REVIEW OF LITERATURE
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The impressive correlation between cardiovascular disease (CVD) and alterations in glucose metabolism has raised the likelihood that atherosclerosis and type 2 diabetes may share common antecedents. Large vessel atherosclerosis can proceed the development of diabetes, suggesting that rather than atherosclerosis being a complication of diabetes, both conditions may share genetic and environmental antecedents, a “Common Soil”. These same adverse environmental conditions associated with hyperinsulinemia and insulin resistance lead to the development in adult life of the dysmetabolic syndrome, consisting of abdominal obesity, impaired fasting glucose, high triglyceride levels low high density lipoprotein levels and hypertension. These constituents may be associated with additional elements such as elevations in small low-density lipoproteins, prothrombic factors and free acids. Taking into consideration that components of this cluster of abnormalities are essentially shared by both diabetes type 2 and atherosclerosis, the American Heart Association stated in 1999 that diabetes is a cardiovascular disease. Inflammation plays an important role in mediating all phase of atherosclerosis, from initially recruitment of circulating cells to the inner arterial layer to weakening of the fibrous cap of the plaque, eventually leading to rupture.65

Oxidative stress has been considered deleterious due to free radical induced oxidation and damage of macromolecules, membranes and DNA. ROS generation by phagocyte cells such as neutrophils is a pivotal component of their antimicrobial actions and as such deleterious for ingested organisms, but is, however clearly beneficial for the host. On the other hand, the restoration of oxygen supply during myocardial reperfusion after prolonged ischaemia is accompanied by a burst of free radical production that is damaging for the heart.133 Oxidative stress induced damage includes
acceleration of cell death through apoptosis and necrosis, mechanisms that may also be of relevance in advanced heart failure.

Among the many health prediction for the new millennium, the most alarming is that of cardiovascular disease (CVD), heart disease and stroke – topping the list for death and disability. While there are undoubted regional differences between the developed countries and other economies, the predictions for India by 2015 show a steady increase since 1985. The projected rate per 100,000 for 1985 for all “circulatory diseases” was 145 males and 126 females for 2000, 253 males and 204 females and for 2015, 295 males and 239 females for 2025 which is higher than that for other causes such as cancer.135

Prevalence:

In the United States an estimated 12 million people have cardiovascular disease and about one half of whom have acute myocardial infarction and half have angina pectoris. For men prevalence of myocardial infarction is 1% at ages 35 to 44 years and 16% at age 75 and over. In women the prevalence is less than 1% at ages 35 to 44 years and 13 percent at age 75 and over.136

Incidence:

In the United States cardiovascular disease cause about 6,50,000 new heart attacks each year and 450,000 recurrent attacks. The incidence in women lags behind that in men by serious clinical manifestation such as myocardial infarction and sudden death. In male predominance is least striking for uncomplicated angina pectoris. In men more angina occurs after myocardial infarction than before. Only 20% of coronary attacks are preceded by long standing angina, the percentage is lower if the infarction is silent or unrecognized.117
Classification of Cardiovascular Diseases

Cardiovascular diseases are the leading cause of mortality in the developing world and are becoming more and more important as the cause of mortality in the developing countries as well, most of them are atherosclerosis.
Definition:

Atherosclerosis is a complex inflammatory fibroproliferative response to retention of plasma derived atherogenic lipoproteins in the arterial intima.

The term atherosclerosis and atheroma are derived from Greek works “athera” meaning gruel, skleros meaning hard and “oma” meaning a moss lesions which characterize the degenerative and inflammatory disease of arteries.\textsuperscript{124}

Classification

Atherosclerosis of coronary arteries causes several major clinical categories largely determined by clinical history, physical examination and laboratory findings. These major clinical categories include angina pectoris stable angina, unstable angina, silent ischemia, syndrome x, and prinzmetal variant angina, myocardial infarction (MI), ischaemic cardiomyopathy and sudden cardiac death due to coronary heart disease (CHD), unstable angina pectoris and MI together with sudden ischaemic death are the main features of so called acute coronary syndrome.\textsuperscript{133}

**Angina Pectoris** is a clinical syndrome caused by the delivery of insufficient oxygen to the heart muscle via the coronary arteries, leading to ischemia. Angina is characterized by episodic chest (pericardial) discomfort pressure or pain lasting up to 15 minutes. Unstable angina is caused by an acute but reversible increase in coronary obstruction due to rupture or fissuring of the fibrous cap of the atheromatous plaque with consequent thrombus formation.\textsuperscript{134}

**Silent Ischemia** is myocardial ischemia detected on ambulatory ECG monitoring or during exercise stress testing, echocardiography or nuclear stress scintigraphy in the absence of chest pain or any other symptoms. It may be categorized into 3 types: type 1 patients are totally asymptomatic, type 2
are those who are symptomatic after a prior documented MI and type 3 patients are those who manifest silent ischemia but also may have symptomatic ischemia. At least 75% of the ischemia occurring in patients with stable angina is clinically silent.

*Syndrome* x is angina, or angina – like chest pain relieved by rest or sublingual nitroglycerin in patients who have an abnormal ECU in exercise test and myocardial lactate production during ischemia but no coronary atherosclerotic lesion proved on coronary angiography. The exact aetiology, of this syndrome is unclear but most likely it represents a heterogeneous group of changes best characterized by a reduced capacity of the coronary circulation to augment blood flow in the face of an increase in oxygen demand.

*Prinzmetal’s (or variant) angina* is angina caused by spasm occurring within 1 cm of an obstruction of the proximal portion of a major coronary artery. It is characterized by chest discomfort at rest which is relieved after sublingual nitroglycerin and by ST segment elevation in ECG during the attack. The changes are usually confined to a single epicardial coronary artery, but multivessel spasm can also occur as well as the spasm at different level within the same vessel.

*Acute myocardial infarction* is ischemic necrosis of the myocardium usually resulting from an occlusion by an acute thrombus of a coronary artery that supplies the damaged area, often after an atheromatous plaque rupture. The patients suffer from a deep chest pain or pressure often with radiation to the left arm or jaw which is similar to the discomfort of angina pectoris.

*Ischemic cardiomyopathy* is predominantly caused by diffuse coronary artery atherosclerotic disease. It is caused by chronic coronary artery stenosis, myocardial fibrosis with diffuse loss of myocytes and result in impaired ventricular systolic function reflected by low ejection fraction. The main symptoms of this chronic disease are effort dyspnea and fatigueness.
Cerebrovascular disease is the first cause of death in Croatia and the third most common cause of death in developed countries. Cerebrovascular disease presents either as transient ischaemic attacks and / or as ischaemic stroke. Thrombi or emboli from ulcerated atheromatous plaques can interrupt intracranial or extracranial arterial blood supply causing brain ischemia and consequent neurological symptoms.

Transient ischaemic attacks are episodes of sudden, focal neurologic dysfunction from a vascular cause i.e. internal carotid, middle cerebral or the vertebrobasilar arterial system.

Stroke can be caused by cerebral ischemia and infarction (85% of all cases), cerebral hemorrhage (10%) or subarachnoid haemorrhage (5%). The hallmark of ischaemic strokes is the sudden onset of focal neurological deficit. The symptoms of ischaemic stroke are related to the location and the volume of brain tissue damaged as well as to the mechanism of injury.

Peripheral artery disease Peripheral artery disease (PAD) caused by atherosclerosis is also increasing as the population ages and as patients survive their myocardial infarctions. PAD can be present as chronic ischemia that is caused by gradual enlargement of an atheromatous plaque and therefore insufficient blood supply.136

Pathophysiology of Cardiovascular disease caused by Atherosclerosis

The process of inflammation is now believed to be the etiological event that precedes the development and the continual process of atherosclerosis. This process, beginning with an injury or change in the endothelial wall of the artery, causes an alteration in the intimal layer that increases leukocyte low density lipoprotein (LDL) and platelet adhesion to the endothelium.143
Endothelial Dysfunction caused by injury and subsequent attraction and adhesion of damaged LDL and macrophage.

