catalysing •OH production.

Fig. 2.4 Schematic diagram showing oxygen reduction reactions. (Marshall WJ. and Bangest SK. 1995)

Controlled leakage in enzymatic reactions

Sources of free radicals within the cell can be derived from leakage of superoxide anions from the mitochondrial electron transport chain. This leakage seems to be an inevitable consequence of operating electron transport chains in the presence of

oxygen.In addition, many compounds will react with molecular oxygen to form superoxide.e.g.adrenaline participates in a complex series of reactions to produce superoxide and hydrogen peroxide. Radicals may be generated during the metabolism of various drugs by the cytochrome P450 microsomal oxidation system, e.g. paracetamol, alcohol. Lastly , some enzymes are known to catalyse the formation of free radicals; for example, xanthine and also oxidizes xanthine to uric acid. In both reactions superoxide and hydrogen peroxide are formed (Fig. 2.5)



Proposed mechanism for free radical injury induced by anoxia–reoxygenation. The conversion of xanthine dehydrogenase to xanthine oxidase is catalysed by Ca^{2+} and by Ca^{2+}-activated neutral protease (calpain). ATP – adenosine triphosphate; AMP – adenosine monophosphate; O_2^- – superoxide radical; H_2O_2 – hydrogen peroxide.

Figure 2.5 Proposed mechanism for free radical injury induced by anoxia-reoxygenation.

Role of reactive oxygen species in pathology

Haemochromatosis(Marshall WJ. and Bangest SK. 1995)

As has been previously stated, under normal circumstances iron is successfully sequestered into a variety of iron- containing proteins, e.g. transferrin and ferritin, to prevent its participation in the Fenton reaction. In genetic haemochromatosis, there is increased uptake of iron at the intestinal mucosa despite adequate iron stores and in tranfusional haemosiderosis there is progressive accumulation of iron from repeated blood transfusions. In both conditions, the hepatocytes become increasingly loaded with iron , in ferritin in the cytosol of the cell and in haemosiderin within distended lysosomes. It is likely that some iron may not be successfully sequestered into these iron storage proteins and that the low molecular weight iron pool may be increased. The evidence for this is equivocal as it is probable that it is the flux of iron through this pool that is increased rather than the size of the pool. It is more likely that there is iron-catalysed lipid peroxidation of the subcellular organelle membranes, especially of the lysosomal and mitochondrial membranes.Lysosomal distention and disruption would occur with the release of the cathepsins and acid hydrolases,leading to cell damage and possibly cell death. Resently, it has also been shown that iron-induced lipid peroxidation causes an increase in collagen gene transcription , indicating that lipid peroxidation may also play an important role in the development of cirrhosis.

Carcinogenesis(Marshall WJ. and Bangest SK. 1995)

Early studies clearly demonstrated that ionizing radiation can cause cancer.It is suggested that the initiation stage in cancer development involves some fundamental change in the genetic material of the cell, brought about by carcinogens including or perhaps in combination with oxygen free radicals. We have already mentioned that DNA strands can be altered or broken by reactive oxygen species. In particularly, hydroxyl radicals will also form base adducts, notably 8-hydroxyguanine, which can cause polymerase induced miscoding on the transcribed DNA strand.

Ischaemic reperfusion damage

One of the more recent advances in free radical research has been the realization that reactive oxygen species may be generated during the reperfusion of ischaemic tissue. This is of importance in organ transplantation.There is considerable evidence that injury to ischaemic tissue occurs almost exclusively during the reperfusion phase and that the damage is caused by the large flux of superoxide radicals which are generated when oxygen is reintroduced to the ischaemic tissue.

Inflammation and immune injury

All kinds of tissue damage, mechanical,physical or chemical trauma(including ischemia), are followed by an inflammatory response. Many other conditions,including autoimmune disorders and inflammatory bowel disease, are also characterized by an inflammatory response. Lymphocytes, granulocytes and macrophages will produce a variety of inflammatory stimulants, including the prostaglandins, reactive oxygen species and free radicals, which may perpetuate tissue dammage.

Neurological disorders

Evidence suggests that in several neurological diseases, for example Parkinson' s disease, Huntington's chorea and Multiple sclerosis, there is iron accumulation secondary to the initial toxic lesion. The reason for this is uncertain,but such iron accumulation could clearly be involved in the exacerbation of the initial lesion by the generation of reactive oxygen species.

