Chapter - II

General review of literature
What is coronary artery disease

Coronary artery disease (CAD) is a type of ischemia or obstructed heart disease. The coronary arteries are blood vessels that carry blood and oxygen to the heart muscle. When these arteries become clogged with fatty deposits called plaques, the flow of blood is reduced giving rise to a condition known as coronary artery disease. CAD is sometimes called coronary heart disease (CHD). As plaques build up, the lumen of artery gradually narrows and thickened to finally become blocked. The artery at this stage also becomes less elastic, called "hardening of the arteries". Blocked arteries can not supply the heart enough blood and oxygen, as a result may cause chest pain which is called angina and if blood clot forms, it can suddenly cut off blood flow in the artery and causes myocardial infarction or heart attack. Over the many years, gradually plaques form in the arteries and this process is called atherosclerosis.

What is atherosclerosis

Atherosclerosis is a slowly progressive hardening of the arteries characterized by cholesterol rich fatty deposits on the intimal or inner lining of the coronary artery (atheromas). The presence of fatty deposits, called plaques, leads to an important loss of arterial elasticity called "hardening of the arteries", with narrowing of the artery. This constriction restricts smooth blood-flow and causes blood clots to form leading to blockage of blood flow. This is a condition which ultimately deprives the heart from getting adequate oxygenated blood supply and may cause acute complications in the forms of myocardial infarction (MI), angina pectoris, stroke and intermittent claudication\textsuperscript{1,2}

Nature of atherosclerosis\textsuperscript{3}

Three types of thickening are recognized. Fatty streaks are slightly raised, yellow, narrow, longitudinally lying areas. Foam cells are their main character, lipid-laden distorted cells that can arise both from endogenous smooth muscle cells and (more
usually) from macrophages. Fatty streaks probably serve as precursors of fibrous plaques. These are approximately round, raised lesions, usually off-white in colour and often a centimeter or so in diameter, slightly obstructing the vascular lumen. A typical fibrous cap obtained in fibrous plaque (is mostly composed of smooth muscle cells and dense connective tissue containing collagen, elastin, proteoglycans and basement membranes) that covers an area rich in macrophages, smooth muscle cells and T lymphocytes. Often there is a deeper necrotic core, which contains debris from dead cells, extra cellular lipid deposits and cholesterol crystals.

Fibrous plaques are very complicated when altered by necrosis, calcium deposition, bleeding and thrombosis. Plaques cause disease by limiting blood flow to a region of an organ such as the heart or brain. Stroke or myocardial infarction occurs when the lumen of an essential artery becomes completely occluded, formation of thrombus is often triggered by plaque rupture, thereby releasing noxious products into the bloodstream. Most frequently plaque disruption occurs where the fibrous cap is thinnest and most heavily infiltrated by foam cells: macrophage-derived protease enzyme may involve.

Atherosclerosis and its relationship to coronary artery disease

Atherosclerosis is a slow progressive disease characterized by the accumulation of cholesterol deposits in macrophages in large and medium-sized arteries. This deposition leads to a proliferation of certain cell types within the arterial wall that gradually impinge on the vessel lumen and impede blood flow. This process may be quite insidious lasting for decades until an atherosclerotic lesion, through physical forces from blood flow, becomes disrupted and deep arterial wall components are exposed to flowing blood, leading to thrombosis and compromises oxygen supply to target organs such as the heart and brain. The loss of heart function as a result of reduced blood flow is termed heart attack, and this clinical manifestation of atherosclerosis is often referred to as coronary artery disease.
The formation of an atherosclerotic plaque and its complications

The normal artery

Structure of blood vessels

In general, the blood vessel wall is composed of three well-defined concentric layers that surround the arterial lumen, each of which has a distinctive composition of cells and extracellular matrix, they are intima, media, and adventitia.

a) The intima: The layer immediately adjacent to the lumen is called the intima. It consists of a single layer of endothelial cells, in close proximity to the internal elastic lamina, lying on a continuous basement membrane composed of type IV collagen and structural glycoproteins.

b) The media: The middle layer is known as the media. It is composed of smooth muscle cells surrounded by a connective tissue matrix made up of collagen fibres (type I and type III), elastic fibres, glycosaminoglycans, and structural glycoproteins.

c) The adventitia: It is the outermost layer containing fibroblasts, adipocytes, mast cells, and an extracellular matrix of collagen fibres, lipids, and structural glycoproteins. Most of the neural input into blood vessels also traverses through the adventitia. At one time, the adventitia was considered inactive with respect to vascular homeostasis, however, recently it has become clear that the adventitia, through the production of reactive oxygen species (ROS) may play an important role in controlling vascular remodelling and nitric oxide (NO) bioactivity.

The concentric layers of elastin demarcated these three layers, known as the internal elastic lamina, which separates the intima from the media, and the external elastic lamina that separates the media from the adventitia. There is a single contiguous layer of endothelial cells that lines the luminal surface of arteries. These cells sit on a basement membrane of extracellular matrix and proteoglycans that is bordered by the internal elastic lamina. Occasionally smooth muscle cells are found in the intima, where endothelial cells are the principal cellular component and form a physical and functional barrier between flowing blood and the stroma of the arterial wall. A wide array of processes including thrombosis, vascular tone, and leukocyte trafficking are regulated.
by endothelial cells. Progressing outwards from the internal elastic lamina, the media consists principally of smooth muscle cells arranged in layers, depending on the arterial size. An extracellular matrix consisting largely of elastic fibers and collagen with a lesser content of proteoglycan holds the smooth muscle cells together. An increasing content of elastin characterizes larger arteries that need to provide considerable elastic recoil during diastole, the time period between ejections of blood from the heart.

The atherosclerotic artery

Primarily atherosclerosis is a disease of the tunica intima of elastic arteries. Coronary, carotid, cerebral, renal arteries and the aorta are most often involved. Some are more prone than others, for reasons that remain largely unknown. Atherosclerotic lesions pass through several stages during development and it was observed that the earliest lesion is a subendothelial accumulation of lipid-laden macrophage foam cells and associated T-lymphocytes, which form a non-stenotic fatty streak. Fatty streaks are with progression; the core of the plaque becomes necrotic, containing cellular debris and form foam cells. This core is bounded on its luminal aspect by an endothelial-sized fibrous cap containing vascular smooth muscle cells embedded in an extensive collagenous extracellular matrix. Inflammatory cells are also present in the fibrous cap, particularly in the shoulder regions, where T-cells, mast cells and especially macrophages have a tendency to accumulate. In advanced lesions, there are also deposits of calcium that make the lesions less compressible and therefore more prone to rupture. There are also numerous immature new blood vessels that facilitate further recruitment of inflammatory cells and tend to predispose to intra plaque hemorrhage. Thus, the composition of atherosclerotic lesions is variable and complex, and it is the interaction between the various cell types within a plaque that determines the progression, complications and outcome of the disease.
Cellular Involvement in Atherosclerosis

Endothelial cells

The endothelial cell lining of the vasculature plays a fundamental role in maintaining vascular homeostasis in health. The size of blood vessels varies extensively from the capillaries to the veins and arteries. All of them have lining in the luminal surface as a single layer of endothelial cells that is supported by an underlying extracellular matrix (ECM). Endothelial cell morphology varies between large and small vessels.

There are tightly packed sheets of polygonal endothelium cells in large veins and arteries. In small venules and capillaries, individual endothelial cells form the vessel through which the blood cells pass in single file.

The integrity of endothelial cells is related to atherogenesis, and this relationship is still in research stage. It is not clear of the doubt that macromolecules of the configuration of LDL can gain entrance to the artery wall via the transport vesicles which traverse the endothelial cells. Nitric oxide has multiple actions in the vasculature, including flow-dependent vasodilatation, inhibition of leukocyte and platelet adhesion and aggregation, anti-proliferative activity, and the inhibition of vessel permeability, which collectively tend to be antiatherosclerotic. Compelling evidence supports a role for endothelial cell dysfunction as a key to early event in the pathophysiology of atherosclerosis. The production or bioactivity of nitric oxide is reduced in conditions that predispose to atherosclerosis such as hypercholesterolaemia, diabetes mellitus, and cigarette smoking.

Therefore, endothelium works as the gatekeeper, regulate the lipoprotein transportation and also have potential to express leukocyte adhesion molecules, attractant protein, and major histocompatibility antigens. Many factors can induce endothelial cell injury and cause its dysfunction, such as:

a) Increased endothelial permeability
b) Increased leukocyte adhesion
c) Accumulation of macrophages in subendothelial intima
d) SMC activation and proliferation

Lipoproteins (mainly LDL-C and also VLDL-C), oxidized lipoprotein, and circulating cells (particularly monocytes and T- lymphocytes) enter the subendothelial space and
form the fatty streaks, then monocytes transform to macrophages and foam cells in the vessel wall, and eventually results in complex advanced lesion of atherosclerosis\textsuperscript{12}.

**Smooth muscle cells**

In the raised fibrous plaque smooth muscle cells (SMC) are the predominant cells\textsuperscript{13}. SMC are the major component of the medial layer in a normal artery, although they may also be present in the intima in some arterial segments. In some pathological conditions SMC are stimulated to migrate and proliferate, when it becomes much more active synthetically and more responsive to stimuli causing cell proliferation. Furthermore, morphological appearance is also changed. Rough endoplasmic reticulum, free ribosomes and the Golgi apparatus become more prominent\textsuperscript{14}. The interaction between LDLs and SMCs has become a very important part of the formation of atherosclerosis\textsuperscript{15}. The arterial SMCs can take up relatively large quantities of LDLs and store some of these in vacuoles, perhaps by an unregulated, non-receptor scavenger mechanism, whereas the quantity of LDL taken up by SMCs under usual circumstances is regulated by the LDL receptor system and its “feedback” mechanisms.

Gross morphology of atherosclerotic lesions.

**Plaque Morphology**

The light microscopic appearance of a prototypical atherosclerotic plaque is depicted. Plaques contain a central lipid core that is most often hypocellular and may even include crystals of cholesterol that have formed in the aftermath of necrotic foam cells. In this late stage of lesion development, residual foam cells may be difficult to see but have often left the core with an abundant quantity of tissue factor\textsuperscript{16}, an important activator of the clotting cascade. This lipid core is separated from the arterial lumen by a fibrous cap and myeloproliferative tissue that consists of extracellular matrix and smooth muscle cells. The junction between the cap and the morphologically more normal area of artery is known as the shoulder region of the atherosclerotic plaque. This area is typically more cellular than other areas of the plaque and may contain a variable composition of smooth muscle cells, macrophages, and even T cells. The shoulder region is most prone to rupture and, may even contain evidence of previously healed fissures.
Human atherosclerotic plaques have been classified by the American Heart Association (AHA) committee on Vascular Lesions according to lesion morphological characteristics. Plaque progression, from fatty streak to the advanced complicated lesion, is divided into six phases which reflect the early, developing, and mature stages of the disease\textsuperscript{17,18}.

a) In lesion-prone arterial sites, adaptive thickening of the intima is among the earliest histological changes. It consists of a small lesion of the type often found in those less than 35 years of age. These plaques can advance over time and are characterized as lesion types II, III, and I.

b) Type II lesions (i.e., foam cells), as macrophages accumulate lipid, form nodular areas of lipid deposition that are also known as “fatty streaks,” and these represent lipid-filled macrophages. It consists of both macrophages and smooth muscle cells surrounded by some extracellular debris and lipid droplets.

c) In type III lesions, foam cell formation more readily progresses, contain small extracellular pools of lipid that can produce lesions. Types II and III lesions are readily apparent through the use of fat-soluble dyes that stain cholesterol esters accumulated in macrophages and the extracellular space. These early lesions are often evident by age 10\textsuperscript{19}, and can occupy as much as one-third of the aortic surface by the third decade. Developing lesions represent the next two types of lesions and, are characterized by significant areas of extracellular lipid that represents the “core” of the atherosclerotic lesion.

d) Type IV lesions, characterized by confluent cellular content with an extensive amount of extracellular lipids whereas type V lesions exhibit fibrous thickening of this structure, also known as the lesion “cap”.

e) These, type IV and V lesions can be found initially in areas of the coronary arteries, abdominal aorta, and some aspects of the carotid arteries in the third to fourth decade of life. Alterations in the shape of the disrupted plaque and organization of the thrombus by connective tissue can lead to the more stenotic and fibrotic type Vb or Vc lesions. Type Vb or Vc lesions may be manifested clinically by angina and evolve into occluding lesions. However, the final occlusion may be silent or subclinical.
f) Type VI lesions, (mature type) exhibit architecture that is more complicated and characterized by calcified fibrous areas with visible ulceration. These types of lesions are often associated with symptoms or arterial embolization. It was once thought that end-organ damage and infarction were due to gradual advancement of these lesions, but now we know the processes involved in precipitating heart attack and stroke are considerably more complex. As a consequence, we direct our attention to the histology of an atherosclerotic plaque.

Role of macrophages in the developing plaque

A cytokine or growth factor produced in the inflamed intima, macrophage colony-stimulating factor, induces monocytes entering the plaque to differentiate into macrophages which is very important for the development of atherosclerosis and is associated with up-regulation of pattern-recognition receptors for innate immunity, including scavenger receptors and toll-like receptors. A broad range of molecules are internalized by scavenger receptors and particles bearing molecules with pathogen-like molecular patterns. Through these pathways bacterial endotoxins, apoptotic cell fragments, and oxidized LDL particles are all taken up and destroyed. If cholesterol derived from the uptake of oxidized LDL particles cannot be mobilized from the cell to a sufficient extent, it accumulates as cytosolic droplets. As a result, foam cell is produced from this cell, the prototypical cell in atherosclerosis. Toll-like receptors also bind molecules with pathogen-like molecular patterns, but in contrast to scavenger receptors, they can initiate a signal cascade that leads to cell activation. The activated macrophage produces inflammatory cytokines, proteases, and cytotoxic oxygen and nitrogen radical molecules. Similar effects are observed in dendritic cells, mast cells, and endothelial cells, which also express toll-like receptors. Stress proteins, bacterial toxins, and DNA motifs are all recognized by various toll-like receptors. Along with it, human heatshock protein 60 and oxidized LDL particles may activate these receptors. Cells in human atherosclerotic lesions display a spectrum of toll-like receptors, and plaque inflammation may partly depend on this pathway. In support of this notion, genetic removal of a molecule in the toll-like receptor-signaling pathway inhibits atherosclerosis in apoE-knockout mice.
**Plaque Rupture**

As early as in 1920s, concepts of atherosclerosis involved progressive luminal narrowing until the blood flow was compromised to the point that organ metabolic needs could no longer be met, producing ischemia and infarction of the subtended tissue such as the heart or the brain. And also known that coronary thrombosis resulted from disruption of the intima. The clinical significance of this finding remained controversial for the next 60 years.