Possible causes of dysfunctional endothelium include free radical damage from environmental exposure, hypertension and its proinflammatory effects (smooth muscle lipoxygenase activity and oxide radical formation), direct toxic effects of homocysteine and advanced glycosylated end products (the result of oxidation reaction with glucose that results in type of oxidant commonly found in the blood of diabetes)\(^\text{145}\).

Oxidized or modified LDL is a recognized source of damage to the endothelium when it becomes immunogenic, (autoantibodies have been
isolated against oxidized LDL) when LDL aggregates form or when LDL undergoes glycation oxidized. LDL becomes a chemo attractant for monocytes because it represents cell damage. Macrophages then bind to the altered LDL via scavenger receptors on the macrophage surface, a mechanism that is part of the innate immune system and is a rapid first line of defense designed to respond to tissue damage. Altered LDL particles continue to undergo oxidation in the lumen of the artery and promote injury to the artery through multiple pathways. The presence of oxidized LDL promotes the expression of growth factors and chemotactic proteins, causing an expanding inflammatory response and up-regulating monocyte replication to increase macrophage populations. The mediators of the inflammatory response interleukin – I (IL - I), tumor necrosis factor – alpha (TNF – 2), interleukin – 6 (IL - 6) macrophage colony stimulating factor etc, all increase the binding of LDL to the endothelium and smooth muscle to further up regulate the inflammatory response. Present at every stage and in every lesion of arteriosclerosis the monocyte turned macrophage responds to an attempt to remove oxidized LDL by binding to it engulfing the modified LDL, and turning into a foam cells.\textsuperscript{146}
Formation of the fatty streak

1. Endothelial cells

2. Endothelial cells express selectin, MCP-1

1. Endothelial cells proliferate in an effort to heal the lesion, causing plaque to form on vessel lining.

2. Cholesterol

Monocytes (rush to sites of injury, causing further inflammation...)

Endothelial injury by homocysteine, endotoxin...........

Smooth muscle cells

LDL

Platelets

Stable plaque with core of lipids
This process signifies the attempt of the macrophages to remove an offending agent. Foam cells are fatty cells that together form the fatty streak which is the first identifiable characteristic lesion of advanced atherosclerosis at the time the increase in the “stickiness” of the endothelial wall also alters its permeability, making it easier for leukocytes macrophages and LDL to migrate into the wall. The adhesiveness and permeability of the endothelial wall is controlled by a variety of chemokines prostacyclin, nitric oxide, angiotensin II, growth factors and monocyte chemotactic proteins released as complex interactions between monocytes T-cells and the endothelial walls. As endothelial permeability becomes increasingly altered, increasing amounts of modified LDL are absorbed along with increasing number of monocots and T cell. The resultant inflammation causes increasing number of macrophages and lymphocytes to be produced and to multiply inside the lesions leading to the production of enzymes, cytokines and growth factors. The inflammatory
response initiates migration of smooth muscles cells into the fatty streak and eventually the inflammatory cells produce a lesion of necrotic cellular debris inside the arterial wall. The lesion then becomes covered with a “fibrous cap” that protects the area. The artery dilates to accommodate this lesion initially and the lumen begins to narrow.  

In areas where the fibrous cap is uneven or thinning and rupture occurs, the contents of necrotic cellular debris are exposed to the lumen of the artery and a thrombus forms. If this thrombus is significant enough to cause a blockage in arterial flow, myocardial infarction is the result. The rupture of what is termed vulnerable plaque and the resulting thrombus is potentially responsible for at least 50 percent of all myocardial infarctions even though it is seen in 10 – 20% of all lesions.

**Diabetes and cardiovascular disease**

As recently expressed in a scientific statement from the American Heart Association “Diabetes is a cardiovascular disease”. Unlike classical microvascular complications, large vessel atherosclerosis can precede the development of diabetes suggesting that rather than atherosclerosis being a complication of diabetes, both conditions have common genetic and environmental antecedent’s i.e. They spring from a common soil.

In the coming decades the burden of cardiovascular disease (CVDs) related to diabetes will increase substantially. Diabetes formerly thought of as a problem most of its harm by effects on the cardiovascular system. Most diabetic’s die of CVD and atherosclerosis accounts for some 80 percent of all diabetic mortality. About three quarters of the cardiovascular deaths from diabetes results from coronary artery disease (CAD). The remaining quarter results from cerebral or peripheral vascular disease.
Atherosclerotic disease causes some three quarters of all hospitalizations for diabetic complications.

The first reports on glucose as a risk factor for cardiovascular complication were published in 1965 in the UK (the Bedford Study) and the US (the Tecumseh study). Although CVD has been a focus of intensive research in patients with type 2 diabetes for decades it is only in recent years that the role of hyperglycemia as a risk factors for CVDs has been clarified. The first study of this series to demonstrate a positive association between glycemic control and CVD was published by Uusitupa et al. 99

What is Diabetes?

Glucose is a simple sugar found in food. It is an essential to the patient that provides energy for the proper functioning of the body cells. After meals food is digested in the stomach and the intestine to glucose and other nutrients. The glucose in digested food is absorbed by the intestinal cells into the blood stream, and is carried by blood to all cells. It needs assistance from insulin to penetrate the cells. Insulin therefore acts as a regulator of glucose metabolism in the body. 66

Insulin is also called the hunger hormone. As the blood sugar level increase following a carbohydrate rich meal, the corresponding insulin level raises with the eventual lowering of the blood sugar level and glucose is transported from the blood into the cell for energy. When the blood glucose levels are lowered the insulin release from the pancreas is turned off. When the blood sugar level drops below a certain level hunger is felt. This often occurs a few hours after the meal. Craving for sweets frequently from part of this cycle which can lead to snaking, often for more carbohydrates. It the cravings are not fulfilled sensations such as hunger, dizziness, moodiness and state of collapse can result. 98
This system of auto regulation and homeostasis is the function of the pancreas and it works around the clock. Dysfunction of this auto regulation system either inability of the pancreas to secrete any or insufficient insulin, or pancreas overload from too much sugar ingested over a long period of time, or over compensatory mechanism, or a combination of these result in the lack of insulin, and hence high blood sugar. This hallmark of diabetes mellitus commonly is called diabetes.

**Diabetes Mellitus is classified into the following categories.**

1) Insulin- dependent diabetes mellitus (IDDM type 1) is the term used to describe the condition in patients for whom insulin therapy is essential because they are prone to develop ketoacidosis. It usually presents during childhood. It has been suggested that many cause follow a viral infection, which has destroyed the β-cells of the pancreatic islets. Subjects most at risk are those with HL-A types DR3 and DR4 of the major histocompatibility.

2) Non Insulin Dependent Diabetes Mellitus (NIDDM2) is the commonest variety. Patients are much less likely to develop ketoacidosis than those with IDDM and though insulin may sometimes be needed, it is not essential for survival. Onset is most usual during adult life. Although no genetic markers have been found, there is a familial tendency. A variety of inherited disorders may be responsible for the syndrome, either by reducing insulin secretion or by causing relative insulin deficiency because of resistance to its action or of post receptor defects, despite high plasma insulin concentrations. Factors increasing the risk of developing NIDDM include obesity, sustained stress and sedentary life style.