Atherosclerosis

The pathogenesis of atherosclerosis is unclear,but it is hypothesized that it is initiated by damage to the vascular endothelium. Endothelial cells are known to be sensitive to damage by reactive oxygen species and lipid hydroperoxides. Thus, any lipid hydroperoxide present in plasma lipoproteins could contribute to initial endothelial damage. Macrophages play an important role in the development of the atherosclerotic lesion. Activated monocytes and macrophages could injure neighbouring endothelial cells by secreting superoxide, hydrogen peroxide and hydrolytic enzymes, while factors

released by macrophages can stimulate proliferation of smooth muscle cells. Macrophages possess receptors for low-density lipoproteins (LDLs), but if LDL has already undergone lipid peroxidation it is recognized by a separate class of receptors known as scavenger receptors. Such modified LDL is taken up with greater efficiency, leading to the rapid accumulation of cholesterol in macrophages and their conversion to foam cells, the characteristic cells of the atheromatous lesion. (Marshall WJ. and Bangert SK.,1995)

Mechanisms of LDL Oxidation

LDL oxidation is generally believed to occur mainly in the intima of artery, in microdomains isolated from antioxidants.LDL can be oxidatively modified in a cell-free system by transition metals such as iron and copper and by all the major cells of the arterial wall such as endotherial cells, smooth muscle cells and monocyte-macrophages. (Steinberg D et al ,1989) Various studies implicate superoxide anion as one agent that promotes oxidation of LDL lipids (Heinecke 1986)

Certain heme protein , myeloperoxidase,secreted by activated phagocytes, may also oxidize lipoprotein by acting as a physiological catalyst. The products of myeloperoxidase action, hypochlorous acid and tyrosyl radical, promote lipoprotein oxidation. Nitric oxide and peroxynitrite are other oxidants relevant to LDL oxidation produced by endothelial cells and macrophages. Thus, LDL can be oxidatively modified by numerous different mechanisms. To date, however, there is no consensus on the predominant mechanism of LDL oxidation in vivo.

Oxidative Modification of LDL

Human LDL is defined as the population of lipoproteins that can be isolated by ultracentrifugation within a density range of 1019- 1.063 kg/l.(Jialal I and Devaraj S,1996)

Each LDL particle contain ~ 1600 molecules of cholesteryl ester and 170 molecules of triglycerides, which from a central lipoprotein core. This core is surrounded by a monolayer of ~ 700 phospholipid molecules, consisting mainly of

lecithin and small amounts of sphingomyelin and lysolecithin and 600 molecules of free cholesterol. Embedded in the outer layer is a large protein , apolipoprotein (apo B100),consisting of 4596 amino acid residues. The total number of fatty acids bound different classes of an LDL molecule is ~ 2700, half of these being PUFAS, mainly linoleic acid. Variations in PUFA content contribute to the difference in oxidation behavior of different LDL samples.

Oxidation of LDL is free radical- mediated process, resulting in numerous structural changes, all of which depend on a common initiating event,the peroxidation of PUFAs in LDL. It is shown in Fig 2.2 .

Biological Effects of OX-LDL

A schema depicting the role of OX-LDL in atherogenesis is shown in Figure 2.6.

During the oxidation of LDL, at first , minimally modified LDL (MM-LDL) is formed in the subendothelial space. Second it is typified by mild lipid peroxidation and uptake by the classical LDL receptor. MM-LDL can induce leukocyte- endothelial adhesion and secretion of monocyte chemotactic protein-1(MCP-1)and macrophage colony- stimulating factor(M-CSF) by the endothelim.This results in monocyte binding and recruitment to the endothelim and subsequent migration into the subendothelial space. Third, macrophages in turn can modify MM-LDL into a more oxidize form. LDL receptor is not recognized OX-LDL and then it is taken by the scavenger receptor on the monocyte-macrophages, which is not regulated by intracellular cholesterol content. At last, this results is foam cell formation.

OX-LDL is cytotoxic, which could promote endothelial dysfunction and the evolution of the fatty streak into a more advanced lesion. It could also promote atherogenesis by altering expression of other genes in the arterial wall and adversely affect the coagulation pathway by inducing tissue factor and plasminogen activator inhibitor-1 synthesis. The products of OX-LDL can impair expression of inducible genes such as tumor necrosis factor and platelet derived growth factor. This could promote further cholesterol accumulation.