But this concept has changed dramatically over the last 25 years; the notion of plaque rupture was included as not only a precipitant of clinical events but also a component of plaque progression in atherosclerosis. In the 1980s, angiographic and angioscopic studies show the importance of plaque rupture and thrombus formation in the development of myocardial infarction and unstable angina. Acute myocardial infarction and crescendo angina, two cardinal manifestations of atherosclerosis, are associated with atherosclerotic plaque rupture and fissuring in the artery with compromised blood flow and also suggest that clinical events are the consequences of an abrupt, catastrophic change in plaque morphology rather than a gradual narrowing of the lumen, hinting that plaque rupture is part of atherosclerotic lesion progression rather than a unique feature of clinical events from atherosclerosis. Given that plaque rupture is implicated as a precipitating event in the clinical manifestations of atherosclerosis, a considerable effort has been directed at understanding the successive events involved in this process.

Mature atherosclerotic plaques can be categorized as either stable or vulnerable to rupture. Smaller lipid core, a thick fibrous cap, and shoulder regions with few inflammatory cells are common character of stable plaques, whereas vulnerable plaques contain considerable lipid in their core, a thin fibrous cap, and a robust population of macrophages and T cells in their shoulder regions. Morphology differences of these plaques suggests vulnerable plaques may be weaker structurally and more likely to rupture in response to the physical forces of flowing blood. This contention is supported by experimental data linking an increased content of macrophages in lesions to
structural weakness\textsuperscript{30}. In summary, atherosclerosis is a major source of morbidity and mortality in the developed world that is characterized by LDL-C deposition in the arterial wall, a process that is stimulated by environmental and genetic factors together with tobacco use, diabetes, and hypertension. This LDL-C deposition occurs primarily within macrophages and ultimately be gets the formation of well defined lesions in the arterial intima. Such lesions then develop and are prone to rupture and, as a consequence, can precipitate the clinical events such as heart attack and stroke. Given the public health implications of this disease, it is not surprising that considerable effort has been devoted to understanding the molecular mechanisms of atherosclerosis and the factors that predispose individuals to clinical events.

**Mechanism of plaque rupture**

Now it’s a great question to scientist that what causes a silent atherosclerotic lesion to rupture. Several types of molecules like inflammatory cytokines, proteases, coagulation factors, radicals, and vasoactive molecules are produced by activated macrophages, T cells, and mast cells at sites of plaque rupture\textsuperscript{31,32,33} which can destabilize lesions. Formation of stable fibrous caps are inhibited by them, attack collagen in the cap, and initiate thrombus formation\textsuperscript{34-37}. All these reactions can conceivably induce the activation and rupture of plaque, thrombosis, and ischemia. Two types of proteases have been implicated as key players in plaque activation: matrix metalloproteinases (MMPs) and cysteine proteases\textsuperscript{38,39}. Several members of these families of enzymes occur in the plaque and may degrade its matrix. At several levels MMP activity is controlled: inflammatory cytokines induce the expression of MMP genes, whereas plasmin activates proforms of these enzymes, and inhibitor proteins (tissue inhibitor of metalloproteinase) suppress their action. In a same way, cysteine proteases are induced by certain cytokines and checked by inhibitors termed “cystatins”\textsuperscript{40}. In the formation of aneurysms, several of these molecules play decisive roles, as shown by experiments in gene-targeted mice. However, mechanistic studies in models of atherosclerosis have yielded complex results, with certain MMPs reducing rather than increasing the size of the lesions. At the same time, these enzymes clearly affect the composition of plaque.
Therefore, they may represent future therapeutic targets. Study of plaque rupture in animal models should be helpful in determining the role of these proteases in the activation of plaque and myocardial infarction.

Overview of current concepts of atherogenesis

It is well accepted that the fatty streak lesion is the earliest visible lesion of atherosclerosis and that most of more advanced lesions can be traced back to the fatty streak as the progenitor lesion. Presumably, then, if we could limit the generation and rate of progression of fatty streak lesions we would reduce the number and severity of the more advanced, clinically consequential lesions as well. One of the earliest responses to hypercholesterolemia is an increase in the adherence of circulating monocytes to arterial endothelial cells. This is due to an increase in the expression of adhesion molecules both on the circulating monocyte and on the endothelial cell surface. The adherent monocytes then migrate between endothelial cells in response to chemoattractant molecules generated and released by cells of the arterial wall (including the endothelial cells themselves). In the subendothelial space the monocyte undergoes a dramatic modification of its prototype to become a "tissue macrophage," expressing a large number of genes that are not expressed on the circulating monocyte, including the scavenger receptors involved in the uptake of ox-LDL-C. Presumably LDL-C is constantly entering the wall of the artery from the plasma compartment and returning during its residence in the subendothelial space. It may undergo some degree of oxidation even a very minimal degree of oxidation is enough to confer proatherogenic properties on LDL (MM-LDL), including the ability to stimulate release from endothelial cells of an important macrophage growth factor. A degree of oxidation is necessary to generate the form of LDL-C recognized by the scavenger receptor, now expressed on the phenotypically altered monocyte population that presumably is the basic for the beginning of lipid accumulation leading to generating of foam cells.
Molecules involved in atherosclerosis

Several important molecular components like the adhesion molecules and some growth factors like the platelet-derived growth factor (PDGF) play an important role in the development of atherosclerosis\textsuperscript{41}.

Adhesion molecules

It is already proved that the expression of adhesion molecules is correlated with the extent of the atherosclerotic lesion\textsuperscript{42,43}. There are several different structural groups of adhesion factors which have been identified on endothelial cells and which interact with receptors on leukocytes and platelets. Intracellular adhesion molecule-1 (ICAM-1) and intracellular adhesion molecule-2 (ICAM-2) are cell surface glycoproteins found on many cell types. ICAM-1 is inducible on cultured endothelial cells by the inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor (TNF), interferon and endotoxin. ICAM-1 can bind lymphocytes, monocytes and neutrophils to endothelium. ICAM-2 is constitutively expressed on endothelial cells and appears to be a truncated form of ICAM-1. Vascular cell adhesion molecule (VCAM) is also induced by cytokines and binds selectively to lymphocytes and to some degree to monocytes, but not to neutrophils\textsuperscript{44}.

In human samples from autopsies and hearts of atherosclerotic patients, ICAM-1 is detected in endothelial cells over plaques, intimal vascular smooth muscle cells, and macrophages. VCAM-1 is detected in luminal endothelial cells in advanced coronary artery plaques and neovascular endothelial cells at base of plaques; it is also found in focal endothelial cells of uninvolved vessels with diffused intimal thickening and in macrophages. These CAMs are likely to mediate leukocyte infiltration\textsuperscript{45}.

Pathogenesis of atherosclerosis

The extracellular matrix is the result of the biosynthetic activity of most cells of the organisms. The vascular walls are relatively rich in extracellular matrix (ECM). ECM
forms the basal lamina in the capillaries and also constitute the major portion of the media and the adventitia in the aorta\textsuperscript{46}. The selection of the quality and expression of the quantity of these ECM macromolecules require a precise "programming" to be able to determine the differentiation of the vascular wall cells. There may be some problems if it deviates any way from this precise "programming". Atherosclerotic development is a good example of such deviations from the normal programming of the biosynthesis of ECM macromolecules. There are two major cell types of the arterial wall: endothelial cells and smooth muscle cells. Many others types of cells also are involved in the process of atherosclerosis such as monocytes, macrophages and platelets and other molecules such as chemotactic factors.

**Endothelial dysfunction and cardiovascular disease**

A primary function of the endothelium is to control the intra- and transcellular traffic of numerous nutrients, hormones and cells. The endothelium can express cell adhesive molecules, which have a key role in the adhesion and subsequent trans-endothelial migration of leukocytes into the vascular wall. This process is crucial in the clearance of LDL-C, and especially ox-LDL, in the arterial wall. LDL-C is cleared by legation to LDL receptors and ox-LDL may also bind to scavenger receptors on monocytes/macrophages in the subendothelial compartment. The uptake of ox-LDL by monocytes/macrophages subsequently leads to formation of foam cells, a primary event in the generation of fatty streak lesions. The endothelium also prevents exposure of the thrombogenic subendothelium to circulating coagulation factors and thus exerts anti-thrombotic actions\textsuperscript{47}. The endothelial function may be impaired by risk factors for CVD such as hypertension, hyperlipidemia (including elevated ox-LDL levels), and diabetes. These risk factors, which are also oxidative stress-related, contribute to an increase in endothelial cell permeability leading to intimal edema and the influx of ox-LDL into the vessel wall. Hypertension is an independent indicator of increased risk of coronary events. EDV (endothelium-dependent vasodilation) has been shown to be reduced in patients with
essential hypertension⁴⁸. Reduced levels of endothelium-derived relaxing factor have been demonstrated in hypertensive patients with left ventricular hypertrophy⁴⁹. Abnormal endothelium-dependent vasodilation has also been demonstrated to be related to hypercholesterolemia in patients without evidence of atherosclerosis⁵⁰.

Increased endothelial cell permeability has been demonstrated in patients with elevated glucose levels and this leads to interstitial edema and may enhance cell proliferation and matrix production⁵¹. There is substantial evidence that EDV is impaired in insulin-dependent and -independent diabetes mellitus⁵². Endothelial dysfunction is an important initial step in the development of atherosclerosis. Endothelial dysfunction has also been shown to be a predictor of future cardiac events⁵³. It has also shown that the development of endothelial dysfunction depends substantially on the degree of oxidative stress⁵⁴,⁵⁵. Moreover, a prolonged severe oxidative stress may lead to greater endothelial dysfunction.

Oxidative Stress: a definition

With an initial focus on oxygen toxicity and X-irradiation the notion of “oxidative stress” in biological systems goes back to the early period of research on oxygen activation⁵⁶. Primarily, the concept of oxidative stress was developed by Sies, (1985)⁵⁷,⁵⁸,⁵⁹ with synonymous terms such as “oxidant stress” and “pro-oxidant stress,” or the related term “reductive stress” receiving comparatively less emphasis. Sies described oxidative stress as a “disturbance in the pro-oxidant/antioxidant balance in favor of the former”⁶⁰. This original denotation has been modified since to the more refined definition of “imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage”⁶¹. This more careful definition accounts for some important operational considerations. For example, an oxidative challenge or a loss of antioxidants alone does not constitute oxidative stress. However, if increased formation of oxidant(s) is accompanied by a loss of antioxidant(s) and/or accumulation of oxidized forms of the antioxidant(s), oxidative stress is approached. The refined definition also conceptually distinguishes oxidative stress from oxidative damage. Thus even a severe oxidative assault that is accompanied by a loss of antioxidants may not
necessarily result in oxidative damage\textsuperscript{62}. The refined definition of oxidative stress and its underlying redox chemistry involving reduction-oxidation reactions implies that any form of “tipping the balance” causes an “imbalance.” This has led to the concept of reductive stress to describe a situation where the balance is altered in favour of reductants\textsuperscript{63}. Reductive stress can be intimately linked to oxidative stress. For example, an overproduction of reducing equivalents such as NAD (P) H may result in increased redox cycling of substances that can undergo repetitive rounds of oxidation/reduction, ultimately leading to the increased generation of superoxide anion radical (O$_2^-$) and secondary oxidants. This has been implicated in the formation of ROS by hypoxia-like metabolic imbalances\textsuperscript{64}. With the increased appreciation of interplay between ROS and reactive nitrogen species (RNS), including their responses in cells, the term nitrosative stress has also been introduced\textsuperscript{65}. Nitrosative stress, defined as increase in S-nitrosated compounds associated with a decrease in intracellular thiols, may be associated with a number of biological responses, some of which are of particular interest to vascular physiology and pathophysiology\textsuperscript{66,67}.

**Free radicals**

A free radical can be defined as any species capable of independent existence that contains one or more unpaired electrons\textsuperscript{68}. In biological systems, a variety of radicals can be generated with their reactivity depending on their nature and the molecule(s) encountered.

If two radicals meet, they can join their unpaired electrons to form a covalent bond in reactions that are often kinetically fast and that lead to nonradical products. Both free radicals and reactive non-free radical compounds are collectively called reactive species (RS). RS are divided into reactive nitrogen species (RNS) – derivates on the basis of nitrogen and reactive oxygen species (ROS) – derivates on the basis of oxygen. ROS are superoxide anion radical (O$_2^-$), hydroxyl (OH$^\cdot$), peroxyl (LOO$^\cdot$), alkoxy (LO$^\cdot$) and hydroperoxyl (HOO$^\cdot$) as free radicals, and hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCl), ozone (O$_3$), singlet oxygen (\textsuperscript{1}O$_2$) and hydroxy alkenes as oxygen-based reactive non-radicals. RNS are nitric oxide (NO$^\cdot$), nitrogen dioxide (NO$_2^\cdot$) and
peroxynitrite (ONOO') as free radicals and nitrous acid (HNO₂), dinitrogen trioxide (N₂O₃) and alkyl peroxynitrites (LOONO) as nitrogen-based non-radicals. There are also hydrogen radical (H'), the carbon-centered radical (R') and trichloromethyl radical (CCl₃)⁶⁹. An example of a very fast reaction between two free radicals is most of O₂' with NO' to form peroxynitrite (ONOO') at the vessel level.

\[
O₂' + \cdot NO \rightarrow ONOO' \quad (I)
\]

Alternatively, a radical may add to a nonradical molecule or abstract a hydrogen atom from a C-H, O-H, or S-H bond of nonradical molecules. These types of radical reactions are common in biological systems where most molecules are nonradical species. The molecules potentially affected include low-molecular-weight compounds like antioxidants and cofactors of enzymes, lipids, proteins, nucleic acids, and sugars. In this case, a new radical is generated, and this can set up a chain reaction. A typical example of such a chain reaction is the process of lipid peroxidation that may be initiated by, for example, a hydroxyl radical (-OH) abstracting a hydrogen atom from a fatty acid side chain (LH) containing carbon atoms with bisallylic hydrogens (reaction 2). The resulting, carbon-centered radical (L'') adds rapidly to O₂ to generate a lipid peroxyl radical (LOO') (reaction 3) that itself can propagate the chain by reacting with a neighbouring lipid molecule to generate another L' and lipid hydroperoxide (LOOH) (reaction 4). In this fashion, many molecules of LOOH may be generated for each initiating radical.

\[
\text{LH} + \cdot OH \rightarrow L' + H₂O \quad (2)
\]

\[
L' + O₂ \rightarrow LOO' \quad (3)
\]

\[
LOO' + LH \rightarrow L'' + LOOH \quad (4)
\]

Whereas, the highly reactive -OH abstracts H atoms almost without discrimination, less reactive radicals, such as LOO' preferentially abstract H atoms from molecules with
weaker bonds, such as the chromanol O-H bond contained in \( \alpha \)-tocopherol (\( \alpha \)-TOH). In this case, the \( \alpha \)-tocopheroxyl radical (\( \alpha \)-TO\(^*\)) is produced and, for LOO\(^*\), a molecule of LOOH (reaction 5):

\[
\text{LOO}^* + \alpha\text{-TOH} \rightarrow \text{LOOH} + \alpha\text{-TO}^* \tag{5}
\]

A radical may be an oxidizing agent, accepting a single electron from a nonradical, or a reducing agent, donating a single electron to a nonradical. As implied above and like other reactions, free radical reactions are governed by thermodynamic and kinetic principles. A thermodynamic parameter commonly applied in free radical chemistry is the reduction potential that determines the feasibility of a compound X to chemically reduce compound Y. Accordingly, the ascorbate/ascorbyl radical system is, for example, capable of reducing the \( \alpha \)-TO\(^*\), H\(^+\)/\( \alpha \)-TOH system (reaction 6) that has a more positive standard reduction potential.

\[
\text{H}^+ + \text{ascorbate}^- + \alpha\text{-TO}^* \rightarrow \alpha\text{-TOH} + \text{ascorbyl radical} \tag{6}
\]

Attack of reactive radicals on membranes or lipoproteins starts lipid peroxidation, which is particularly implicated in the development of atherosclerosis\(^7\). If hydroxyl radicals are generated close to DNA, they can attack the purine and pyrimidine bases and cause mutations\(^7\). Free radicals do not only exert disadvantageous effects, but are also formed deliberately in the body for useful purposes and have important physiological functions. One of the well-defined roles of free radicals is when activated phagocytic cells produce superoxide anion radicals and hydrogen peroxide as one mechanism to kill bacteria and fungi and to inactivate viruses\(^7\). In a biological system free radicals attack takes place in the presence of an unbalanced ratio between free radicals and antioxidants.
Chemistry of Free radicals

In modern terminology a free radical is defined as any species capable of independent existence (hence the term ‘free’) that contain one or more unpaired electrons. These molecules having at least one unpaired electron in the outermost orbital that alters the chemical reactivity of the atoms or molecule, usually making it more reactive than the non-radical. Free radicals have cationic, anionic or neutral characteristics and are extremely reactive, having rate constants of the order of $10^5 - 10^{10} \text{ M}^{-1} \text{ S}^{-1}$.