Metabolic syndrome, insulin resistance, prediabetes and overt type 2 diabetes mellitus are associated with an accelerated atherosclerosis & atheroscleropathy. This quartet is also associated with multiple metabolic toxicities resulting in the production of reactive oxygen species. The redox
stress associated with these reactive oxygen species contribute to the development, progression and the final fate of the arterial vessel wall and diabetic atheroscleropathy. \(^\text{101}\)

**How Diabetes Promotes Atherosclerosis; Molecular Mechanism**

Diabetes induces a large number of alterations at the cellular level of vascular tissue that, potentially accelerate the atherosclerotic process.

There major mechanisms that encompass most of the pathological alterations observed in the diabetic vasculature are.

1. Non enzymatic glycosylation of proteins and lipids.
2. Oxidative stress
3. Protein kinase C activation.

Importantly, these mechanisms are not independent. For example, hyperglycemia induced oxidative stress promotes the formation of advanced glycosylation end products and PKC activation. \(^\text{108}\)

**Advanced glycosylation end products**

One of the important mechanisms responsible for the accelerated atherosclerosis in diabetes is the non enzymatic reaction between glucose and proteins or lipoproteins in arterial walls collectively known as Maillard, or browning reaction. Glucose form chemically reversible early glycosylation products with reactive amino groups of circulating or vessel wall proteins (Schiff base), which subsequently rearrange to form the more stable Amadori-type early glycosylation products. Equilibrium levels of Schiff-base and Amador products (the best known of which is hemoglobin A1C) are reached in hours and weeks, respectively. \(^\text{89}\)
Some of the early glycosylation products on long – lived proteins (e.g. vessel wall collagen) continue to undergo complex series of chemical rearrangement to form advanced glycosylation end products (AGE’s). Once formed, AGE – protein adducts are stable and virtually irreversible. Although AGE’s comprise a large number of chemical structures, carboxymethyl-lysine protein adducts are the predominant AGE’s present in vivo. 18

AGE’s accumulate continuously on long lived vessel wall proteins with aging and at an accelerated rate in diabetes. The degree of non-enzymatic glycation is determined mainly by the glucose concentration and time of exposure. However, another critical factor to the formation of AGE’s is the tissue microenvironment redox potential. Thus situations in which the local
Oxidative stress, lipoproteins in cardiovascular dysfunctions

redox potential has been shifted to favor oxidant stress AGEs formation increases substantially. 81

AGE’s can accelerate the atherosclerotic process by diverse mechanism, which can be classified as “non-receptor dependent” and “receptor mediated”.

Non-receptor mediated mechanisms

Glycosylation of proteins and lipoproteins can interfere with their normal function by disrupting molecular conformation, altering enzymatic activity, reducing degradative capacity, and interfere with receptor recognition. Thus, changes in the normal physiology of proteins that are relevant to atherogenesis, may promote atherosclerosis in diabetic individuals.

The glycosylation process occurs both on the apoprotein B and phospholipids components of LDL, leading to both functional alternations in LDL clearance and increased susceptibility to oxidative modifications.

Glycosylation of LDL ape B (the surface protein of LDL) occurs mainly on a positively charged lysine residues within the putative LDL receptor binding domain which are essential for the specific recognition of LDL by the LDL receptor. LDL glycosylation is increased in correlation with glucose levels, and AGE -Apo B levels are unto 4 – fold higher in diabetic patients. Glycosylation of Apo- B result in a significant impairment of LDL - receptor mediator uptake decreasing the in vivo clearance of LDL compared to native LDL. In contrast to fibroblasts, human monocytes derived macrophages recognized glycated LDL47 by these cells however, is not mediated by the LDL receptor pathway, but by a high capacity, low affinity receptor pathway. Thus, gyrated LDL are poorly recognized by the specific LDL receptor and are preferentially recognize by a nonspecific (scavenger) receptor present on human macrophages. Because LDL glycosylation
enhances its uptake by human-aortic intimal cells and monocyte derived macrophages. With stimulation of foam cells formation, the recognition of gyrated LDL by the scavenger receptor pathway is thought to promote intracellular accumulation of cholesteryl esters and promote atherosclerosis.

**Potential mechanism by which LDL glycosylation increases its atherogenicity.**

Another atherogenic effect of glycation is to confer increased susceptibility of LDL oxidative modification. Oxidation reactions occur normally during glycation can oxidize the amine containing phospholipids component of LDL. Independently of transition metals or exogenous free radical-generating systems LDL oxidation following AGE-LDL formation occurs in direct proportion to glucose concentration and can be inhibited by the AGE formation inhibitor amino guanidine. Thus, glycation confers increased susceptibility of LDL to oxidative modification which is considered a critical step in its atherogenicity. 20

**Receptor – Mediated Mechanisms**

The cellular interactions of Ages are mediated through a specific receptor for AGE receptor (RAGE), a member of the immunologic super family of receptors has been demonstrated in all cells relevant to the atherosclerotic process including monocyte derived macrophages, endothelial cells, and smooth muscle cells. The macrophage AGE receptor system is closely field to AGE turnover, and is thought to represent a mechanism that responds to raising AG levels with along and degrade senescent proteins. 22

In pathological lesions, abundance of RAGE expressing cells is usually associated with sites of accumulated RAGE legends. In diabetic vasculature,
cells expressing high levels of RAGE are often proximal to areas in which AGEs are abundant.  

Binding of soluble AGEs to RAGE bearing monocots induces chemotaxis. Followed by mononuclear infiltration through an intact endothelial monolayer. Pathological studies of human atherosclerotic plaques showed infiltration of RAGE expressing class in the expanded intimae. Monocyte macrophage interaction with Ages result also in the production of mediators such as interleukin –1, tumor necrosis factor α, platelet-derived growth factor, and insulin growth factor-I which have a pivotal role in the pathogenesis of atherosclerosis.

The potential role of RAGE in the atherogenic process in diabetes has been demonstrated by park and associates. In this model of atherosclerosis-prone mice due to homozygous deletion of apolipoprotein E (apo E) gene, the induction of diabetes using streptozotocin resulted in atherosclerosis of increased severity. Compared to euglycemic apoE controls. The development of vascular was more rapid with the formation of more complex lesions (fibrous caps, extensive monocyte infiltration etc) and atherosclerosis extending distally in the aorta and major arteries. Increased expression of RAGE and the presence of AGEs in the vessel wall especially at sites of vascular lesions were also evident. Blockade of AGE-RAGE interaction using a truncated soluble extra cellular domain of RAGE resulted in a striking suppression of lesions in diabetic mice, with lesions largely arrested at the fatty streak stage and a large reduction in complex lesions.
Protein Kinase C

The metabolic consequences of hyperglycemia can be expressed in cells in which glucose transport is largely independent of insulin. The resulting intracellular hyperglycemia has been implicated in the pathogenesis of diabetic complications through the activation of the protein kinase C (PKC) system.

High ambient glucose concentrations activate PKC by increasing the function of diacylglycerol (DAG), the major endogenous cellular cofactor for PKC activation, for glycolytic intermediates such as dihydroxy-acetone phosphate and glyceraidehyde-3- phosphate. The elevation of DAG and subsequent activation of PKC in the vasculature can be maintained chronically.

Oxidative stress in diabetes as pathogenic mechanism for atherosclerosis.

Oxidative stress is widely invoked as a pathogenic mechanism for atherosclerosis. Among the sequelae of hyperglycemia, oxidative stress has been suggested as a potential mechanism for accelerated atherosclerosis. Hyperglycemia can increase oxidative stress through several pathways. A major mechanism appears to be the hyperglycemia- induced intercellular reactive oxygen species (ROS), produced by the proton electromechanical gradient generated by the mitochondrial electron transport chain and resulting in increased production of superoxide. Two other mechanisms have been proposed that may explain how hyperglycemia causes increased ROS formation.