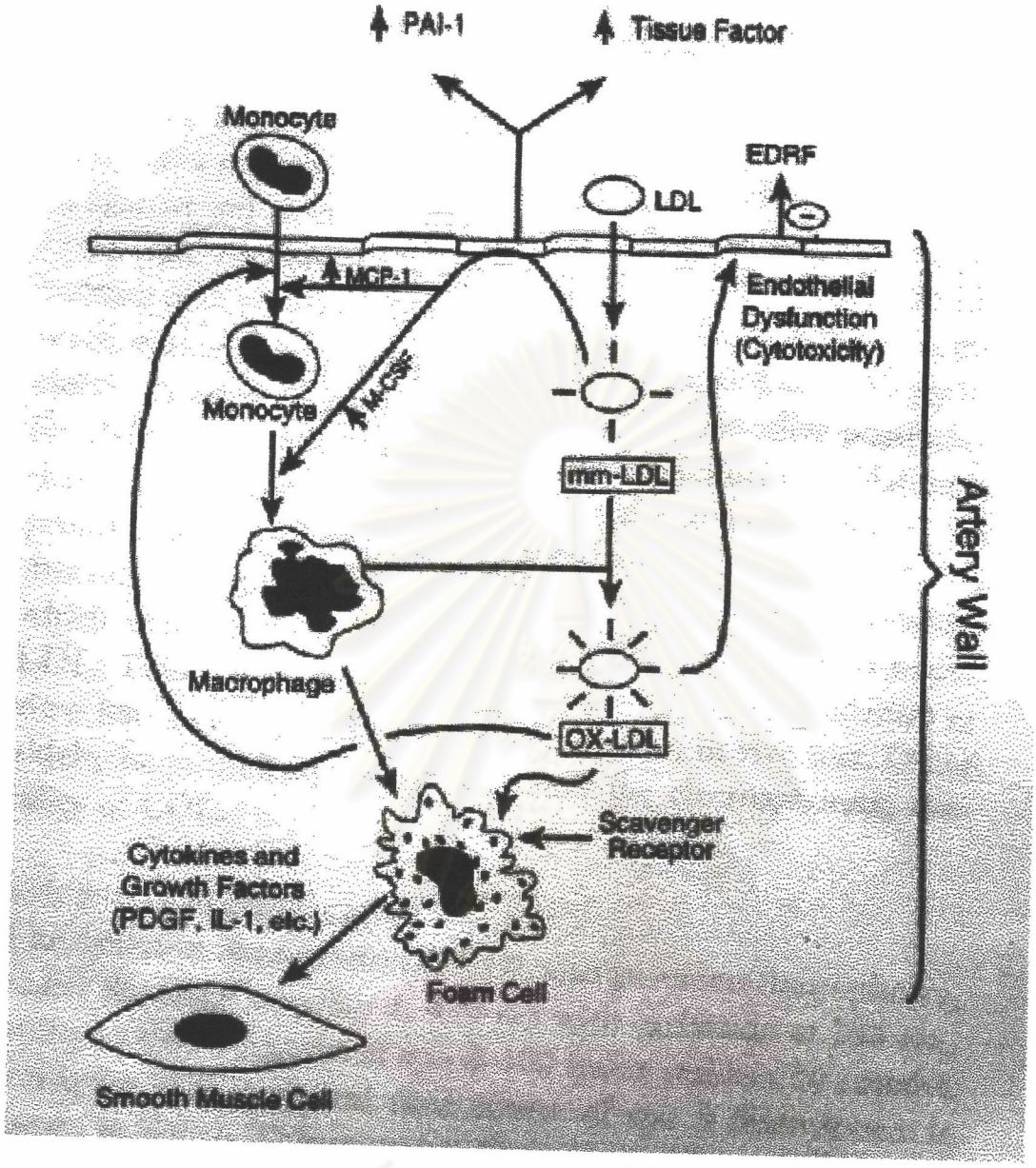


Figure 2.6 The role of OX-LDL in atherogenesis. (Jialal IS and Devaraj S,1996)

Measurement of oxidative stress in humans

Free radicals have a very short half-life, which makes them very hard to measure in the laboratory. Multiple methods of measurement are avaiable today, each with their own benefits and limits.(Halliwell B. and Chirico S. 1993, C Larkson PM and Thompson H 2000, Jialal IS and Devaraj S,1996)

One of the most widely used methods for monitoring LDL oxidation in vitro was the measurement of conjugated diene. This method is so called conjugated dienes, which is rapid and easily performed.(Ahotopa M et al ,1996)

Vasankari T. et al 1994 investigated the three methods which is the most representative in measuring oxidative stress after physiological stimulus (physical exercise) in man. They suggested that diene conjugation as a measure of the early phase of lipid peroxidation may be less affected by protective antioxidant functions than fluorescent chromolipids and reactive material methods, which both measure and products of lipid peroxidation. Therefore diene conjugation may be the most sensitive of the three methods to estimate serum lipid peroxidation induced by exercise in man.