Sources of Free radicals

Free radicals are produced in cells by enzymatic or non-enzymatic electron transfer reactions. The sources of oxidants or free radicals are from

1. **Endogenous sources**
   - Autooxidation of biomolecules
   - Enzymatic oxidation
   - Respiratory burst by phagocytic cells
   - Sub cellular organelles
   - Mitochondrial respiratory chain
   - Intracellular enzymes such as NADPH oxidase, Xanthine oxidase etc.

2. **Exogenous sources**
   - Drugs
   - Radiations
   - Tobacco smoke
   - Transition metals ions
   - Ischemic-reperfusion injury
   - Inorganic particles such as asbestos, quartz, silica etc.
   - Gases such as ozone
   - Other agents such as fever, excess glucocorticoid therapy, photochemical air pollutants as pesticides, solvents, anaesthetics, exhaust fumes etc.
Oxygen is required to transform various substrates for the release of energy, to oxidize endogenous compounds and to detoxify xenobiotics. During this process, oxygen acts as a terminal 4-electron acceptor and is eventually converted to more stable chemical state, water. Some biological reduction of oxygen occurs by the monovalent pathway and necessarily produces first superoxide anion radical (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) and then, these products are all reactive substances and would not be well tolerated by living cells. The hydroxyl radical, in particular is incredibly reactive and its production must be minimized. Since it is the third intermediate in the monovalent pathway of oxygen reduction, its production can be avoided by efficiently removing the first two namely O$_2^-$ and H$_2$O$_2$. Molecular oxygen or dioxygen is a stable triplet diradical in the ground state, with a kinetic preference for undergoing radical reaction such as initial univalent reduction in enzymatic reaction where H$_2$O$_2$ is formed.

A significant kinetic constraint exists on dioxygen in its role as an oxidant. The electronic structure of dioxygen described by the one electron molecular orbital diagram that the ground state $^3\Sigma_g^+$, has two unpaired electrons are the reflection of the Paulis principle.

In contrast the first two excited states, $^1\Delta_g$ and $^1\Sigma_g^-$ have all electrons paired, like most other compounds. This crucial difference between dioxygen and most other molecules provides a kinetic barrier to reaction since a concomitant change in spin state is required. This change is slow relative to lifetime of the collision complex. These constraints do not apply to reactions with single electrons, hydrogen atoms or other atoms or molecules containing unpaired electrons.

The relationship between these molecules in the oxidation state diagram provides a convenient method of displaying the oxidation-reduction potential, measured relative to the element in its standard state. The more positive the gradient of the line joining the points representing two species, the more powerful the couple is as an oxidant.
Lipids, definitions, biochemistry and peroxidation

Lipids are usually divided into two main classes: polar lipids (PL) and neutral lipids (NL). NL consists mainly of triacylglycerols (Tg), and minor amounts of mono- and diacylglycerols, whereas PL includes mainly phospholipids. Tg serve mainly as an energy source, whereas phospholipids are mostly constituents of biological membranes. Fatty acids (FA) are involved in determining the physical and chemical properties and capacities of biological membranes, and also serve as precursors in the synthesis of several different chemical messengers and eicosanoid hormones, as well as other regulating factors. Fatty acids consist of carbon chains with a methyl (CH₃) group at one end and a carboxyl (COOH) group at the other. The length of the carbon chain, and number of double bonds determine the properties of the fatty acid (FA). Saturated FA (SFA) lack double bonds, whereas, unsaturated FA can contain up to six. Those having one double bond are called monounsaturated FA (MUFA), whereas those with two or more double bonds are called polyunsaturated FA (PUFA). The main forms in which FA are present in lipids are: Free FA (FFA), acylglycerols (FA bound to a glycerol), phospholipids (diacylglycerols including a phosphatic acid derivative), glycolipids (diacylglycerols including a mono-, di-, tri or tetra saccharide) and sphingolipids (containing sphingosine).
Whereas, terrestrial and aquatic plants contain the necessary desaturases and elongases to synthesize 18:2 n-6 and 18:3 n-3 and their longer derivatives of the n-3 and n-6 family. However mammals lack 12 and 15 desaturases that are essential for insertion of double bonds at n-3 and n-6. It is very known that mammals are able to elongate and desaturate 18:2 n-6 and 18:3 n-3 in significant amounts towards 20:4 n-6 and 20:5 n-3, 22:5 n-3 and 22:6 n-3.
Desaturation of n-3 fatty acids

18:3 n-3
\[ \xrightarrow{\Delta-6 \text{ desaturase}} \]

[ + elongase ]

18:4 n-3 \[ \rightarrow \] 20:4 n-3

\[ \xrightarrow{\Delta-5 \text{ desaturase}} \]

[ + elongase ]

20:5 n-3 \[ \rightarrow \] 22:5 n-3 \[ \rightarrow \] 24:5 n-3

\[ \xrightarrow{\text{peroxisomal oxidation}} \]

22:6 n-3 \[ \rightarrow \] 24:6 n-3

Desaturation of n-6 fatty acids

18:2 n-6
\[ \xrightarrow{\Delta-6 \text{ desaturase}} \]

[ + elongase ]

18:3 n-6 \[ \rightarrow \] 20:3 n-6

\[ \xrightarrow{\Delta-5 \text{ desaturase}} \]

[ + elongase ]

20:4 n-6 \[ \rightarrow \] 22:4 n-6 \[ \rightarrow \] 24:4 n-6

\[ \xrightarrow{\text{peroxisomal oxidation}} \]

22:5 n-6 \[ \rightarrow \] 24:5 n-6

Fig 2: Schematic of the desaturation and elongation steps involved in the conversion of 18:3 n-3 to 22:6 n-3 and 18:2 n-6 to 22:5 n-6. Vertical and horizontal lines represent desaturation and elongation steps, respectively (Sprecher, 2000)
In a process when a free radicals attack on polyunsaturated fatty acids (PUFA) is called lipid peroxidation (LP). Initiation of lipid peroxidation is caused by attack of any species that has sufficient reactivity to remove a hydrogen atom from a PUFA. Since a hydrogen atom is a free radical with a single unpaired electron, its removal leaves behind an unpaired electron on the carbon atom. The carbon-centered radical is stabilized by a molecular rearrangement to form a diene conjugates (DC), followed by reaction with oxygen to give a peroxyl radical. Peroxyl radicals are capable of abstracting a hydrogen atom from another adjacent fatty acid side-chain to form a lipid hydroperoxide (LOOH), but can also combine with each other or attack membrane proteins. When the peroxyl radicalabstracts a hydrogen atom from fatty acid, the new carbon-centered radical can react with oxygen to form another peroxyl radical, and so the propagation of the chain reaction of lipid peroxidation can continue. The length of the propagation chain depends on several factors, e.g. the oxygen concentration and the amount of chain-breaking antioxidants present. Extensive LP is often reflected in increased levels of LP products in blood, whereas lipid peroxides formed at a primary site may accumulate in lipoproteins and be transferred through the circulation with consequent LP propagation. The peroxidation of membrane lipid may lead to enhanced membrane permeability and increased intracellular calcium. LP products may contribute to endothelial injury and may be involved in intensive oxidative modifications of LDL and in the development of atherosclerosis.

**LDL-C Oxidation**

It is very much known that oxidatively modified LDL exists in atherosclerotic lesions. Yet, it remains largely unknown where precisely within the vessel wall, how, and to what extent, LDL becomes oxidized during atherogenesis. In addition to intrinsic properties of the lipoprotein, factors that prolong the life/residence time of LDL may also be conducive to oxidation. It is generally upheld that LDL oxidation occurs in the arterial wall rather than the circulation, as lipoprotein lipids in plasma are well protected from oxidation due to the robust antioxidant defenses. It is noteworthy that
LDL is the major transport vehicle for most of the plasma TOH. Furthermore, oxidized lipoproteins that may exist or form in plasma are diluted rapidly by either hepatic clearance\textsuperscript{92} or accumulation and subsequent degradation in the arterial wall\textsuperscript{93}. Initiation begins with either abstraction of hydrogen atoms from polyunsaturated fatty acid (PUFA) within LDL by various ROS or by direct enrichment of the LDL with lipoperoxides from cells. Both the mechanisms lead to loading of LDL with lipoperoxides which change into more reactive intermediates that can initiate oxidation in neighboring PUFAs or PUFAs in nearby LDL particles. In vitro, the decomposition of lipoperoxides to more reactive peroxy radicals is dependent on the presence of transition metals (eg. copper or iron) and can be inhibited by metal chelators. Importantly, the oxidation process is autocatalytic such that, a single hydrogen abstraction could lead to oxidation of the entire LDL particle as well as neighboring LDL particles. The oxidized fatty acids released by phospholipase A2 are more mobile and presumably may help spread the oxidation process to other areas of the LDL particle. Initially, the oxidation process proceeds slowly, but eventually the antioxidant content within LDL is depleted and the number of fatty acid lipoperoxides amplify such that the oxidation process rapidly accelerates (the propagation phase). Eventually, PUFAs are cleaved into a variety of reactive aldehydes, ketones and other short chain fragments. These in turn may bind to apoprotein B-100 in LDL that leads to decreased recognition and binding by the LDL receptor. In addition, new epitopes are formed that lead to recognition and enhanced uptake of modified LDL by the scavenger receptor of macrophages. There is in fact a family of “scavenger” receptors whose function is to remove modified or altered proteins, including LDL. Oxidation of LDL may be initiated by a number of different mechanisms. However, cell mediated oxidation of LDL may be the most relevant to our understanding of LDL oxidation \textit{in vivo}. In tissue culture, all the cells normally present in the artery wall, including endothelial cells, smooth muscle cells, macrophages and lymphocytes can oxidize LDL. The mechanisms by which cells initiate oxidation of LDL are poorly defined.
Atherogenic Properties of Oxidized LDL

Oxidized LDL takes on a variety of properties that make it more atherogenic than unmodified LDL. Every early step in the initial stages of atherosclerosis is the focal adherence of monocytes to the artery wall. This may result from expression of specific leukocyte adherence molecules such as VCAM-1 on endothelial cells. These adherence proteins are expressed prior to monocyte binding to the endothelium and accumulate over areas of the artery wall, which later become sites of foam cell formation. LDL that has been minimally oxidized stimulates the expression and secretion of many different cytokines. In vitro, adding minimally modified LDL to endothelial and smooth muscle cells stimulates their secretion of monocyte chemoattractant protein (MCP-1). Presumably, this cytokine will attract monocytes to the endothelium where they bind to specific adherence proteins. During LDL oxidation, phosphatidylcholine is formed and can stimulate expression of the adherence molecule VCAM-1, and is one example of the many products of modified lipoproteins that likely influence monocyte recruitment.

Epidemiological study on CAD

According to World Health Organization (WHO) estimates, cardiovascular disease killed 14.7 million individuals in 1990 and 17 million in 1999. It is noteworthy that the principal cardiovascular disorder responsible for the global rise in mortality is no longer rheumatic heart disease, but rather atherosclerotic vascular disease. Ischemic heart disease is the leading cause of death in the world, and cerebrovascular disease is the second leading cause. Cardiovascular diseases are responsible for 30% of all deaths worldwide each year. It is often assumed that atherosclerosis is a disease of affluent, industrialized countries. However, 80% of these deaths occur in low-to-middle income countries of varying size like China, Russia, Poland, Mauritius, Argentina, and India.

The prevalence of coronary artery disease has been increasing in India over the past few decades. Cardiovascular disease will be the leading underlying cause of
deaths in India by 2010\textsuperscript{104}. In 1990, there were 783 000 deaths due to CAD in India and this is projected to double by the year 2015.