One mechanism involves “the transition metal catalyzed auto oxidation of free glucose “as described in cell free systems. Through this mechanism glucose itself initiates autoxidative reaction and free radical production yielding superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$).
The other mechanism involves “the transition metal catalyzed autoxidation of protein bound Amadori products”, which yields superoxide and hydroxyl radicals and highly reactive dicarbonyl compounds.

Two alternative mechanisms by which glucose may induce structural changes in proteins have recently become prominent. Monosaccharides are oxidized when catalyzed by trace amounts of transition metals, generating free radicals, hydrogen peroxide and reactive dicarbonyls directly. The process of glucose oxidation in a transition metal dependent reaction can lead to protein damage by free radicals and by covalent binding of the carbonyl products to protein components.

**Pro-atherogenic mechanisms of diabetes associated with hyperglycemia**
Mechanism of glucose oxidation

The Maillard reaction describes the non-enzymic glycation of proteins. Glucose is considered to be toxic because of its ability to behave chemically reversible, early glycosylation products with protein (Schiff bases) at a rate proportional to the glucose concentration. These Schiff bases then rearrange to form the more stable Amadori type early glycosylation products. Protein which has been glycated in vitro is conformationally altered. The amount of early glycosylation products in vivo in diabetes, whether on haemoglobin or basement membrane, increases when blood glucose levels are high and returns to normal after the glucose levels are normalized by treatment.

The subsequent degradation or glycoxidation of protein bound Amadori products in a transition metal dependent process can yield further oxidants and protein reactive aldehydes. Thus some of the early glycosylation products on collagen and other long-lived proteins of the vessel wall undergo a slow, complex series of chemical rearrangement to form irreversible advanced glycosylation end products. A number of this irreversible end product is capable of forming covalent bonds with amino groups on other proteins, forming cross-links. It has been proposed that hyperglycemia in diabetes may involve covalent cross linking of extraverted plasma lipoproteins to matrix lipoproteins by advanced glycosylation end-products, retarding the rate of cholesterol efflux and accelerating the development of vascular disease.

Oxidative stress may also be involved in the activation of DAG-PKC in vascular tissue oxidants produced in the setting of hyperglycemia can activate PKC.
The Involvement of Free Radicals in the Pathogenesis of Atherosclerosis.

The earliest recognizable lesion of atherosclerosis is the fatty streak, an aggregation of lipid-rich macrophages and T lymphocytes within the intima, the innermost layer of artery wall. The hypothesis that Ldls that have undergone oxidative damage are considerably more atherogenic than native LDLs was promulgated by the group of Steinberg et al (1989) who proposed that oxidized LDL and certain other modified forms are ligands for scavenger receptors on macrophages, and can convert them to the cholesterol laden foam cells characteristic of the fatty streak.

Evidence is accumulating from in vitro studies that LDL can be oxidatively modified by a variety of systems and agents, and is subsequently recognized and rapidly taken up by scavenger receptors on target macrophages.

a) Cell Induced Modification

Evidence from cellular studies in vitro initially showed how oxidative processes could play a central role in the pathological changes involved in the genesis of atherosclerosis. LDL can be oxidatively modified in culture by a range of cell types including endothelial cells, arterial smooth muscle cells, as well as macrophages and monocytes and is subsequently taken up by scavenger receptors on target macrophages.

Human neutrophils have also been shown to be capable of mediating LDL oxidation such that it becomes cytotoxic, but these cells are not a common constituent of atheromatous lesions. The relative contributions of the different cell types may achieve different levels of importance at the various stages in the development of the lesion. Normal arterial wall contains endothelial cells and smooth muscle cells, whereas atherosclerotic lesions may also contain macrophages and T lymphocytes.
Atherosclerotic lesions may also contain macrophages and T lymphocytes. Thus when the atherosclerotic lesion develops, what is the mechanism by which LDL becomes oxidized in vivo? Stimulation of endothelial cells, smooth muscle cells or macrophages may induce the secretion of components capable of promoting mechanisms of initiation of LDL oxidation. Initiating oxidation may be formed or propagation of peroxidation may occur subsequent to lipoxygenase - mediated hydroperoxide formation. If the former, what is the probable initiating agent, where is it located and what activates it? If the latter are the lipoxygenases derived or from other cell sources? 55
Cell – induced modification of LDL invitro has been demonstrated to be mediated by free radicals. All the cell types mentioned have been shown to release superoxide radicals, albeit by different mechanisms and at different rates. However it is known that superoxide radicals will not initiate the oxidation of polyunsaturated fatty acids unless protonated.  

It is suggested that endothelial cells can initiate the oxidation of LDL through a superoxide independent pathway that involves lipoxygenase and this pathway may predominate in endothelial cells. Monocyte lipoxygenase products, which induce release of superoxide radical from the monocytes. Thus, of the cells present in the arterial wall, activated macrophages, endothelial cells and smooth muscle cell, all of which secrete superoxide radical, hydrogen peroxide and hydrolytic enzymes have been reported to oxidize LDL but the superoxide released from these cells and hydrogen peroxide generated these form are not very reactive towards polyunsaturated fatty acids. Their reactivity may in principle, be amplified through protonation of superoxide or in the presence of: 

1. Available delocalized haem proteins generating ferryl haem protein derived radicals. 
2. Transition metal ions generating hydroxyl radical or 
Oxidative stress
Antioxidant and Metabolic Control of ROS Levels and Actions

The influence of metabolizing or scavenging systems on the levels of individual ROS (and RNS) may be a key aspect that determines the expression of signaling processes regulated by these species.

Auntie Oxidant kicks out the Free Radicals.
Superoxide Dismutase

Superoxide dismutase destroys the free radicals superoxide by converting it to peroxide that can in turn be destroyed by catalase or GPx reaction. SOD converts the highly reactive superoxide radical to less reactive hydrogen radical.

\[
\text{SOD} \quad O_2^- + O_2^- + 2H_2 \rightarrow H_2O_2 + O_2^-
\]

Another function of superoxide dismutase is to protect dehydratases (dihydro acid dehydratase, aconitase, 6-phosphoglyconate dehydratase) and fumarase A & B against inactivation by the free radical superoxide.\(^{11}\)

Four classes of SOD have been identified containing either a dinuclear Cu, mononuclear Fe, Mm or Ni cofactor, FeSODs & Mn – SODs show homology and passes identical metal chelating residues at the active site, substantial sequence and three dimensional structural homology, while the superoxide dismutase are structurally unrelated. In humans there are three types of SOD, and extra cellular EC-SOD, SOD catalyses the dismutation of \(O_2^-\) by successive oxidation and reduction of the transition metal ion of the active site in a Ping Pong type of mechanism with remarkably high reaction rates.\(^{58}\)

Manganese Superoxide Dismutase

Mn-SOD is a homotrimer (96 KDa) containing one manganese atom per subunit that cycles from Mn (III) to Mn II and back to Mn III during the two step dismutation of superoxide. The respiratory chain in mitochondria is a major source of oxygen radical. Mn –SOD is a nuclear - encoded primary antioxidant enzyme that functions to remove this superoxide radical.
The expression of Mn-SOD is essential for the survival at aerobic life and the development of cellular resistance oxygen radical mediated toxicity. 

**Copper, Zinc superoxide dismutase**

CuZn-SOD (SOD-1) are another class of enzyme conserved throughout evolution, which usually have two identical subunits of about 32 Kda, each containing a metal cluster and a zinc bridged by a common ligand. This Cu-Zn-SOD is believed to play a major role in first line of antioxidant defense radicals to form hydrogen peroxide and molecular oxygen. 