Conjugated dienes

Oxidation of polyunsaturated fatty acid side chains of LDL is accompanied by the formation of dienes that absorb ultraviolet light at 234 nm. Measurement of this UV absorbance is useful in studies of pure lipids and it measure an early stage in the peroxidation process. (Halliwell B. et al ,1993) However, conjugated dienes may also appear in diets, thereby confounding whole tissue peroxidation. The use of conjugated dienes as a marker of oxidative stress in humans must therefore be interpreted with caution.

However, there is no single biomarker that is considered the "gold standard" of lipid or protein oxidation. It is recommended that ≥ 2 techniques should be used for more accurate and consistent evaluation of oxidative stress in humans. (Clarkson PM., 2000)

Thiobarbituric acid

One of the most commonly applied assays is the thiobarbituric acid (TBA) test. This is relatively cheap and simple. The test sample is heated with TBA at low pH and the pink chromagen (a TBA- MDA adduct) is measured by its absorbance at 532 nm or by its fluorescence at 553 nm. The TBA test supposedly measures malondialdehyde formed in peroxidizing lipid systems. However, aldehydes other than MDA can form chromagens,

some of which will absorb at 532 nm. The TBA test rarely measures the free MDA content of the lipid system, most of the MDA being generated by the decomposition of lipid peroxides during the acid heating stage. Such decomposition is enhanced by the presence of iron in the reagents or the sample, while inhibition can occur if chelating agents are present. Peroxide decomposition produces radicals that themselves can peroxidize other fatty acids during the assay procedure. Furthermore, the greater the lipid content, the greater the TBA reactivity; conversely, the higher the amounts of the chain-breaking antioxidants, the lower the TBA reactivity. For such reasons the TBA test has been modified and a new HPLC method has been developed which eliminates many of these artefacts. Amplification of peroxidation during the heating at low pH is prevented by the addition of the chain-breaking antioxidant butylated hydroxytoluene and HPLC is used to separate the authentic TBA- MDA adduct from other chromagens absorbing at 532 nm. Even so, problems still remain with this test; for example, the sugar deoxyribose can also react with TBA to form adducts such that an overestimate of the amount of peroxide present in the tissue can still occur.

Volatile hydrocarbons

Volatile hydrocarbons such as pentane and ethane can be formed during the decomposition of lipid peroxides and their measurement in expired air has provided a popular approach to the assessment of free radical activity in *vivo*. It is a non- invasive technique and artefacts are not generally introduced during sample preparation. However,rigorous controls are needed as hydrocarbons are produced by intestinal bacteria and can also be air pollutants. Hydrocarbon gas production depends upon the presence of metal ions to decompose lipid peroxides and therefore may not give an adequate index of the overall peroxidation process if such ions are only available in limited amounts. (Marshall WJ. and Bangert SK.,1995)

Exercise guidance

The benefits of exercise are too numerous to list, which include weight control cardiovascular disease prevention, control of diabetes, lower blood pressure, enhanced

immune response, prevention of osteoporosis and bone loss, improved mental health and well-being, enhanced self-esteem, longevity and optimum quality of life.

American heart association recommends that low to moderate aerobic exercise with intensity 50-60% VO_{2max}, duration 30 minutes and frequency 3 cession/day can also help sedentary men to improve their healthy and safety from oxidative stress, Further more aerobic exercise for at least 30 minutes per session. It takes a while to liberate fatty acids from adipose tissue and really start burning much fat.

From these review literatures aerobic exercise can induce reactive oxygen species via electron leak in the mitochrondria and reperfusion state during physical activity. Increasing maximum or minimum of oxidative stress depends on intensity and duration of the exercise program.

Hence this study would like to determine whether the widely used program exercise used for untrained people, such as moderate exercise 50-60% VO_{2peak} for 30 min duration could increase oxidative stress due to the exercise.

Furthermore, we would like to detect the level of lipid peroxidation in subject who perform exercise program by LDL diene conjugate method. This investigation of the acute effect of moderate exercise will be the fundamental information for future studies.