In the absence of reliable mortality data, estimates of the burden of disease have mostly been based on morbidity indicators from population based cross-sectional surveys. Morbidity surveys involve problems of sample design, sample size, standardization, and measurement errors. Indian CHD epidemiological studies have been reviewed earlier\textsuperscript{105}. In the urban population the prevalence increased from 1.05\% (Agra, 1962)\textsuperscript{106} and 1.04\% (Delhi, 1962)\textsuperscript{107} to 6.60\% (Chandigarh, 1968)\textsuperscript{108}. In recent years a consistent high prevalence of CHD has been reported from Delhi (9.67\%, 1990)\textsuperscript{109}, Jaipur (7.8\%, 1995)\textsuperscript{110}, Chennai (9.0\%, 2001)\textsuperscript{111}, Jaipur (8.1\%, 2002)\textsuperscript{112}, and Panjim (13.2\%, 2004)\textsuperscript{113}. In semi-urban populations of Haryana and Kerala the prevalence has increased from 3.6\% (1975)\textsuperscript{114} to 7.4\% (1993)\textsuperscript{115}. In rural populations, its prevalence increased from 2.06\% (Haryana, 1974)\textsuperscript{116} and 1.69\% (Vidarbha, 1988)\textsuperscript{117} to 2.71\% (Haryana, 1989)\textsuperscript{118}, 3.09\% (Punjab, 1994)\textsuperscript{119}, 3.46\% (Rajasthan, 1994)\textsuperscript{120} and 5.00\% (Himachal, 2002)\textsuperscript{121}. Rural-urban comparison shows that while prevalence has increased two-fold in rural areas (2.06\% in the 1970s to 4.14\% in the 1990s) the prevalence in urban areas has increased nine-fold (1.04\% in the early 1960s to 9.45\% in the mid 1990s)\textsuperscript{122}. There is evidence of CHD growth from rural to semi-urban and urban areas with the highest prevalence reported from metropolitan Delhi and Chennai. This clearly shows the importance of socioeconomic factors associated with CHD epidemic in India. Analyses of prevalence studies in various decades in India provide significant information regarding the absolute number of CHD cases. Decadal variations indicate that the prevalence has increased in urban areas from about 2\% in 1960 to 6.5\% in 1970, 7.0\% in 1980, 9.7\% in 1990 and 10.5\% in 2000 while in rural areas it increased from 2\% in 1970 to 2.5\% in 1980, 4\% in 1990 and 4.5\% in 2000. In terms of absolute numbers there is a very steep increase in CHD cases in both urban and rural areas. In urban populations, the numbers have increased from 0.5 million in 1960 to 4.5 million in 1970, 5.6 million in 1980, 9.7 million in 1990 and 14.1 million in the year 2000. In rural populations the numbers have increased from 4.1 million in 1970 to 6.4 million in 1980, 11.8 million in 1990 and 15.7 million in 2000. Thus epidemiological studies show that there are at present 29.8 million CHD patients in this country. So in summary, since
1960, life expectancy in India has increased by 20 years to 61 years of age\textsuperscript{123}. From 1960 to 1995, the prevalence of CAD in adults increased from 3% to 10% in urban Indians and from 2% to 4% in rural Indians, with women having rates similar to men\textsuperscript{124}. Although the prevalence of CAD in rural India is half that of urban India, this is still two-fold higher than the overall CAD rates in the US and several-fold higher than in rural China \textsuperscript{125}. These statistics are similar to that derived from GBD studies. As epidemiological studies exclude many patients with silent and asymptomatic CHD, the actual numbers may be much greater.

**Prevalence of CAD in Indian immigrants**

At the threshold of the new millennium coronary artery disease (CAD) is looming large as the new epidemic afflicting Indians at a relatively younger age with severe and diffuse form of lesions. Recently, the subject of CAD in Indians (referred as immigrants or Asian Indians or South Asians when outside India) has become a challenge for many research centers worldwide \textsuperscript{126,127}. In some studies from India, the percentage of patients below the age of 45 years suffering from acute myocardial infarction (AMI) is reported as high as 25-40\%\textsuperscript{128,129}. In Great Britain the first AMI among Indians at age less than 40 years is reported 10 times higher than local Whites\textsuperscript{130}. The first report to highlight the high prevalence of CAD among Indian expatriates came from an autopsy study done in Singapore\textsuperscript{131}. Coronary artery disease with myocardial involvement was 7 times more common in Indians when compared to Chinese males. Subsequently, other studies from Singapore\textsuperscript{132}, Uganda\textsuperscript{133}, South Africa\textsuperscript{134} and Fiji\textsuperscript{135} confirmed a three-fold higher prevalence of CAD in Indians compared to the respective native populations. The St James Survey from Trinidad found that major Q waves on the ECG (Minnesota codes 1-1, 1-2) were seen in 14\% of Indian men under the age of 55 years\textsuperscript{136}. In the age group 35–54 years, this worked out to an odds ratio of 3.8 for the Indians when compared to the other ethnic groups. Further, between 1977 and 1985, the age-adjusted relative risk of death from cardiovascular causes was 2.6 in Indians compared to people of African descent\textsuperscript{137}. Balarajan\textsuperscript{138} analyzed the mortality data for England and Wales (classified by country of birth) for the periods 1970–72 and 1979–83. Between 1970–
72 and 1979–83, while all the other ethnic groups studied showed either no change or a decline in mortality due to CAD, immigrants from the Indian subcontinent experienced a rise in mortality (6% in men and 13% in women) despite having had the highest rates in 1970–72.

The risk of CAD in Indians is 3-4 times higher than White Americans, 6-times higher than Chinese, and 20-times higher than Japanese. In Singapore, mortality from CAD below 30 years of age is 10 times higher in Indian than Chinese population of the same age group. Angiographically, Indians have 15 times higher rate of CAD than Chinese and 10 times higher rate than local Malays below the age of 40 years. Young patients from other communities do not show extensive disease. The disease pattern is severe and diffuse. Premature CAD is defined as cardiac events occurring before the age of 55 in men and 65 in women. In its severe form it is defined as CAD occurring below the age of 40 years. CAD is affecting Indians 5-10 years earlier than other communities. Indians also show higher incidence of hospitalization, morbidity, and mortality than other ethnic groups. The prevalence of CAD is two-times higher (10%) in urban than in rural India. South Indians have higher prevalence, 7% in rural and 14% in urban areas. The vulnerability of urban Indians to CAD is possibly related to different nutritional, environmental, and life-style factors. Migration from rural to urban environment and migration from India to industrialized countries is another special risk factor for our people. Migration is usually associated with stress of seeking and maintaining the new job, stress of coping with the new job-expectations, and stress of competing with the peer-group who is in the organization longer. New affluence is associated with sedentary life-style and higher consumption of calories, saturated fats, salt, tobacco, and alcohol. These factors contribute to obesity, dyslipidaemia, hypertension, hyperuricaemia, and diabetes mellitus. Also the India has pointed to differences in the prevalence of coronary risk factors across different geographic territories of the country.
Prevalence of CAD in native Indian

The growth of urbanization in the western countries during the 20th century was accompanied by an increase in the rate of coronary heart disease (CHD). This epidemiological trend went hand in hand with changes in lifestyle, specially an increase in the consumption of processed, energy-dense food and dependence on machines for physical work. Similar changes are now occurring in the developing world, including India. At present, 30% of India’s population lives in urban areas, a figure which is bound to increase in the coming decades. The use of machinery in the rural sector is also on the rise. The important question is whether these changes are likely to give rise to an epidemic of CHD in India and other developing countries. Systematic epidemiological studies are required to monitor the trend in the prevalence of CHD, as well as the risk factors, in various geographical areas over a period of time. Much of our understanding of CHD epidemiology has been derived from studies carried out in the western countries, where urbanization has reached its zenith. Although a few studies have been conducted in India, diverse definitions of CHD and the varying survey methods used make it difficult to estimate the trends over time, or the variations between different geographical regions. Some of these studies consider only urban areas, while others focus exclusively on rural areas. Few studies have covered urban–rural differences. The rates of CHD were reported to be quite low in rural areas, but among some of the urban populations, they were found to be as high as in the developed countries.

Since 1960, life expectancy in India has increased by 20 years to 61 years of age. From 1960 to 1995, the prevalence of CAD in adults increased from 3% to 10% in urban Indians and from 2% to 4% in rural Indians, with women having rates similar to men. Although the prevalence of CAD in rural India is half that of urban India, this is still two-fold higher than the overall CAD rates in the US and several-fold higher than in rural China. In 1990, there were 783,000 deaths due to CAD in India and this is projected to double by the year 2015, and cardiovascular diseases could be the most important cause of mortality in India. Epidemiological studies show that there are at present 29.8 million CHD patients in this country. The prevalence of coronary artery
disease increased from 1% in 1960 to 9.6% in 1995 in urban populations, and in rural areas it has almost doubled in the last decade\textsuperscript{162-168}. Studies from rural areas\textsuperscript{169-171} have demonstrated a lower prevalence compared to studies\textsuperscript{172-175} derived from a few studies. A recent review of Indian studies has concluded that the rates of CHD, hypertension, diabetes and obesity are very low among the rural population of India, and high in many of the metropolitan cities\textsuperscript{176}. Beginning in the 1960s through the 1990s, investigators in India have estimated the prevalence of CAD in several urban\textsuperscript{177-183} and rural\textsuperscript{184-189} populations. Overall, prevalence estimates obtained from the studies performed in the last decade range between 7.6% and 12.6% for urban populations, and 3.1% to 7.4% for rural populations. The difference in prevalence between the urban and rural populations has been accounted for by the prevalence of different risk factors in these two groups. The largest study, by far, was the one by Chadha \textit{et al.}\textsuperscript{190} who collected data from over 13500 urban dwellers in Delhi. Using clinical and ECG criteria, the prevalence rate of CAD was 9.7%, but major Q waves were seen in only 80 (1.4%) of the 5621 ECGs examined. In a rural population in Rajasthan, Gupta \textit{et al.}\textsuperscript{191} found a 3.5% prevalence of CAD. The prevalence of major Q waves was, however, very low, being present in only 2% of the highest risk subgroup. In an analysis of the available data, Gupta and Gupta\textsuperscript{192} found that the prevalence of CAD had increased significantly over the last four decades. However, this inference has to be weighed against the potential confounding influences of the different methodologies used and the various ethnic groups studied. More recently, in an urban population in South India, Mohan \textit{et al.}\textsuperscript{193} found a prevalence of 11%. Again, the prevalence of documented MI or major Q waves was only 2.5%. This appears to be lower than the prevalence of CAD detected using similar criteria in immigrant Indian populations (14% in the Trinidad study\textsuperscript{194}, 4% in the Southall study\textsuperscript{195} and 5.2% in the SHARE study\textsuperscript{196}). It is unclear whether these variations are due to the small sample sizes or the different demographic characteristics of the populations studied. Another more plausible explanation might be that the epidemiologic transition has not yet had its full impact among urban Indians\textsuperscript{197}. 
CAD Rates in Rural India:

In rural populations the number of CAD rate has increased from 4.1 million in 1970 to 6.4 million in 1980, 11.8 million in 1990 and 15.7 million in 2000.

Prevalence increased from 2.06% (Haryana, 1974)\textsuperscript{198} and 1.69% (Vidarbha, 1988)\textsuperscript{199} to 2.71% (Haryana, 1989)\textsuperscript{200}, 3.09% (Punjab, 1994)\textsuperscript{201}, 3.46% (Rajasthan, 1994)\textsuperscript{202} and 5.00% (Himachal, 2002)\textsuperscript{203}. Rural-urban comparison shows that while prevalence has increased two-fold in rural areas (2.06% in the 1970s to 4.14% in the 1990s) the prevalence in urban areas has increased nine-fold (1.04% in the early 1960s to 9.45% in the mid 1990s)\textsuperscript{204}.

Despite higher rates of smoking, studies from rural areas\textsuperscript{205-207} have demonstrated a lower prevalence compared to studies\textsuperscript{208-211} from urban areas. CAD rates contributes in rural India are about one-half those in urban India\textsuperscript{212}. A cross-sectional survey done in rural Haryana in 1998 revealed a CAD prevalence rate of 6% in rural Indians aged 35-64 years\textsuperscript{213}. This CAD rate is 2-fold higher than contemporary U.S. rates and 3-fold higher than the 2.1% reported in 1974 from the same village\textsuperscript{214,215}.

CAD Rates in Urban India:

The prevalence of CAD in urban India is about double\textsuperscript{216}, the rate in rural India\textsuperscript{217} and about 4-fold higher than in the U.S. The rates appear to be higher in south India with Kerala having a prevalence of 13% in urban areas\textsuperscript{218} and 7% in rural areas\textsuperscript{219}.

In the urban population the prevalence increased from 1.05% (Agra, 1962)\textsuperscript{220} and 1.04% (Delhi, 1962)\textsuperscript{221} to 6.60% (Chandigarh, 1968)\textsuperscript{222}. In recent years a consistent high prevalence of CHD has been reported from Delhi (9.67%, 1990)\textsuperscript{223}, Jaipur (7.8%, 1995)\textsuperscript{224}, Chennai (9.0%, 2001)\textsuperscript{225}, Jaipur (8.1%, 2002)\textsuperscript{226}, and Panjim (13.2%, 2004)\textsuperscript{227}.

In semi-urban populations of Haryana and Kerala the prevalence has increased from 3.6% (1975)\textsuperscript{228} to 7.4% (1993)\textsuperscript{229}. The prevalence of CAD has increased in urban areas from about 2% in 1960 to 6.5% in 1970, 7.0% in 1980, 9.7% in 1990 and 10.5% in 2000 while in rural areas it increased from 2% in 1970 to 2.5% in 1980, 4% in 1990 and 4.5% in 2000.
In urban population, the numbers have increased from 0.5 million in 1960 to 4.5 million in 1970, 5.6 million in 1980, 9.7 million in 1990 and 14.1 million in the year 2000.

Overall there has been a more than 3-fold increase from 3% prevalence 30 years ago in urban India\textsuperscript{230}. In Sri Lanka, between 1980 and 1988, the CAD mortality rates have doubled and now have a prevalence of 10%, similar to India. CAD in India appears to follow the same pattern that is observed in the U.S., where high rates of CAD first appeared in the urban and affluent, followed by the poor and rural Americans. Higher rates of CAD in urban India compared to rural India suggest important roles for nutritional and environmental factors, or nurture. There is a significantly higher body mass index (BMI) in urban India compared to rural India (BMI, 24 versus 20 in men and 25 versus 20 in women). There is also a higher rate of abdominal obesity among the urban population, with urban men having a waist to hip ratio (WHR) of 0.99 compared to 0.95 among rural men. This increase in BMI and WHR results in significant dyslipidemia and insulin resistance and a 3-fold increase in diabetes.

**Prematurity of CAD**

The excess risk of CAD in Asian Indians appears to be greater at younger ages\textsuperscript{231}. In the U.K, the CAD mortality in Asian Indian men compared with Whites is 3.3 between the ages of 20-29 as opposed to 1.36 overall\textsuperscript{232}. In Singapore, compared with Chinese, the CAD mortality in Asian Indian men between ages 30-39 is 12.5 in contrast to 3.0 between the ages of 60-69. In an angiographic study in Malaysia, Asian Indians under 40 years of age had a 15-fold higher rate of CAD compared to Chinese and a 10-fold higher rate compared to Malays\textsuperscript{233}. About 25% of acute MI in India occurs under the age of 40 and 50% under the age of 50\textsuperscript{234}. One center reported a 47-fold increase in the incidence of first MI under the age of 40 in the last 20 years\textsuperscript{235}. In general, MI develops 5-10 years earlier in Asian Indians than in other populations\textsuperscript{236,237}, and its occurrence in patients under 40 is 5 to 10-fold higher\textsuperscript{238}. 
Severity of CAD

The earliest report of high rates of CAD in Asian Indians was based on 9,568 autopsies undertaken between 1950 and 1954 in Singapore. This study showed a 7-fold higher rate of coronary atherosclerosis in Asian Indians compared to Chinese. Among those studied by coronary angiogram, three-vessel disease is seen among half of all Asian Indians and one third of premenopausal women. Unlike in Whites, CAD in young Asian Indians is known to be severe, extensive, and malignant. This is attributed to an accelerated atherosclerotic process that begins early in life. Contrary to common belief, the size of the coronary arteries is not different in Asian Indians when adjusted for BMI.