**Extra cellular superoxide dismutase**

E-C - SOD is a secretary, tetramer, copper and zinc containing glycoprotein with a high affinity for certain glycosaminoglycans such as heparin and heparin sulfate found in the interstitial spaces of tissues and also in extracellular fluids accounting for the majority of SOD activity of plasma lymph and synovial fluid. EC-SOD is not induced by its substrate or other oxidants (xanthine oxidase plus hypoxanthine paraquot, pyragallal, Fe$^{2+}$ ions, Cu$^{2+}$ ions, buthionine, selenite), and high oxygen partial pressure and its regulation in mammalian tissues primarily occurs in a manner co-ordinated by cytokines, rather than as a response of individual cells to oxidant.

**Nickel Superoxide dismutase**

Ni-SOD has been purified from the cytosolic fraction of streptomycin species and streptomytces cellular. It is composed of four identical subunits of 13.4 Kda stable at PH 4.0-8.0 and up to 70 Celsius degrees. It is inhibited by cyanide and H$_2$O$_2$ but little inhibited by aside. Amino acid composition is different from iron manganese and zinc-copper SODs. The apoenzyme, lacking in nickel, had no ability to mediate the conversion of superoxide indicating that Ni$^{III}$ plays a main role in the activity.
Role of superoxide dismutase in cardiovascular disease.

The production of ROS often begins with 1-electron reduction of molecular oxygen to superoxide anion ($O_2^-$) by various oxidases. Superoxide anion is negatively charged free radicals that undergo rather selective chemical reactions with the components of biological systems. Although $O_2^-$ reacts with itself with a rate constant of $8 \times 10^4 \text{ mol}^{-1}\text{ L. S}^{-1}$ from H$_2$O$_2$ and $O_2^-$ superoxide dismutase (SOD) enzymes function to accelerate the removal of $O_2^-$ as a result of their rate constant of $2 \times 10^{-1} \text{ L. S}^{-1}$ forms the reaction with $O_2^-$.

\[
\begin{align*}
\text{oxidase} & \\
O_2^- + \text{electron} & \rightarrow O_2^- \\
\text{SOD} & \\
O_2^- + O_2^- & \rightarrow \text{H}_2\text{O}_2 + O_2^- \\
O_2^- + \text{NO} & \rightarrow \text{O NOO}^- 
\end{align*}
\]

Vascular tissue contains a cytosolic copper-zinc form of SOD (CuZn-SOD) a mitochondrial manganese form of SOD (Mn-SOD) and an extracellular CuZn-SOD. One of the most important role is the prevention of the reaction of $O_2^-$ with NO. It has been demonstrated that CuZn-SOD permits NO release from the endothelial and NO-mediated vascular smooth muscle (VSM) relaxation whereas extracellular SOD appears to protect NO during its diffusion from endothelium to VSM. 94

The widespread occurrence in biology of the enzyme superoxide dismutase (SOD), which catalyses the dismutation of the superoxide anion, has led to the hypothesis that this radical superoxide is toxic so that it leads to the formation of toxic metabolites. The SOD enzyme exists in a number of different forms which presumably serve different functions. For example the
mitochondrion, a major source of superoxide radicals within the cell, possesses a manganese-containing SOD. On this context, it is interesting to note that extracellular forms of SOD have also been described. There are three types A, B and C with type C having the highest affinity for heparin-binding sites on the endothelium. An admittedly teleological argument suggests that extra cellular SOD-C may have evolved to prevent the formation of peroxynitrite in the artery wall. In support of this idea, extracellular SOD-C is distributed throughout the interstitium and prevents the superoxide dependent loss of EDRF activity in isolated rabbit aortic rings.\textsuperscript{95}

Wilkins G.M. et al hypothesized that superoxide radical is responsible for cell mediated oxidation depends on two major sets of observations. The first rely on the inhibitory activity of superoxide dismutase (SOD). Many studies have shown that the addition of SOD can cause substantial inhibition of LDL oxidation by a number of cell types.\textsuperscript{160}

SOD can both chelate copper in a redox-inactive form and act as a peroxyl radical scavenger, since the cell mediated LDL oxidation in the systems studied using SOD is usually, metal dependent the observed inhibitor activity of SOD may be catalytic dismutation of superoxide variabilities in the efficiency of inhibition of cell mediated oxidation measured in the present of SOD could depend on the relative amounts of SOD protein and redox-active metal present in each cause.\textsuperscript{137}

Salvermini, utilized the oxygen free radical scavenger SOD and the H$_2$O$_2$ degrading enzyme catalase to decrease myocardial infract size in the canine heart other studies utilizing SOD alone, and SOD conjugated to polyethylene glycol have obtained similar results.\textsuperscript{137}

Guzzocrea.S.observed that SOD serves to protect the tissue from free radical attack are depleted during the ischemic insult and are not available to defend the cell against associated with the reintroduction of molecular oxygen during myocardial reperfusion.\textsuperscript{38}
Glutathione Peroxidase

The selenium containing peroxidase being more important example glutathione catalase the reduction of a variety of hydroperoxides (ROOH and H₂O₂) using GSH, thereby protecting mammalian cell against oxidative damage.

\[
\text{ROOH} + 2 \text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H₂O}
\]

There are at least five GPx isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type cytosolic and mitochondrial glutathione peroxidase (GPx or GPx1) reduces fatty acid hydroperoxides and H₂O₂ at the expense of glutathione GPx1 and the phospholipids hydro peroxide glutathione GPx1 peroxides Gap 4 (or PH Gap) are found in most tissues. GPX 4 is located in both the cytosol and the phospholipid hydroperoxides, fatty acid hydroperoxides and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins. GPx1 is predominantly present erythrocytes.

GPX (80 KDa) contain one selenocysteine residue in each of the fourth identical subunits, which is essential for enzyme activity. Although GPx shares the substrate, H₂O₂ with catalase, it alone can react effectively with lipid and other hydroperoxides. The glutathione redox cycle is a major source of protection against low levels of oxidant stress, whereas CAT becomes more significantly in protecting against severe oxidant stress. In animals cells and especially in human erythrocytes, the principal antioxidant enzyme for the detoxification of H₂O₂ has for a long time been considered to be GPx as catalase has much low affinity for H₂O₂ than GPx.

GPx equally protects against the oxidation of dihydroamine 123 can indicator dye by peroxynitrite requiring GSH as reluctant. Thus there is also
Oxidative stress, lipoproteins in cardiovascular dysfunctions

a function of GPx and potentially of other selenoproteins containing selenocysteine or selenomethionine in the GSH dependent medicated oxidations as a peroxynitrite reductase.

**Role of Glutathione peroxidase in cardiovascular disease**

In human erythrocytes the principal antioxidant enzyme for the detoxification of H$_2$O$_2$ as for long time been considered to be GPx as catalase has much lower affinity for H$_2$O$_2$ than Gap.

Evidence has accumulated that H$_2$O$_2$ can produce vascular relaxation by stimulating soluble guanylate catalase (SGC). The properties of stimulating SGC by H$_2$O$_2$ suggest that its expression is likely to be modulated by competing processes, including the efficiency of H$_2$O$_2$ metabolism by glutathione peroxides (GPX) and by the levels of the physiological modulators of peroxide metabolism by catalase that inhibit SGC stimulation, including O$_2$, NO, and the tissue derived electron donors for compound 1 of catalase, which remain to be identified.

\[
\text{Catalase} + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{compound} \rightarrow \text{cGMP}
\]

\[
\text{Compound} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2^- + \text{H}_2\text{O} + \text{catalase}
\]

GPx metabolizes H$_2$O$_2$ and other biological peroxide by reducing them with the use electrons derived from the oxidation of GSH to its disulfide form (GSSG)

\[
\text{GPX} \rightarrow \text{ROOH} + 2 \text{GSH} \rightarrow \text{ROH} + \text{GSSG}
\]
One of the major muscle antioxidant is the glutathione peroxidase / reductase system, by providing a labile pool of reducing equivalents, it is able to consume oxidants and protect against oxidative stress. One of the central functions of this system is to degrade peroxides. However, during the ischemic phase, reduced glutathione is depleted from the myocardium and this antioxidant defense system is therefore compromised. The myocyte contains glutathione peroxidase but relatively little catalase. Therefore loss or impaired function of intracellular antioxidants during ischemia as well as depletion of glutathione predisposes the myocardium to further injury upon reperfusion by the reducing the ability of the cell to defend itself against the deleterious effects of radical formation.\textsuperscript{151}
Ascorbic Acid

This water soluble antioxidant is also found in specific intracellular locations, particularly muscle. The most important chemical property of ascorbate is its ease of oxidation either by one – or two electron transfers.

The important of the antioxidant nutrients vit C in maintaining health in contributing to a decreased incidence of disease and in protecting against. The recurrence of pathological events. Thus it is been suggested that ability of ascorbate to cooperate in the protection of lipid against peroxidation. 36

Vitamin C, or ascorbic acid, is a water soluble antioxidant vitamin and an essential cofactor for many enzymes. It is a six – carbon lactones that is synthesized from glucose by most animals but not by humans. It was discovered in the 1920’s and popularized in the 1970s as a method of treating the common cold. 31

The biological actions of vitamin C are attributed to its antioxidant properties. Vitamin C sequentially donates electrons from the double bond between the second and third carbons on the 6-carbon molecule, becoming oxidized to the ascorbyl radical in the process. The ascorbyl radical is generally much less reactive than the free radical that was quenched. The ascorbyl radical can also be recycled back to vitamin C through three separate enzymatic pathways as well as by reducing compounds such as glutathione. Vitamin C can also aid in the recycling of other antioxidants, most notably vitamin C compounds related radicals (superoxide, hydroxyl radical, peroxyl radicals) sulphur radicals, reactive compounds such as hypochlorous acid, nitrosamines, and other nitrosating compounds and many others. 52

In general, oxidative stress occurs in three general classes of biomolecules-lipid, protein and DNA vit. C has the potential to prevent oxidative stress in all of these areas although results depend on study design and greater effect is generally seen in situations where health is compromised.
Lipid peroxidation occurs when lipids interact with reactive oxygen species (ROS), and these lipid peroxides further react with oxygen to form peroxyl radicals which then result in lipid hydroperoxides. This process is called radical propagation, and Vit C can prevent this by reducing the initiating ROS. Studies both in vitro and in animals have found vitamin C to reduce lipid peroxidation. Proteins can be oxidized in a variety of ways, which also leads to radical propagation and in this case vitamin C also inhibits the initiating step. DNA can be oxidized either directly or indirectly through protein or lipid oxidation, and reactive nitrogen species can also damage proteins needed for DNA repair, Vit. C can help prevent the formation of reactive nitrogen species, and vit. C has also been found to reduce DNA oxidation from a variety of causes both in vitro and in vivo.

Vitamin C also acts as an electron donor for eight known enzymes. Out of these, three the stability of the collagen hydroxylation, which increases the stability of the collagen structure, two participate in the synthesis of carnitine, one participates in the biosynthesis of non epinephrine from dopamine, one adds amide groups to peptide hormones to increase their stability, and one modulates tyrosine metabolism.

Under certain conditions Vit. C can also act as a pro-oxidant. This is particularly the case when vit C Reacts with transition metals such as iron.

**Role of vitamin C in cardiovascular disease**

There is a very large body of literature and the use of vit. C in the treatment and prevention of cardiovascular disease, most commonly in conjunction with vit. E. Vit. C has numerous effects that are beneficial to the cardiovascular system. First it can protect endothelial nitric oxide (NO) from oxidation and increase synthesis of NO, leading to improved endothelial function. Second as discussed above, vitamin C is an inhibitor of lipid peroxidation. It inhibits the buildup of oxidized LDL in arteries, a major
Contributing factor to atherosclerosis. Third vitamin C status has been correlated with the level of homocysteine, a cardiovascular disease risk factor. Finally synergism with vitamin E may play a strong role in the cardioprotective properties of vitamin C.\textsuperscript{56}

In addition to the epidemiologic evidence above, a number of other studies have been conducted. In animals, co-administration of vitamin C and E decreases atherosclerosis in LDL-receptor deficient mice, cholesterol led primates vitamin C reduces blood pressure in blood pressure in both salt-fed and fructose fed rats. In healthy humans, vit C can restore vasodilatation that is impaired by acute hyperglycemia, and another trial found that reduced serum LDL and total cholesterol and raised HDL cholesterol. A six year trial with 100 mg of vit E and 250 mg vit. C found that the vitamins slowed the progression of atherosclerosis.\textsuperscript{35}
Alpha Tocopherol

α Tocopherol is the major lipid-soluble chain-breaking antioxidant in plasma and in cell membranes. It inhibits amplification of peroxidation by intercepting propagating lipid chains by reducing peroxyl radicals to hydroperoxides. The suitability of ascorbate and tocopherol as chain-breaking antioxidants is exemplified by the fact that they are effective in relatively small amounts and their radical states are relatively uncreative, in either reducing or oxidizing capacities.²⁹

Role of Vitamin E in Preventing Lipid Peroxidation

Vitamin E has been termed as lipid-soluble chain-breaking antioxidant. Although this is not the only means by which vitamin E prevents lipid peroxidation. Vitamin E both quenches and reacts with singlet oxygen and thus protects against the peroxidation initiated by this radical. It reacts with superoxide also. The proton on the hydroxyl group of the chromane ring is readily donated by vitamin E to superoxide and consequently vitamin gets converted into a vitamin E radical.¹⁰⁶

Most of the superoxide generating enzymes are membrane bound and hence ample opportunities exist for the generation of superoxide close to membrane and if it is not scavenged immediately, it can initiate peroxidation through the formation of hydrogen peroxide. In vitro models also support the capability of the vitamin E to scavenge superoxide radical. It has been found that the superoxide generated by xanthin-xanthin oxidize system deoxycholate and the reaction can be inhibited by addition of superoxide dismutase.¹¹⁴

A water soluble model compounds of alpha tocopherol, 6 hydroxy 2,5,7,8 tetra methyl 2 chroman carboxylic acid, a compound where C-16, isoprenoid chain at C-2 is replaced by COOH group also gets oxidized by xanthin-xanthin oxidize system.
However many counter arguments also have put forward with respect to superoxide scavenging function of tocopherol. First the rate constant for reaction between tocopherol and superoxide being $5.9 \times 10^{3}$ m$^{-1}$ s$^{-1}$ is so slow that this reaction is unlikely to be significant in biological system. Second at physiological pH the peroxidation of liposome’s prepared from dimyristryl phosphotidyl chlorine has shown to be considerably low in radiation induced generation of free radicals, when formate was induced form and reconverts all the radicals into superoxide radical.

Third the oxidation of water soluble model compound (mentioned above) may be due to small amount of hydrogen peroxide produced by xantin – xanthin oxidize system, this can give rise to hydroxyl radical. The model compound also does not prevent the reduction of cytochrome – C by xanthin – xanthin oxidase system which is mediated by superoxide.