Burden of CHD in India

Indian subcontinent suffers from a tremendous loss of productive working years due to CVD deaths: an estimated 9.2 million productive years of life were lost in India in 2000, with an expected increase to 17.9 million years in 2030 (almost ten times the projected loss of productive life in the United States). The Global Burden of Disease (GBD) study reported that of a total of 9.4 million deaths in India in 1990, cardiovascular diseases caused 2.3 million deaths (25%); 1.2 million deaths were due to coronary heart disease (and the number is expected to almost double to 2.03 million by 2010 and 0.5 million due to stroke. The study also reported the estimated mortality from CVD in India at 1.6 million in the year 2000. It has been predicted that by the year 2020 there will be an increase by almost 75% in the global cardiovascular disease burden. Extrapolation of these numbers estimates the burden of CHD in India to be more than 32 million patients and there would be a 111% increase in cardiovascular deaths in India. This increase is much more than 77% for China, 106% for other Asian countries and 15% for economically developed countries. Another study reported that mortality from cardiovascular diseases was projected to decline in developed countries from 1970 to 2015 while it was projected to almost double in the developing countries. While the situation in India is more alarming. In 2003, the prevalence of CHD in India was estimated to be 3-4 per cent in rural areas (two-fold higher compared with 40 years ago), and 8-10 per cent in urban areas (six-
fold higher compared with 40 years ago), with a total of 29.8 million affected (14.1 million in urban areas, and 15.7 million in rural areas) according to population-based cross-sectional surveys\textsuperscript{248,249}. Epidemiological studies also show a sizeable burden of CHD in adult rural (3-5\%) and urban (7-10\%) populations. Thus, there could be 30 million patients with CHD in India of whom 14 million are in urban and 16 million in rural areas. This number is similar to that derived by the GBD study. This estimate is comparable to the figure of 31.8 million affected, derived from extrapolations however, whereas about one-quarter of all cardiovascular disease deaths occurred in persons who were under 70 years of age in the developed world, more than about half of these deaths occurred in those under 70 years in the developing world.

The burden of CHD can be measured as (a) population impact measured by premature mortality and disability, (b) burden on healthcare systems, and (c) burden on economy.

**Burden on Health care system:**

There is a significant burden of CHD on healthcare systems. Pattern of various cardiovascular diseases in hospitalized patients has been reported by many workers. From 1940s to 1960s CHD formed 5-20\% of all heart disease admissions in big hospitals in Delhi, Mumbai and some other cities. Wasir \textit{et al.}\textsuperscript{250} reported an increasing trend and significant burden of CHD cases in cardiology outpatient department and medical admissions to a Delhi-based tertiary care hospital. During 1966-70, CHD was present in 18.4\% of all heart disease cases seen at All India Institute of Medical Sciences, Delhi. This changed to 16.5\% in 1971-75, 15.2\% in 1976-80 and 19.7\% in 1981-85. In the same duration, proportion of CHD cases admitted in hospital increased from 20.8\% to 21.0\%, 20.3\% and 23.9\%, respectively. Pooled data from the states of Assam, Madhya Pradesh, Punjab, Kerala and Karnataka reveal that proportion of all cardiac admissions to various government hospitals, and incidence of CHD increased from 14\% in 1970 to 19\% in 1985. There are substantial regional variations in cardiovascular mortality in different parts of the country but all these studies report an increasing burden of CHD on healthcare system, especially in urban hospitals, in all regions of India.
Social and Economic Burden:

Coronary artery disease (CAD) has assumed epidemic proportions in India. The disease occurs at a younger age in Indian subjects compared to western developed nations. The Global Burden of Diseases (GBD) study reported the estimated mortality from CAD in India at 1.6 million in the year 2000. Extrapolation of these numbers estimates the burden of CAD in India to be more than 32 million patients. Epidemiological studies show a sizeable burden of CAD in adult rural (3-5%) and urban (7-10%) populations. Thus, there could be 30 million patients with CAD in India of whom 14 million are in urban and 16 million in rural areas. This number is similar to that derived by the GBD study. There is a significant burden of CAD on healthcare systems. In urban primary health clinics 1-1.5% of all patients have CAD while in general internal medicine clinics CAD prevalence is 10-20%. In a rural internal medicine practice it was reported that 8% of all patients have CAD. Hospital statistics reveal that 20-25% of all medical admissions are due to CAD. The admissions due to acute myocardial infarction (AMI) are increasing in India. The economic costs of CAD are poorly understood. It is roughly calculated that annually India spends about Rs. 100 billion as direct costs of treatment. The magnitude of indirect costs is unknown and could be another Rs. 100 billion. The sum is equal to 0.8% of the Indian gross national product.

From the year 1995 to 2000, India has been spending about 5% of its gross domestic product (GDP) on health. Of this, direct private expenditure on health is about 82-83% and the subsidized general government expenditure is 17-18%\textsuperscript{251}. Therefore, any disease that is as widespread as CAD would entail substantial economic burden on the population. The National Family Health Surveys report that direct medicine costs are about 45-50% of medical treatment costs in India\textsuperscript{252}. Therefore we conclude that this is a waste as almost 80% of the heart attacks can be prevented by appropriate management and prevention strategies\textsuperscript{253,254}. Numerous reports and anthropological statements have shown that premature CAD causes significant social burden in terms of loss of support for young children, women and the elderly. Exact cost to a family of such a catastrophe
is difficult to calculate. The calculations include burden of premature morbidity on the individual and clearly shows that CAD contributes a large burden in India. Formal studies that measure individual and societal burden of CAD on social structures are needed.

**Risk factors of CAD**

Ischaemic heart disease (IHD) and stroke are among the most common causes of death and disability in the world. The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanka, and Nepal) has among the highest rates of cardiovascular disease (CVD) globally. There are many well known risk factors of IHD, such as smoking, high blood pressure and dyslipidaemia. Having diabetes, being overweight and/or a sedentary lifestyle are other favourable risk factors, as is old age. Epidemiologic studies have identified important cardiovascular risk factors including hypertension, diabetes mellitus, a sedentary lifestyle, obesity, cigarette smoking, elevated low-density lipoprotein (LDL) cholesterol and depressed high-density lipoprotein (HDL) cholesterol.

Many of these risk factors, including blood lipids, are modifiable and amenable to treatment. Disparities in cardiovascular and other health outcomes across geographical regions are common, and as yet not well understood. The prevalence of coronary heart disease (CHD) is known to be high in people of south Asian descent (subjects originally from India, Pakistan and Bangladesh). Moreover, CHD among them is often premature and occurs a decade earlier than that seen in Europeans and/or Americans. However, its precise etiology and mechanisms remain incompletely understood. Although prevalence of conventional risk factors such as smoking, hypertension and hypercholesterolemia is not higher in South Asians as compared to other ethnic groups, yet it is quite clear that some metabolic abnormalities are more prevalent among them, including high triglyceride (Tg) concentration, increased total cholesterol (TC) and high-density lipoprotein (HDL-C) ratio (TC/HDL-C), diabetes mellitus (DM) and central or visceral obesity. In Asian populations,
morbidity and mortality from CHD is occurring in people with lower body mass index (BMI)\textsuperscript{280}.

**Age:** Compared with the age group 34–44, CAD mortality among women increases 40-fold by the age of 80, when its incidence becomes identical in men and women. Women are about 10 years older than men at first manifestation of CAD, although they have a similar plaque burden.

**Obesity:** Obesity is one of the risk factors for CAD\textsuperscript{281}. The World Health Organization (WHO) defines a body mass index (BMI) (calculated as weight in Kg/height in meter\textsuperscript{2}) of 25-29.9 as overweight, and of > 30 as obese. Various studies revealed positive correlation with BMI and CAD mortality\textsuperscript{282,283} with a stronger association in non-smokers than smokers, and in white populations compared to black\textsuperscript{284}. A three to four times higher risk of morbidity from coronary artery disease is observed in greater than 28 BMI than the lesser \textsuperscript{285}. In addition to the total body weight, total body fat and the pattern of fat distribution is a critical factor in the relationship between obesity and metabolic abnormalities, with a positive association between abdominal/visceral fat and CAD risk\textsuperscript{286,287} and type 2 diabetes\textsuperscript{288}. Waist circumference is an indicator used to determine abdominal fat/obesity\textsuperscript{289}. BMI and visceral fat have also been found to be associated with impaired endothelial function\textsuperscript{290-292}. Obesity substantially increases the occurrence and risk of various other CAD risk factors including: insulin resistance, hyperglycemia, hyperinsulinemia, hypertension, and dyslipidemia\textsuperscript{293} and the combination of these pathologies is frequently referred to as the metabolic syndrome or syndrome X\textsuperscript{294,295}. Although the close link between obesity and its related complications has been well documented, yet the exact mechanism linking one to another is still not clearly understood. It has been hypothesized that in obesity (especially abdominal obesity), insulin resistance occurs due to the increased concentrations of circulating free fatty acids, which inhibit efficient glucose uptake by cells\textsuperscript{296}. Moreover, a number of hormones produced by adipose tissue are increased in obese people. Leptin, the hormone responsible for regulation of food intake and energy expenditure, is also a marker for body fat content and metabolic activity\textsuperscript{297}. Other hormones including tumour necrosis factor alpha (TNF-\textalpha), plasminogen activator
inhibitor-1 (PAI-1), and resistin have been shown to induce obesity related insulin resistance, impaired lipid profiles and diabetes under experimental conditions\textsuperscript{298,299}. Adiponectin, another protein secreted by adipose tissue, is reduced in obesity and is closely related to the degree of insulin resistance and hyperinsulinemia\textsuperscript{300,301}. Animal studies have shown delayed clearance of circulating free fatty acids, increased concentrations of TNF-\(\alpha\), severe high fat diet induced insulin resistance and reduced activity of insulin receptors in adiponectin knock-out rats\textsuperscript{302}. Conversely, data from human studies indicate that weight loss, through diet, drug/surgical means or physical activity, increases adiponectin\textsuperscript{303}, improves insulin sensitivity\textsuperscript{304,305}, lipid profile\textsuperscript{306,307} and decreases the incidence of diabetes\textsuperscript{308,309}.

**Hypertension:**

Hypertension is even more prevalent (20-40% among urban and 12-17% among rural adults)\textsuperscript{309}, and was affecting an estimated 118 million inhabitants in India in 2000; this number is projected to almost double to 214 million in 2025\textsuperscript{310}. Hypertension remains a standard-risk factor associated with CAD have shown a positive association between insulin and blood pressure in both normotensive and hypertensive people\textsuperscript{311-314} as well as in obese individuals with greater visceral fat deposition\textsuperscript{315}, though the mechanism by which obesity and insulin resistance or hyperinsulinemia may cause hypertension is not clear. Prevalence of hypertension is increasing in urban population, as compared to rural population. Hypertension confers a 4-fold risk of CAD in women compared to a 3-fold in men. Hypertension tends to be more common in women than in men after 45 years of age (White women 60% and Black women 79%). The systolic blood pressure (BP) continues to increase disproportionately in women until the age of 80. Hypertension is closely correlated with obesity and is 6-fold higher in women with a BMI \(>30\) versus BMI \(<20\). Conversely, a weight reduction of 9 kg can lower systolic BP by 6 mmHg and diastolic BP by 3 mmHg in hypertensive patients\textsuperscript{107}. In women, however, total cholesterol has been suggested to be of less importance than in men, but instead high blood pressure has been shown to convey a higher risk in women than in men\textsuperscript{316-318},
It is well known that the incidence of CHD is increased in hypertensive patients compared to normotensive controls\(^3^{20}\), also when hypertension is treated\(^3^{21}\). Hypertension may promote the development of atherosclerosis by causing impaired endothelial function\(^3^{22}-^{3^{24}}\), and may also be a trigger of acute plaque disruption by inducing mechanical stress on the arterial wall\(^3^{25},^{3^{26}}\). Hypertension has also been associated with impaired fibrinolytic activity, as evaluated by an impaired capacity for stimulated release of tissue plasminogen activator from vascular endothelium\(^3^{27}\), and may thereby promote thrombosis formation.

**Diabetes:** The Indian subcontinent has a higher prevalence of diabetes mellitus than any other region in the world, and 2-3 times the reported prevalence in Western countries\(^3^{28}\). In India alone, an estimated 19.3 million people had diabetes in 1995, and this is expected to almost triple to 57.2 million in 2025\(^3^{29}\). The Indian Council of
Medical Research (ICMR) estimates that the prevalence of diabetes is 3.8 per cent in rural areas, compared with 11.8 per cent in urban areas. Approximately 80% of deaths in diabetic patients are attributable to CVD, which in turn is highly correlated with dyslipidemia. Diabetic dyslipidemia consists of elevated Tg, low HDL-C, and an increased proportion of small dense LDL-C. Recently, the NCEP ATP III has also recommended an LDL-C goal of <100 mg/dl in diabetic patients, irrespective of the presence or absence of CAD.

**Crucial Role of Dyslipidemia**

**Total cholesterol (TC):**

After the results of the Scandinavian Simvastatin Survival Study in 1994 hypercholesterolemia, and especially elevated levels of LDL-cholesterol, are generally accepted, as strong risk factors for CHD. Total cholesterol levels in women compared to men are about 10 mg/dl lower before the age of 45 and 10 mg/dl higher after the age of 65. A 20% difference in TC level is associated with a 50%-60% difference in CAD risk over a lifetime. After menopause, the blood lipid profile changes with a decrease in high-density lipoprotein (HDL-C) cholesterol, and increases in low-density lipoprotein (LDL-C) cholesterol and triglycerides. Low HDL cholesterol has been shown to be a risk factor for CAD in both younger and older women and a stronger predictor of CAD mortality in women than in men.

**Low density lipoprotein cholesterol (LDL-C):**

The LDL-C fraction of TC is a strong predictor of CAD mortality in women as well as in men. Unlike in men whose LDL-C levels plateau at the age of 50 years, the LDL-C levels in women increase steadily by an average of 2 mg/dl/year between the ages of 40 and 60 (total of 40 mg/dl). In addition to elevated levels of LDL-C, high amounts of small dense LDL-C increases the risk of CHD. Small dense LDL-C has a poor affinity for binding to LDL receptors and hence stays in plasma for longer periods of time. It interacts with proteoglycans of the arterial wall matrix, leading to retention...
and accumulation in the arterial intima. Small dense LDL-C rich in triglycerides have lower antioxidant concentration, reduced free cholesterol, and increased content of polyunsaturated fatty acids, which is susceptible to increase of LDL-C to oxidation. As a result endothelial dysfunction increased and, vasorelaxation is reduced.