Vitamin E has shown to scavenge the perhydroxyl (HO$_2$) radical, the protonated form of superoxide and hydroxyl radicals is artificially prepared liposomes. In fact it scavenges more efficiently these radicals than superoxide, the reactivities being with perhydroxyl more than that of superoxide. But at physiological pH perhydroxyl can not occur since it is formed at pH around 4.8, the pka for superoxide. The only chance left for tocopherol is to react with hydroxyl radical (provided the alpha tocopherol is not a scavenger of superoxide). This may be generated by hydrogen peroxide by Fentons reaction or by iron catalyzed Haberweies reaction. Hydrogen peroxide itself is produced from dismutation of superoxide. Moreover superoxide is not an inhibitor of lipid peroxidation but hydroxyl radical is the most potent initiator of lipid peroxidation of all the oxygen free radicals. Thus vitamin E is highly effective in protecting membrane lipids against peroxidation. To play its role as a scavenger a critical level of Alpha tocoperol should be present in the membrane. Below this level protection is poor and Alpha tocopherol will be rapidly consumed. Above this level it effectively scavenges the radical and
rate of consumption is very low. Perhaps the most important fact concerned with Alpha tocopherol as antioxidant is that it can react with lipid peroxyl radicals to form vitamin E radical which are insufficiently reactive to abstract hydrogen atom from membrane lipids.75

Vitamin E radical thus produced is highly stable because the unpaired electron on the oxygen atom can continuously be localized from one atom to other in the heterocyclic ring thus making it stable. In this course of delocalization various resonance structures of Alpha tocopherol are obtained.

Animal models also provide evidence for the protective function of vitamin E against lipid peroxidation. When animals are made vitamin E deficient, the peroxidation process is increased as judged by ethane evolution.

It has been proposed that the chromanol ring of tocopherol is located at the polar surface of membranes and phytol chain interacts with hydro-carbon side chains of membrane lipids. It is further proposed that tocopherol is adjacent to membrane bond enzymes that generate free radicals. This localization in the close vicinity of the surface of free radicals makes it possible to scavenge the radicals immediately. It is also possible that tocopherol is an effective membrane radical scavenger because it is able to move very rapidly through the no polar portion of membrane. As well as the primary defenses (scavenger enzymes and metal-ion sequestration), secondary defenses are also present. The cell membranes and plasma lipoproteins contain ξ - tocopherol is an ‘OH’ group whose hydrogen atom is easily removed. Hence, proxy and alloy radicals generated during lipid predation preferentially combine with the antioxidant e.g.

\[
\begin{align*}
\text{Tocopherol} - \text{OH} + \text{-- C --} & \rightarrow \text{tocopherol} - \text{O}_2^- + \text{-- C --} \\
\text{O}_2^- & \text{O}_2\text{H}
\end{align*}
\]
Instead of an adjacent fatty acid chain. This therefore terminates the chain reaction. Hence the term chain breaking antioxidant. It also converts the $\alpha$-tocopherol into a new radical, tocopherol-O, which is poorly reactive and unable to attack adjacent fatty acid side chains, consequently stopping the chain reaction. Evidence exists that the tocopherol radical can migrate to the membrane surface and convert to $\alpha$-tocopherol by reaction with ascorbic acid (vit C). Both vitamin C and vitamin E seem to minimize the consequences of lipid peroxidation in lipoproteins and in membranes should this process begin. Some tiny compounds, such as GSH, might also be involved in regenerating $\alpha$-tocopherol from its radical in vivo.

The terms "$\alpha$-tocopherol" and "Vitamin E" are often used synonymously, which is not strictly correct. Vit – E is defined nutritionally as a factor needed in the diet of pregnant female rats to prevent resorption of fetus and compounds other than $\alpha$-tocopherol (e.g. $\beta, \gamma$ and $\delta$ tocopherols) have some effect in this assay. However, $\alpha$-tocopherol is the most effective and it seems to be the most important lipid – soluble chain breaking antioxidant in vivo in human. The content of $\alpha$ tocopherol in circulating low density lipoproteins helps to determine their resistance to lipid per oxidation and thus may affect the development of atherosclerosis, a disease in which lipid per oxidation is involved. Low plasma levels of $\alpha$ tocopherol and vitamin C correlate with an increased incidence of myocardial infarction and of some forms of cancer.

Tocopherols are present in tissues, largely is subcellular membrane such as mitochondrial, microsomes and chloroplasts. Electron transport system located in these membranes have been shown to produce $\overline{O}_2$. Therefore, it is possible that $\alpha$-tocopherol scavenges $\overline{O}_2$ generated within the membranes, functioning as a defence against $\overline{O}_2$. 

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53
Tissue destruction and degeneration can result in increased oxidative damages, by such process as metal ion release, phagocyte activation, lipoxygenase activation and disruption of mitochondrial electron transport chains, so that more electrons escape to oxygen to form $\cdot O_2$. \(^{74}\)

It follows that almost any disease is likely to be accompanied by increased formation of reactive oxygen species. It is not therefore surprising that the list of disease in which their formation has been implicated as long as is growing longer. For atherosclerosis, rheumatoid arthritis. Some forms of ARDS deoxygenating injury and traumatic or ischemic damage to the central nervous system, there is reasonable evidence to suggest that free radical reaction make a significant detrimental contribution to the pathologic process. As previously stressed, it is equally likely that in some (perhaps most) diseases, the increased ROS formation is an epiphenomenon, making no significant contribution to the progression of the disease. Each proposal must be subject to stringent examination because the likely clinical value of “antioxidant therapy” will depend on how well the exact role of reactive oxygen species is known. \(^{48}\)

Antioxidants can be defined in various ways often the term implicitly restricted to chain breaking antioxidant, inhibitors of lipid peroxidation such as vitamin E, a broader definition for an antioxidant is any substance that when present at low concentrations compared with those of oxidisable substrate, significantly delays or prevents oxidation of the substrate. The term “oxidizable substrate includes, almost everything found in living cell, including proteins, lipids, carbohydrates and DNA. \(^{111}\)

The vitamin E inhibits both ethane and evolution argues for an intimate relationship between vitamin E and cyclic peroxidation of PUFA. Diplock and Lucy have proposed close conformational association between vitamin E and arachidonic acid. \(^{111}\)
A scheme combining these concepts is peroxidation toxicity. Free radical initiation is likely to occur by abstraction of one of the hydrogen’s of the methylene group. Initiation could be from a variety of natural or unnatural means. Ozone, nitrogen, dioxide, organic solvents and anesthetic agents containing halogen are also well known as agents for initiating lipid peroxidation in the liver.  

The initial radical is unlikely to survive due to the rapid rate of reaction with molecular oxygen. The peroxyl free radical resulting from reaction with molecular oxygen becomes a key intermediate determining the lesion ultimately observed, although it may be transient itself. It is long supposed that such peroxyl free radicals abstract hydrogen from other relatively stable compounds and have little prostaglandin like activity. Either the hydroperoxide or the peroxyl free radical may be reduced by glutathione peroxidase to the corresponding alcohol as a detoxification mechanism. Indeed, glutathione peroxidase activity increased after exposure of rats to ozone, suggesting the induction of a protection mechanism. Glutathione peroxidase, a soluble enzyme, may, however, be restricted in its activity on membrane–bound peroxidase.  

In the presence of little or no vitamin E in the tissue, β, γ allylic peroxyl free radicals may spontaneously cyclise to form either bicyclic or monocyclic peroxides. The cyclization reaction need not be enzymatically catalyzed and the stereo chemistry need not be identical to the enzymatic product. Spontaneously formed endoperoxide have significant potency as noted above for at least two classes of receptors, smooth muscles and platelets. Tocopherol can’t react with the peroxyl free radical to form a hydroperoxide in preference to calcification to an endoperoxide. In this view, vitamin E provides its essential function by direction of peroxidation to hydro peroxides. While still reactive, hydro peroxides are more stable than endo peroxides and are over 3-10 times less potent than are endoperoxides is not fully known,
but they may serve as chemotactic agents for phagocytes, hydroperoxide decomposition could lead to polymerization of lipids, thereby drastically altering membrane viscosity. Hydroperoxides would also be substrates for glutathione peroxidase. The vitamin E cycle.\(^{153}\)

Vitamin E acts catalytically, being efficiently reduced from its free radical form, its form after quenching radicals, back to its native state. This catalysis occurs through the inter reactions between water and lipid soluble substances by both non-enzymatic and enzymatic mechanisms, which regenerate vitamin E from its radical tocopheroxyl back to tocotrienol and tocopherol respectively. Under conditions where these auxiliary systems act synergistically to keep the steady state concentration of vitamin E radicals low the loss or consumption of vitamin E is prevented.\(^{107}\) The thiocytic acid/dihydrolipoic acid couple (TA/DHLA) is a unique antioxidant system. Normally covalently bound lipoamide exists in small amounts as the cofactor of \(\alpha\) ketodehydrogenas in animals.