High-density lipoprotein cholesterol (HDL-C):

HDL-C protects LDL-C against oxidation, possibly through a combination of mechanisms. HDL-C contains an enzyme called paraoxonase-1, which may act at specific points in the lipid peroxidation cascade and help prevent LDL-C oxidation. HDL-C inhibition of vasoconstrictor ET-1 maintain endothelial function, via the synthesis of vasodilators NO and prostacyclin. Furthermore, increased serum HDL-C concentrations by drug treatment e.g. nicotinic acid, or infusion of synthetic HDL-C improves the endothelial function by increasing NO bioavailability. Low HDL-C is an important risk factor even if TC and Tg levels are normal. It is a stronger predictor of CAD in women than in men, especially after the age of 65; indeed, the protective effect of HDL-C is twice as important as the atherogenic effect of LDL-C. High density lipoprotein levels are about 10 mg/dl higher in premenopausal women than in men. Among women, the HDL-C levels vary markedly depending on the ethnicity, with Indian women having the lowest levels. The HDL-C level among Indian women (45 mg/ dl) is about 10 mg/dl lower than in Whites (55 mg/dl) and 20 mg/dl lower than in Blacks, the Chinese and Japanese (65 mg/dl). These high levels of HDL-C among black, Chinese and Japanese women also parallel their low rates of CAD, whereas the low levels of HDL-C in Indian women parallel their high rates of CAD. The National Cholesterol Education Programme (NCEP) ATP III has classified HDL-C <40 mg/dl as low HDL-C and more than 60 mg/dl as high HDL-C. In India, 32% of urban and 18% of rural women have HDL-C levels less than 40 mg/dl. Many experts consider HDL-C less than 50 mg/dl to be low in women. In the Coronary Artery Disease in Indians (CADI) study, 70% of Indian women had HDL-C levels less than...
50 mg/dl. If the level is less than 35 mg/dl, it confers a 8-fold higher CAD risk than an HDL-C of more than 75 mg/dl in women\textsuperscript{361}.

**Total cholesterol/HDL-C (TC/HDL-C) ratio:**

This ratio is now widely recognized as the single best predictor of CAD. At any given level of TC/HDL-C ratio, the CAD risk is virtually identical in men and women\textsuperscript{362}. Indian women worldwide have a high TC/HDL-C ratio by virtue of low HDL, even when TC levels are not elevated\textsuperscript{363}. The optimum TC/HDL-C ratio is 3 and the average ratio is 4. A TC/HDL-C ratio more than 5 appears to be a strong predictor of CAD, and is observed in 25% of industrial and 32% of urban female populations in India\textsuperscript{364}.

**Triglycerides:**

Association between Tg-rich lipoproteins and CAD was reported as early as 1953 by Gofmann et al.\textsuperscript{365} The role of elevated triglyceride (Tg) levels in the pathogenesis of atherosclerotic cardiovascular disease has remained a controversial issue\textsuperscript{366,367}. Increased serum Tg levels are associated with at least four pathogenic conditions: decreased serum HDL cholesterol levels, increased remnant lipoproteins, increased small dense low-density lipoprotein (LDL-C), and increased thrombogenesis, all of which are believed to expedite atherosclerosis\textsuperscript{368}. High triglycerides levels (fasting and postprandial) are associated with increased susceptibility of LDL-C to oxidation in healthy subjects\textsuperscript{369} and type 2 diabetics\textsuperscript{270}.

A high Tg level is a stronger predictor of CAD in women than in men. An increase in Tg level of 90 mg/dl increases the CAD risk by 75% in women versus 30% in men\textsuperscript{271}. A high Tg level was significantly associated with cardiac and total mortality in a 20-year follow-up of Swedish women\textsuperscript{372}. Conversely, low Tg (<97 mg/dl) and high HDL-C (>57 mg/dl) is associated with very low risk of CAD\textsuperscript{373}, but is uncommon among Indians. A low level of HDL-C often accompanies a high Tg. The optimum Tg level is <150 mg/dl.
Low socioeconomic status (SES):

Ischaemic heart disease and stroke are the two most common causes of death worldwide. Over 80 per cent of deaths and 85 per cent of disability from cardiovascular disease (CVD) occur in low- and middle-income countries. Low socioeconomic status is another psychosocial factor associated with an increased risk of CAD. In the Whitehall II study, low control at home was predictive of CAD in women, but not in men, and data suggested that low control at home among women resulted from a lack of material and psychological resources to cope with excessive household and family demands. Being divorced, or employed without a college degree, has been shown to contribute to predict mortality from AMI in women. It might be that being divorced involves presumed risk factors of CAD such as low social support and/or social isolation. In light of the association between marital stress, less social integration and support, are worse prognosis in women with CAD. However, it seems reasonable to presume that not all available social contacts entail social support, but sometimes are negatively demanding.

Role of Oxidative stress in CAD:

Oxidative stress is the inappropriate exposure to reactive oxygen species (ROS) and results from the imbalance between prooxidants and antioxidants leading to cell damage (damage of lipids, proteins, carbohydrates and nucleic acids) and tissue injury.

Sources of Reactive Oxygen Species:

Reactive Oxygen Species (ROS) represents a variety of diverse species including superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (HO$^-$). Some of these species (HO$^-$, O$_2^-$) are known as radicals (molecules containing unpaired electrons) and are extremely unstable, while others like hydrogen peroxide are freely diffusible, relatively stable and longlived. ROS are produced during normal intracellular
metabolism (endogenously) and from exogenous substances. Endogenous sources include mitochondria, xanthine oxidase, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation (macrophages, neutrophils, eosinophils).

Some diseases like cardiovascular diseases, cancer, diabetes mellitus, cause stressful conditions which lead to the formation of excessive free radicals which are a major internal threat to cellular homeostasis of aerobic organisms. Free radicals are formed in human body both in physiological and pathological conditions in cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes. These free radicals are extremely reactive and unstable chemical species, which react with proteins, lipids, carbohydrates and nucleic acids in the body.

Increasing oxygen consumption potentially initiates enhanced formation of reactive oxygen species (ROS). This in turn leads to oxidative stress and cellular damage if not properly counteracted. The increase in malondialdehyde (an oxidative stress marker), released after intracoronary platelet aggregation might be a biochemical marker of coronary artery disease. Oxygen derived free radicals after temporary coronary occlusion causes myocardial stunning. Hypothetically, free radicals may either directly depress contraction or do so by increasing cytosolic calcium, for example by stimulating sodium hydrogen transport with subsequent sodium calcium transport inhibition. Thus free radicals interact with calcium ions.

Oxidative stress is the resultant consequence of one of the following three factors: (i) an increase in oxidant generation, (ii) a decrease in anti-oxidant protection, or (iii) a failure to repair oxidative damage. Oxidative stress-mediated cell damage occurs, in part, via reactive oxygen species. In the vascular system, the formation of ROS from endothelial cells, smooth muscle cells (SMCs) and macrophages seems to be of major relevance in atherogenesis, in part due to their reaction with nitric oxide (NO). NO, perhaps the most important endothelium-derived vasorelaxing factor, is scavenged by ROS. The half-life of NO under physiological conditions is very short and NO can react with superoxide anion to produce peroxynitrite anion, which can rearrange to form nitrate and the highly reactive OH radical, which is toxic to tissues and cells. Nitric oxide (NO) is formed together with L-citrulline from molecular oxygen and L-arginine (L-Arg) in an enzyme-catalysed reaction. It has a number of effects in the body ranging
from the cardiovascular and nervous system to being involved in the host defense system. The physiological actions of NO were first shown in the vasculature by Furchgott and Zawadzki in 1980\textsuperscript{398}. There are three known isoforms of NOS, the neuronal isoform (nNOS or NOS I), the inducible isoform (iNOS or NOS II) and the endothelial isoform (eNOS or NOS III). The nNOS and the eNOS are said to be constitutively expressed in the tissue and are Ca\textsuperscript{2+} dependent, while the iNOS is inducible and Ca\textsuperscript{2+} independent. Inhibition of NOS with an unselective blocker results in an increased blood pressure, due to an increase in vascular resistance.

NO is a potent vasodilator and there is unequivocal evidence demonstrating that there is basal NO-dependent vasodilatation in humans, which, at least in part, counters the effects of the renin-angiotensin, sympathetic nervous and other vasoconstrictor systems\textsuperscript{399}. This vasodilator tone plays an important role in regulation of blood flow in healthy humans\textsuperscript{400}.

Inflammation of the vessel wall is an important event in early atherosclerosis\textsuperscript{401}. NO has been shown to blunt monocyte adhesion to the endothelial surface\textsuperscript{402,403}. Furthermore, by reducing oxidative stress NO may inhibit the transcription of MCP-1 and VCAM-1\textsuperscript{404-406}, proteins that are of central importance in initiating inflammation of the vascular wall.

Migration and proliferation of vascular smooth muscle cells (VSMC) play an important role in the pathogenesis of the atherosclerotic plaque, effects that may also be inhibited by NO\textsuperscript{407}. Supportive evidence for this has been shown in in vitro studies of human VSMC\textsuperscript{408}.

Oxidative stress has been identified throughout the process of atherogenesis, beginning at the early stage when endothelial dysfunction is barely apparent\textsuperscript{409}. As the process of atherogenesis proceeds, inflammatory cells, as well as other constituents of the atherosclerotic plaque release large amounts of ROS, which further facilitate atherogenesis. In general, increased production of ROS may affect four fundamental mechanisms that contribute to atherogenesis: oxidation of LDL, endothelial cell dysfunction, and vascular SMCs, growth and monocytes migration\textsuperscript{410}.

A number of studies suggest that ROS oxidize lipids and that the oxidatively modified LDL is a more potent proatherosclerotic mediator than the native unmodified LDL\textsuperscript{411}.
The suggestion is based on the observations that high plasma levels of ox-LDL are present in patients with atherosclerosis and that antibody to ox-LDL is detected in plasma of most patients with atherosclerosis. Strong evidence in favor of a pro-atherosclerotic role for ox-LDL comes from a number of studies demonstrating the noxious effects of ox-LDL on various components of the arterial wall. For example, ox-LDL causes activation of the endothelial cells lining the arterial wall, resulting in the expression of several adhesion molecules that facilitate the adhesion of monocytes/macrophages. More over ox-LDL also activates inflammatory cells and facilitates the release of a number of growth factors from monocytes/macrophages.

Stimuli for increased ROS generation

ROS production is induced under several pathological conditions by various stimuli. Risk factors for atherosclerosis, such as hypertension and hyperlipidemia, are also associated with increased generation of ROS, and it is likely that cigarette smoking and diabetes mellitus share oxidative heritages. Increasing evidence shows that ischemia-reperfusion, which frequently occurs in narrowed atherosclerotic arteries, increases ROS generation. At the molecular level, signaling in response to pro-atherogenic agents requires as well as causes generation of ROS. Proatherogenic agents comprise a large variety of molecules. It has been identified that cytokines, including tumor necrosis factor-γ (TNF-γ), interferon-γ (IFN-γ), interleukin-1, IL-6 (IL-1, IL-6), and angiotensin II (Ang II), stimulate intracellular generation of ROS. High levels of low-density lipoprotein (LDL), especially in the form of oxidized low-density lipoprotein (ox-LDL), have also been shown to increase intracellular ROS generation. In addition, growth factors, such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) as well as vascular endothelial growth factor (VEGF), and hormones, such as insulin, all greatly induce intracellular ROS generation.
Role of antioxidant in CAD

Oxidative stress plays an important role in the development of atherosclerotic disease, while antioxidants may delay or prevent various steps in atherosclerosis. Both enzymatic and non-enzymatic pathways leading to formation of reactive compounds by one electron reduction or oxidation generate free radicals. Role of free radicals has been proposed in the pathogenesis of many diseases involving different organs such as breast, gastric, colon, multiple myeloma, and ovarian and oral cancer. ROS are cleared from the cell by enzymatic systems including superoxide dismutases (SODs), catalase, and glutathione peroxidase, or the nonenzymatic system including alphatocopherol (vitamin E), ascorbic acid (vitamin C), glutathione, and uric acid etc. prevents free radical chain reaction. When these antioxidant defence system get exhausted or generation of free radicals exceed to their scavenging capacity, free radical mediated damage results. Antioxidant defences can be classified into enzymatic and non-enzymatic systems.

Nonenzymatic defences

GSH is a tripeptide, present in high amounts in all cells, including hepatocytes. In the mitochondria, GSH is mainly found in the reduced form. Mitochondrial GSH depletion may compromise mitochondrial function and sensitizes cells to diverse oxidant-induced toxicity, leading to cell death. Vitamin C (ascorbate), E (α-tocopherol) and carotenoids (vitamin A precursor) exert their antioxidant action as free-radical scavengers. Tocopherols and lavonoids inhibit peroxidation by acting as chain-breaking peroxy radical scavengers. Other substances such as bilirubin, melatonin and uric acid have been proposed to act as antioxidant systems.

Antioxidant defences of enzymes

There are two broad classes of antioxidants:

1. Preventive
2. Chain-breaking
1. **Preventive antioxidants:** Antioxidants reduce the rate of chain initiation or intercept oxidizing species before damage could be done. Preventive antioxidants act by deactivating metals (e.g., transferring, ferritin, desferal, ethylene diamine tetra acetic acid, etc.), removing hydroperoxides (e.g., catalase, glutathione peroxidases, pyruvate, etc.) and quenching singlet oxygen (e.g., β-carotene, lycopene, bilirubin, etc.).

2. **Chain-breaking antioxidants:** These antioxidants retard or stop oxidative processes after they began, by intercepting the chain-carrying radicals. It can be donor antioxidant, e.g., tocopherol, ascorbate, uric acid, etc., and sacrificial antioxidant like nitric oxide.

**Characteristics of chain breaking antioxidants:**

a.) Both antioxidant and antioxx should be relatively un-reactive.
b.) Antioxx -decays to harmless products.
c.) Does not add O₂ to make a peroxyl radical.

*Propagation:*

\[
\begin{align*}
L^* + O_2 & \xrightarrow{3 \times 10^8 M^{-1}s^{-1}} LOO^* \\
LOO^* + L-H & \xrightarrow{10 M^{-1}s^{-1}} L^* + LOOH
\end{align*}
\]
The donor antioxidant reaction:

\[ k = 10^4 - 10^8 \text{ M}^{-1}\text{s}^{-1} \]

\[ \text{LOO}^* + \text{AntioxA} \rightarrow \text{LOOH} + \text{AntioxB} \]

In free-radical reaction superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one di-oxygen.

\[ \text{SOD} \quad \text{O}_2^{2-} + \text{O}_2^{2-} + 2\text{H}^+ \leftrightarrow \text{H}_2\text{O}_2 + \text{O}_2 \quad (K_{\text{catalytic}} = 2-4 \times 10^5 \text{ M}^{-1}\text{s}^{-1}) \]

The dismutase removes the free superoxide anion radical by accelerating its bimolecular recombination. In this dismutation reaction one molecule of superoxide undergoes 1-e⁻ reduction to H₂O₂, the other undergoing 1-e⁻ oxidation back to molecular oxygen. The enzyme turnover number is very high, estimated at 3 x 10⁶ moles min⁻¹ (mole enzyme)⁻¹ with a Kₘ for superoxide of 5 x 10⁻⁴ M. Coefficiency is given as Kₖₑₚ / Kₘ. Fridovich §6 reported a bimolecular rate of 10⁶ M⁻¹ sec⁻¹, a value that is slightly less than the diffusion limit. As a catalyst, superoxide dismutase simply accelerates the nonenzymatic rate of chemical dismutation. These nonenzymatic disproportion of two molecules of anion is relatively slow probably due to electrostatic repulsions.

Superoxide dismutase (SOD) acts as a defence against the endogenous superoxide radical. The enzyme acts to scavenge the molecules of superoxide anions that are formed during biological reductions but escape from a specific active site of enzyme, thus generating a potential oxidizing agent of cellular constituents.
Superoxide dismutases are purified from both prokaryotic and eukaryotic sources.

<table>
<thead>
<tr>
<th>Procaryotic Cells - SOD</th>
<th>Subunits</th>
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<tr>
<td>FeSOD 40,000 dimer</td>
<td></td>
</tr>
<tr>
<td>MnSOD 40,000 dimer</td>
<td></td>
</tr>
<tr>
<td>80,000 tetrramer</td>
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</table>

Table 1

The prokaryotes have been found to contain superoxide dismutases based on manganese or iron but not on copper. The copper zinc enzyme is considered to be characteristic of eukaryotes. The bovine erythrocyte enzyme has two Cu\(^{2+}\) and two Zn\(^{2+}\) ions and has a molecular weight 32,000 Da. The Zn and Cu atoms are in close proximity, sharing a common imidazole group of a histidine residue as ligand. The metals are thought to be involved in both binding and electron transfer between oxygen species. Fridovich\(^{437}\) also reported a reasonable scheme for redox function of copper, zinc not being essential for activity.

<table>
<thead>
<tr>
<th>Eucaryotic Cells - SOD</th>
<th>Subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSOD 88,000</td>
<td>4</td>
</tr>
<tr>
<td>CuZnSOD 32,000</td>
<td>2</td>
</tr>
<tr>
<td>EC (CuZn) SOD 135,000</td>
<td>4</td>
</tr>
<tr>
<td>EC MnSOD 150,000</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 2

Three major forms of SODs reported are, based on their structure, localisation, inducibility and metal ion requirements.
Intracellular location of characteristic SOD’s are present in prokaryotes and eucaryotes.

In case of Procaryotes:
MnSOD is present in inner matrix of membrane, and FeSOD is present in outer membrane.

In case of Eucaryotes:
CuZnSOD is present in cytoplasm, nucleus, lysosomes, MnSOD is present in mitochondrial matrix and EC(CuZn) SOD is found in plasma and extracellular membrane.

Thus three members of SOD family have been identified in eukaryotes. They are copper-zinc containing SOD (CuZnSOD), manganese containing SOD (MnSOD), and extracellular SOD (ECSOD).

1. CuZnSOD, a homodimeric protein (mol.wt. 32.5 KDa), large, acidic pH 4-6, 150 - 155 a. a. residues per SOD subunit, localized in the cytosol, and requiring both Cu and Zn at its active site for its activity. In 1938, Mann and Keilin described a blue-green protein containing copper (haemocuprein) that they had isolated from bovine blood. In 1953 a similar protein was isolated from horse liver and was named as hepatocuprein. Other proteins of this type were later isolated, such as cerebrocuprein from the brain. In 1970, it was discovered that the erythrocyte protein contains zinc as well as copper. No enzymic function was detected in any of these proteins, so it was often suggested that they served as metal stores. However, in 1969 McCord and Fridovich reported that dead erythrocyte protein was able to remove the superoxide radical catalytically, i.e., it functioned as a superoxide dismutase enzyme.

Gene expression of CuZnSOD altered during hypoxia and other oxidative states. However, unlike the mitochondrial MnSOD, CuZnSOD has not been found essential for normal development and survival in mice. CuZnSOD can exhibit dual properties as a superoxide reductase and superoxide oxidase as well. Therefore, over-expression of this form of SOD may be deleterious to the host in view of its non-specific peroxidase
activity. Also, CuZnSOD in conjunction with nitric oxide (NO) radical generate a potent peroxynitrite anion (ONOO•) which may inactivate several proteins and cell surface receptors by nitration of their tyrosine residues. Guinea pigs pre-treated with recombinant SOD, showed protection against cigarette smoke-induced nuclear transcription factor-kappa β (NF-κβ) - mediated cytokine release and subsequent leukocyte inflammation. SOD has also been shown to inhibit redox cycling, and therefore, induced free radical generation of polycyclic aromatic hydrocarbons (PAH) and o-quinones. This inhibition may be lost in the presence of copper and iron metals. This suggests that SOD might not be an effective antioxidant in the presence of these metals.

CuZnSODs are present virtually in all eukaryotic cells. In the animal cell it is located in the cytosol, but some appear to be present in lysomes and nucleus. Peroxisomes has also been reported to contain some CuZnSOD. CuZnSODs have usually been thought to be much less common in prokaryotic cells. The first to be discovered was a CuZnSOD in the luminescent bacterium *Photobacterium leiognathi*. This organism exists in symbiotic relationship with the ponyfish. CuZnSODs had since been detected in many other bacteria. Thus, the free-living (non-symbiotic) bacterium *Caulobacter crescentus* CB15 contains CuZnSOD.

The copper-zinc SOD was first reported in *Photobacterium leiognathi* by Puget and Michelson. Some bacterial species contain Fe-SOD, apparently located in the periplasmic space between the inner and outer cell walls. Some other bacteria contain MnSOD and still others have both, but none has a Cu-ZnSOD. Since *Photobacterium leiognathi* was symbiotic and had been isolated from a special gland of the pony fish, it was supposed that it inherited the genetic information coding for Cu-Zn SOD from its host fish.

Cu-ZnSOD, one of the most stable proteins, is not dissociated by SDS alone (breaks apart H bonds). But it is disassociated by: SDS + β-mercaptoethanol or EDTA + heat 40-55°C.
Catalytic activity of CopperZinc SOD

The CuZnSOD enzyme, so far isolated from eukaryotes has a relative molecular mass of about 32000 KDa and contains two protein subunits each of which has an active site containing one copper and one zinc ion\(^{443}\).

All CuZnSODs catalyse the same reaction: they greatly accelerate the dismutation of \(O_2^*\):

\[
O_2^{*-} + O_2^{*-} + 2 \text{H}^+ \rightleftharpoons \text{H}_2\text{O}_2 + \text{O}_2
\]

Whereas the overall rate constant for the uncatalysed dismutation of \(O_2^*\) depends strongly on the pH of the solution and is about \(5 \times 10^{-5} \text{ M}^{-1} \text{s}^{-1}\) at physiological pH. The reaction, catalysed by bovine erythrocyte CuZnSOD, is almost independent of pH in the range 5.3-9.5 and the rate constant for reaction of \(O_2^*\) with the active site is about \(1.6 \times 10^9 \text{M}^{-1} \text{s}^{-1}\).

The copper ions in CuZnSODs appear to function in the dismutation reaction by undergoing alternate oxidation and reduction, as elucidated below:

**Catalytic Mechanism** \((E = \text{enzyme})\)

\[
\begin{align*}
\text{E-Cu}^{2+} + O_2^{*-} & \rightarrow \text{E-Cu}^{+1} + O_2 & \text{electron transfer} \\
\text{E-Cu}^{1+} + O_2^{*-} + 2\text{H}^+ & \rightarrow \text{E-Cu}^{2+} + \text{H}_2\text{O}_2 & \text{proton and e}^{-} \text{ transfer}
\end{align*}
\]

2. MnSOD: Manganese superoxide dismutase is considered to be one of the most important antioxidant component. It is a homotetrameric enzyme (mol.wt. 88 KD) and requires manganese at its active center. MnSOD constitutes of about 10-15% of the total SODs and is localised in the mitochondria of type II pneumocytes, alveolar macrophages and bronchial epithelium in rats, and least in the bronchial epithelial cells of human lungs. MnSOD mRNA is prominently expressed in cells in airway walls, the septal tips of alveolar ducts, and in arteriolar walls located adjacent to airways. Altered cellular redox states, inflammatory cytokines such as interleukin-1 and -6 (IL-1 and IL-6), interferon-\(\gamma\), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and hyperoxia induce MnSOD gene
expression. MnSOD has been reported to be crucial for survival and even 50% expression has been found to provide resistance to hyperoxia. Mice with depleted MnSOD or knocked out MnSOD gene has been found to survive for 2-3 weeks only. However, over expression of this enzyme antioxidant in transgenic mice has yielded inconclusive results as to the ability of these animals to resist oxygen toxicity. On the other hand, administration of SOD to reperfused tissues following hyperoxia has been found to be protective against oxygen toxicity.

The Mn-SOD has a dimer of 40 KD molecular weight with one manganese ion apparently as Mn per subunit\textsuperscript{444}. It has the same spectrum of activity as the enzyme from eukaryotic cytoplasm. Subsequent studies established that eukaryotic mitochondria contain a manganoenzyme analogous to the major \textit{E.coli} superoxide dismutase.

**Structure of MnSOD**

Each newly synthesized human MnSOD subunit contains 223 amino acids. After transport into mitochondria, each mature MnSOD subunit contains 198 amino acids. One subunit is of dimensions of about $40 \times 47 \times 49$ Å and can be divided into two distinct domains: an N-terminal helical hairpin domain and a C-terminal \( a/b \) domain, containing a three-stranded antiparallel sheet and five helices. Two subunits of MnSOD pair into a dimer with the active site manganese atoms near the dimer interface. Residues D159, H163, H26, H74, and a water molecule from each subunit contribute to the metal-binding site. For human MnSOD, two dimers further associate into a homotetramer with the dimensions of about $60 \times 79 \times 79$ Å.

Superoxide dismutase enzyme suppresses reactions caused specifically by \( O_2^- \) and has effectiveness in the ng ml\(^{-1}\) concentration range. It has an extremely sensitive and specific assay system for detecting whether \( O_2^- \) formed during some biological processes. Among the systems thus reported to produce superoxide are

1. Aldehyde oxidase, sulphite oxidase
2. Flavoenzyme dehydrogenases during their slow reoxidation by \( O_2^- \)
3. Reduced iron-sulphur chromophores being auto-oxidised in ferredoxins
4. A dioxygenases (tryptophan 2, 3-dioxygenase) is inhibited by superoxide dismutase
5. In fruit, ethylene acts as a ripening hormone; its production is attended by formation of $O_2$.

Fridovich\textsuperscript{445} proposed the general superoxide theory of oxygen toxicity. This enzyme protects oxygen metabolizing enzyme against the deleterious effects of free $O_2^-$ such as sulphhydryl oxidation or unsaturated lipid oxidation. It has long been known that the obligated anaerobes are killed on exposure to oxygen. Fridovich suggested that this oxygen toxicity might arise from the absence of superoxide distmutase in the anaerobes. During their normal metabolism, they do not generate $O_2^-$ and thus they have no need for a scavenging device. Indeed, in a species survey, all aerobic bacteria examined have been found to be a dismutase. Hewitt and Moriss\textsuperscript{446} found some SOD in 14 out of 16 obligate anaerobes. Some of these, in \textit{Chlorobium thiosulphatophilum} and \textit{Clostridium perfringens}, have about one third of the activity found in anaerobically grown \textit{E.coli}; others like \textit{Clostridium Acetobutylicum} and \textit{Clostridum Pasteurianum}, have only trace amounts of activity. Tally \textit{et al.}\textsuperscript{447} addressed the question of the oxygen tolerance of obligate anaerobes and classified 22 strains on the basis of this tolerance-strains which are very sensitive to oxygen lethality have little or no SOD, whereas, oxygen tolerant anaerobes do contain SOD. Halchikian \textit{et al.}\textsuperscript{448} reported SOD in several strains of \textit{Desusulph ovibro} but not in others. They considered the possibility that SOD in anaerobes might be a recent acquisition perhaps via plasmid transfer, rather than having ancient inherited characteristics. Aerobic organisms evolve this enzyme in response to the challenge of oxygen production generated when green plants begin photosynthesis and to convert $H_2O_2$, to $O_2$\textsuperscript{449}

3. **ECSOD**: It is an extracellular SOD (ECSOD), also known as the major extracellular SOD of the pulmonary fluids and interstitial spaces of the lungs. Lungs, the major organ express this enzyme in both rats and humans. ECSOD is obtained abundantly in blood vessels and airways. It accounts for about 70\% of the total SOD in certain pulmonary and systemic blood vessels. It is known as a secretory, tetrameric glycoprotein (mol.wt. 135KDa) and contains Cu and Zn in its active site. Characteristically, ECSOD exhibits heterogeneous affinity for heparin, regulates nitric oxide (NO) bioavailability and
modulates NO levels. Alveolar macrophages and neutrophils express high amounts of ECSOD and have been found to be resistant to hyperoxia and virus mediated and post-haemorrhage lung damage. Although animals expressing low levels of this enzyme may be otherwise healthy, however, they are more susceptible to hyperoxic injury. In human lungs, ECSOD has also been found to be expressed by bronchial epithelial cells, type alveolar cells, alveolar macrophages, chondrocytes and pulmonary endothelial cells. Higher localization of the enzyme has been found in the extra cellular matrix, predominantly around the larger blood vessels and airways and around the alveolar and the capillary regions. ECSOD in conjunction with GPx constitutes a major first line defence against the inhaled oxidants.

Control of the biosynthesis of SOD

Greater resistance towards oxygen toxicity has been conferred by elevated intra cellular levels of SOD as a consequence of increased exposure to oxygen. The phenomenon has been observed in Streptococcus faecalis, E.coli B, E.coli K12, and rat liver. In this mechanism of O$_2^-$ induced intracellular SOD elevation there exists some doubt as to whether the actual inducer is O$_2$ or O$_2^-$, or some other compound uniquely derived from O$_2^-$.

Circumstances leading to induction of SOD at fixed pO$_2$ exclude the possibility of O$_2^-$ itself being the inducer. Under three very different sets of conditions, induction of the manganese containing SOD (MnSOD) of E.coli has been observed with increase in the rate of production of O$_2^-$ at fixed pO$_2$.

Under different sets of conditions, as change of respiration rate in glucose limited chemo state culture, shifts between fermentative and oxidative metabolism by substrate exhaustion switch over technique and methyl violegen electron shunting technique between normal electron transport pathway and cyanide-insensitive respiration and O$_2$, in E.coli cultures under fixed pO$_2$ result in profound induction of SOD, which is dramatically absent in anaerobic conditions and leads to the conclusion that O$_2^-$ itself or some unique product of CV is the inducer rather than O$_2$.\textsuperscript{450,451}
Glutathione peroxidase

Glutathione Peroxidases (GPx) are a family of selenium dependent and independent antioxidant enzymes and can be divided into two groups, cellular and extracellular. In general GPx is a tetrameric protein (mol.wt. 85 KD). It requires 4 atoms of selenium bound as selenocysteine moieties that confer the catalytic activity. Glutathione Peroxidases remove \( \text{H}_2\text{O}_2 \) by coupling its reduction to \( \text{H}_2\text{O} \) with oxidation of reduced glutathione, GSH as shown in the equation.

\[
\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + 2\text{H}_2\text{O}
\]

GPx was first discovered (in animal tissues) in 1957\(^{452}\) and was reviewed in subsequent years\(^{453,454}\). Flohe\(^{455}\) summarized the essential characteristics of the enzyme which is still under investigation. Thereafter various possible roles of GPx in biological systems are reported to reveal that the function of GPx might be relevant to both acute and chronic alteration of mammalian tissue. GPx is not generally present in higher plants or bacteria, although they have been reported, to be found in all eukaryotes and a few algae and fungi. Glutathione peroxidases can be inhibited on incubation with mercaptosuccinate\(^{456}\). In human body, it is present in high levels in liver, moderately in heart, lung, brain, and in low levels in muscle.
Fig. 4

Rate limiting enzyme of pentose phosphate cycle is G-6-P dehydrogenase. NADP & GSSG both overcome NADPH inhibition of G-6-P dehydrogenase. Where, GSH = reduced glutathione; y-Glu-Cys-Gly = y-glutamylcysteinylglycine,

It is observed that H₂O₂-degrading GPx enzymes are widely distributed in animal tissues and are specific for GSH as a hydrogen donor. However, they can act on peroxides other than H₂O₂. Thus, they can catalyse GSH-dependent reduction of fatty acid hydro peroxides:

\[
\text{LOOH} + 2\text{GSH} \rightarrow \text{GSSG} + \text{H}_2\text{O} + \text{LOH}
\]
GPx is unspecific for hydroperoxides but specific for and can be about anything from H₂O₂ to peroxidized membranes and DNA.

There are five known forms of GPx are observed:

1. Cytosolic GPx (GPX-1): Bovine erythrocytes are usually studied;
   i.) It is soluble tetrameric protein of mol.wt = 85 KDa,
   ii.) rat liver mol.wt = 75 KDa,
   iii.) human erythrocyte = 95 KDa,
   iv.) human placenta = 85.5 KDa;
   v.) Each subunit contains selenium, no other metal.
   vi.) Active site contains a selenocysteine.

2. Mitochondrial GPx: never been isolated. It may be a related enzyme such as thioredoxin/peroxiredoxin.
3. Human Plasma GPx:
   i) Tetramer 21.5 to 22.5 KDa per subunit.
   ii) One Se per subunit.
   iii) Synthesized and secreted by kidney.
   iv) Distinct from cytosolic (49% homology) and phospholipid GPx.

4. GSHPx –GI: Tetrameric protein localized in cytosol.
   i) Monomer mol.wt. = 22 KDa, 190 amino acids
   ii) Similar substrate specificities as cytosolic GPx (GSHPx – 1)
   iii) Both reduce H$_2$O$_2$, tert-butylhydroperoxide, amino hydroperoxide, and linoleic acid hydroperoxide, but not phosphatidylcholine hydroperoxide

   i) The rest of GPx’s require phospholipase to clip hydroperoxides
   ii) Rat liver PH-GP-x needs detergent for activity, pig heart does not.
   iii) Rat liver – monomer, mol.wt = 22 KDa, Pig heart – monomer, mol.wt = 20 KDa.
   iv) It contains selenium. Active site is conserved, but the rest of the protein is quite different.
   v) Homology is 25% for plasma EC-GPx and 35% for GPx (with PH-GPx) in terms of amino acids
During the catalytic mechanism GSX, a selenol which reacts with peroxide to give a selenic acid:

\[
E - CysSe^- + H^+ + ROOH \rightarrow E - CysSeOH + ROH
\]

\[
E - CysSeOH + GSH \rightarrow E - CysSe^- + SG + H_2O
\]

\[
E - CysSe^- + GSH \rightarrow E - CysSe^- + GSSG + H^+
\]

GPx is irreversibly inhibited by CN, unless GSH is present together with iodoacetate. Both GPx and CAT are also inhibited by \(O_2^-\). Traces of selenium are essential in the diet of animals: an important role of dietary selenium is to provide the selenium-containing cofactor for the glutathione peroxidase enzyme family. However, selenium is essential for protein synthesis and enzymatic activity of GPx. Animal cells lose GPx if put on a Se-deficient diet. Increased GPx occur on selenium supplementation. Selenite, selenomethionine, and selenocysteine also can be used.

Due to deficiency of selenium some symptoms can occur i.e., liver necrosis, exudative diathesis, failure to grow and reproduce and degenerative heart disease (Keshan disease).

Due to overdose of selenium it may lead to increased lipid peroxidation and cellular toxicity. Accumulation of selenium in plants used as fodder can poison cattle.

Superoxide dismutase, catalase and glutathione peroxidase protect cells from oxidative damage. Various enzymes also repair oxidatively damaged macromolecules. Treatment of cells with oxidants induce the synthesis of heat shock proteins which increase the resistance of these cells to oxidants. The evolution of these defence mechanism are probably critical to the survival of aerobic life forms.

In vitro, GPx prevents the oxidative break down of unsaturated lipids of biomembranes. Flohe et al.\(^\text{457}\) reported that this enzyme plays defensive role against oxidative damage of organisms living in aerobic conditions. This view is categorically based on the following observations of various investigators in different laboratories:

a) GPx can reduce hydroperoxy fatty acids\(^\text{458}\)
b) Endogenous mitochondrial GPx prevents lipid peroxidation and irreversible high amplitude swelling of rat liver mitochondria\textsuperscript{459}.

c) In isolated inner membranes of rat liver mitochondria purified GPx prevents the oxidative degradation of phospholipids and the concomitant formation of malondialdehyde\textsuperscript{460}.

d) Bovine blood GPx added to illuminated chloroplasts inhibits swelling and malondialdehyde formation\textsuperscript{461}.

e) In vitro inhibition of GPx by repeated administration of cadmium salts resulting in an accumulation of degradation products of unsaturated lipid in rat testes\textsuperscript{462}.

f) Conditions requiring a high rate of lipid peroxide removal, such as the ingestion of lipid peroxides or exposure to ozone, lead to increased GPx activity\textsuperscript{463}.

Monitoring of GPx activity in different physiological conditions is considered to be a helpful tool to assess oxidative state of the individual. Thus, low GPx activity is reported in alcoholic patients\textsuperscript{464} and cystic fibrous patients\textsuperscript{465}. Low plasma levels of GPx may lead to the incidence of cardiovascular diseases such as Keshan diseases\textsuperscript{466} and atherosclerosis\textsuperscript{467}.

**Catalase**

Thenard in 1818 discovered and observed that oxygen liberates with the decomposition of hydrogen peroxide by both fibrin and animal tissues, and that certain finely divided metals influence the same action. This erroneous view was rectified to be a general property of all enzymes by subsequent workers\textsuperscript{468}. Decomposition of hydrogen peroxide must be attributed to a specific enzyme, which was made clear by O. Loew\textsuperscript{469} and named it catalase.

Isolation and purification experiments on catalase were done by different workers in the following years. The catalase is a protein was first suggested by Waentig and Gierisch.
Warburg\textsuperscript{471}, as early as in 1923, had postulated that catalases were iron enzymes because their activity was inhibited by cyanide. The catalase was first isolated by Sumner and Dounce and obtained in crystalline form from beef liver\textsuperscript{472} and later from blood and other sources\textsuperscript{473}.

Dismutation of \( \text{O}_2^{*} \) generated by several oxidase enzyme in vivo, including xanthine, urate and D-amino acid oxidases\textsuperscript{474}. Hydrogen peroxide is usually removed in aerobes by two of enzymes. The catalases directly catalyse decomposition of \( \text{H}_2\text{O}_2 \) to ground state \( \text{O}_2 \)

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

Peroxidase enzymes remove \( \text{H}_2\text{O}_2 \) by using it to oxidize another substrate

\[
\text{SH}_2 + \text{H}_2\text{O}_2 \rightarrow \text{S} + 2\text{H}_2\text{O}
\]

Catalase activity is present in most aerobic cells\textsuperscript{475}, although a few do not have it such as the bacterium \textit{Bacillus popilliae}, \textit{Mycoplasma pneumoniae}, the green alga Euglena, several parasitic helminths (liver fluke), and the blue green alga \textit{Gloeocapsa}. Most animal cells and organs have catalytic activity. Catalases had been crystallized from three animal sources: erythrocyte, kidney, and especially concentrated in liver. The catalase activity of tissues varies greatly; it is highest in liver and kidney, and low in connective tissue. In tissues it is mainly particle bound such as mitochondria and peroxisomes, whereas, it exists in a soluble state in erythrocytes. Human erythrocytes is normally rich in catalase. The catalase activity in blood is practically all due to the erythrocytes.

Catalase in erythrocytes protect them against \( \text{H}_2\text{O}_2 \) generated by dismutation of \( \text{O}_2^{*} \) generated by haemoglobin auto-oxidation. Since \( \text{H}_2\text{O}_2 \) diffuses readily, erythrocytes can also protect other tissues against oxidative damage by 'absorbing' \( \text{H}_2\text{O}_2 \). The brain, heart and skeletal muscle contain lower levels of catalase than liver contains.

All kinds of plant materials contain catalase activity. The amounts of catalase always seem to be low, compared to those in liver or erythrocytes. The methods for
crystallising animal catalases are not applicable to plant catalases. The catalase content varies greatly in different parts of plants. Sprouted seeds contain maximum concentration of catalase after 3 to 6 days. In general catalase seems to be ubiquitous in aerobic cells; where a cytochrome system is present, catalase is also present.

A few anaerobic bacteria, such as *Propionionibacterium shermennai*, contain catalase, but most others do not.

There are four protein subunits in animal catalase, each of which contains a ferric haem group bound to its active site. The haem groups are buried in non-polar pockets, connected to the surface by narrow channels, thus preventing most molecules larger than H$_2$O$_2$ from gaining access. Each subunit usually contains one molecule of NADPH bound to it. Dissociation of catalase into its sub-units which easily occurs on storage, freeze-drying, or exposure of the enzyme to acid or alkali, leading to loss of catalase activity.

Catalase and glutathione peroxidase seek out hydrogen peroxide and convert it to water and diatomic oxygen. Together with the SODs and GSH and related enzymes, it forms one of the major intracellular antioxidants which protect against oxidative stress. Although catalase catalyses the dismutation of H$_2$O$_2$ to water and oxygen, but it is not as effective as an antioxidant like glutathione peroxidase which can detoxify a greater number of peroxide radicals including organic and lipid hydroperoxides.

Enzymes preferring H$_2$O$_2$ as substrate are called catalases, and those preferring alkyl peroxides are called peroxidases although each type can use either H$_2$O$_2$ or alkyl peroxides. Catalase acting in the catalytic mode is most effective at a relatively high concentration of H$_2$O$_2$, whereas peroxidases are most effective at low levels of H$_3$O. Catalase has the advantage of not consuming any reductant other than H$_3$O. Catalase has a double function because it catalyses the following reactions:

(i)  Decomposition of H$_2$O$_2$ to give H$_2$O and O$_2$ (catalase activity),

\[
\text{CAT} \quad 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2
\]
(ii) Oxidation of donors: for example, methanol, ethanol, formic acid, phenols with the consumption of 1 mole of peroxide (peroxidase activity).

iii) Peroxidative first substrate is $H_2O_2$

$$ROOH + HQOH \rightarrow QO + ROH + H_2O$$

All catalases contain the prosthetic group Protohematin IX. Lemberg and his associates (1949)\textsuperscript{477} identified a blue substance isolated from impure catalase preparation and also from pure crystallised beef liver catalase as biliverdin. There is an inverse relation between the biliverdin content, protohematin content and the catalase activity. It is found to be free of biliverdin in erythrocyte catalases. The catalase in the horse liver contains little or no biliverdin. But a pathological horse liver gave a high biliverdin value. Isotope experiments indicate a comparatively short life cycle of liver catalase. The oxidation of protohematin to ferribiliverdin could then be a normal intermediate step in the destruction of catalase\textsuperscript{478}.

Beef liver catalase is a tetramer of 284 KDa mol. wt. with a turnover number of $2.8 \times 10^6$ moles min\textsuperscript{-1}. It qualifies as one of the most efficient enzymic catalyst known ($K_{cat}/k_m = 4 \times 10^7$ M\textsuperscript{-1} sec\textsuperscript{-1}). The enzyme contains 4 ferriprotoporphyrin groups per molecule which corresponds to a protohaem content of 1.1 % and iron content 0.09%. The extinction at 405 nm is used to determine the concentration of catalase. The extinction coefficient is 3804.00 per mole of enzyme or 100 per haem group. The specific activity and the ratio $E_{405}/E_{280}$ can be used as an index of purity\textsuperscript{479}.

The kinetics of catalase does not obey the normal pattern. On the one hand, it is not possible to saturate the enzyme with 'substrate' within the feasible concentration range (up to $5M H_2O_2$) and, on the other hand, there is rapid inactivation of catalase at $H_2O_2$ concentration above 0.1 M when the active enzyme-$H_2O_2$ complex is converted to the inactive complex.

It has also been shown that catalases are good antigens; rabbits developed anticatalases on injection of beef-, lamb-, horse-, and dog liver catalases. The anticatalases were not
entirely specific for the same antigen; precipitation sometimes occurs with catalases other than the one used as an antigen, though always to a lesser extent.

The exact role of catalase on physiological function is still a matter of conjecture. Possibly the catalase located in the cell organelles plays the role of a specific peroxidase. The enzyme pattern of the peroxisome is noteworthy for the simultaneous presence of H$_2$O$_2$-producing (e.g. D-amino oxidase, uricase) and consuming enzymes like catalase. Catalase-like glutathione peroxidase exerts a protective function in erythrocytes for haemoglobin and other SH-protein (enzymes, stroma), the importance of which can vary with the species and the experimental conditions. When the catalytic activity of erythrocytes is lower, the more effective is the action of oxidizing agents (H$_2$O$_2$, ascorbic acid, methyl hydrazine) or X-rays on methaemoglobin formation$^{479}$. 
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