However, large amounts of fed TA confer protection in tissues and membrane against oxidative damage. After absorption, TA may be reduced enzymatically or nonenzymatically to form an active antioxidant. It acts as a “double edged sword”, in that it appears to interact directly with the membrane to reduce tocopheroxyl radicals (weak effect) or to reduce ascorbate which in turn acts at membranes to reduce tocopheroxyl radicals (strong effect.) Thus, the TA/DHLA couple works in recycling vitamin E both in membranes and in low density lipoproteins (LDL), where it acts to stabilize them.\(^{142}\)
SELENIUM

More than 75% of selenium in erythrocytes is present in this enzyme. Glutathione peroxides activity is erythrocytes and other tissues decrease in direct proportion to decreases in dietary intake and deficiency of this enzyme is believed to account for many of these manifestations of selenium deficiency. The enzyme catalyzes the transfer of reducing equivalents from reduced glutathione to hydrogen peroxide or to lipid peroxides, thus serving to protect cells and membranes against oxidative damage.\textsuperscript{129}

Selenium is an essential mineral founding trace amounts in the human body. It works as an antioxidant, especially when combined with vit. C by scavenging damaging particles in the body known as free radicals. These particles occur naturally in the body but can damage cell membranes, interact with genetic material, and possibly contribute to the aging process as well as the development at a number of conditions including heart disease and cancer. Antioxidants such as selenium can neutralize free radicals and may reduce or even help prevent some of the damage they cause.\textsuperscript{152}

Selenium is needed for the functioning of the immune system and for the production of prostaglandins (substances that affect blood and inflammation in the body). Low levels of selenium may worsen arteriolosclerosis (plaque buildup in arteries which can lead to heart attack and/or stroke) and can lead to premature aging. Selenium deficiencies have also been linked with certain types of cancer.\textsuperscript{64}

Many of the benefits of selenium are related to its role in the production of the glutathione peroxides. This enzyme is responsible for detoxification in the body. Chronic exposure to environmental toxins,
including chemotherapy drugs radiation and other toxic medicines, increases the requirement of selenium.

Cigarette smokers have lower level of selenium. There are several reasons to this Tobacco decreases absorption of selenium in the digestive tract. In addition, many smokers have poorly dietary habits and eat less food containing selenium. Alcohol also lowers selenium levels. 73

Selenium and cardiovascular disease

Epidemiological evidence among the human population has shown a significantly increase incidence of heart disease and thrombosis in areas low in selenium compared to selenium rich areas. It is believed that the role of selenium in the prevention of heart disease steam of its activity to protect, via glutathione peroxidase, blood platelets and cell against oxidative injury. Studies on animals have shown that during graded selenium depletion glutathione peroxidase activity decreases stepwise with lesser dietary intake and increases when selenium is added to the diet. It is obvious therefore that in order to protect against the development of heart disease everyone, particularly those living in selenium-poor areas, ought to take an additional selenium supplement. 98
ZINC

In addition to being a constituent of more than a hundred enzymes Zn has several other functions, including a protective activity against oxidative stress as demonstrated by several in vitro and in vivo studies. A positive correlation has been shown between cytosolic SOD activity and plasma Zn concentration and dietary Zn deficiency has been shown to cause a decrease in CuZn-SOD activity and an increased susceptibility to oxidative damage. Thus, Zn depletion in rats is associated with an increase in free radical induced lipid peroxidation both in plasma and organs and an increase in lipoprotein oxidation. 134

The Chinese probably are the first to extract zinc metal, though it was first discovered in the year 1597 in India. Zinc was used as an alloy with copper in its manufacture which candidate the earliest record of investigation. 134

The most important discovery regarding the biological role of zinc was done by Kielin and Mann in 1944. The isolation and purification of an enzyme carbonic anhydrate contain 0.33 percent zinc as part of its molecule. Zinc was demonstrated the essential to the mechanism of action of their enzyme, which catalyze the dehydration of carbonic acid and participation in the elimination and incorporation of carbon dioxide. 15

Role of zinc in cardiovascular disease.

As regards the relationship of zinc to atherosclerosis, a few soviet investigator studied the behavior of their element in the aorta wall of atherosclerotic subjects. The results however were contradictory. Some author reported that zinc concentration in aorta increases in atherosclerosis lesions or other found that it decreases. The beneficial effect of zinc therapy in atherosclerotic patient has been reported. 42
Zinc appears to be related also to myocardial infarction. The concentration of their mencial disease in the injured heart tissue. This decrease is perhaps related to the disappearance of lactic dehydrogenates a zinc enzyme, from the infracted heart tissue. It decreases also in serum of infracted patients.

Bailey R.R. et al (1967) studied the response to the stress the an acute myocardial infarction the studies were found to correlated with serum enzymatic changes suggesting that the adrenocortical response is governed by the amount of myocardial infarction. 41

And tested the allegedly beneficial effect or zinc protect against hypertension, so also he observed changes in the mencial content in atherosclerotic and cardiac subjects and compared with healthy control. In atherosclerosis trust zinc content of heart and aorta and plasma zinc level decrease while in myocardial infarction zinc concentration zinc level decrease.
COPPER

Synthesis of Cu-Zn –SOD and its activity depend on Cu status and also been shown to vary as a function of Cu intake- studies of the influence of Cu on cardiac susceptibility to ischemia and reperfusion showed conflicting results. Allen and Sari reported an improvement of post ischemic function of in vitro cardiac preparations isolated from Cu-deficient rats, whereas other studies have shown that Cu deficiency enhance oxidative stress in various tissues and on the basis of animal experiments, it has also been suggested that Cu deficiency or an imbalance of Cu and Zn in the diet might be a risk factor for IHD.

The copper containing protein, copper, zinc super oxide disputes is the primary antioxidant defense in the human body. Higher levels of CuZn are a primary factor in longer life spans in animals. 45

However because copper is usually is short supply in the human body CuZnSOD has only about 50% of its needed copper (zinc supplies are usually adequate) and this markedly reduces CuZnSOD’s antioxidant power and is another reason why more dietary copper would be beneficial. Harris pointed out that while copper, zinc, superoxide dismutase requires two, copper & zinc, only copper, seems to regulate the expression of functional anti-oxidant activity. Restricting dietary copper quickly impairs the catalytic function CuZnSOD in numerous tissues. 46

Under some biochemical circumstances such as after traumatic tissue injury, copper (as well as other metals) can reverse its normal antioxidant role and cause damaging cellular oxidation. This has led some delighted amateur nutritionists to propose restricting dietary copper to reduce damaging oxidation in the body. But controlled animals studies have found the opposite to be true, a reduced copper intake actually increases
deleterious cellular oxidation and promotes a wide variety of the types of degenerative diseases associated with aging. On the other hand, a higher dietary copper intake in animals reduces overall damaging cellular oxidation. 46

**Role of copper in cardiovascular disease.**

Humans and animal studies demonstrated that copper deficiency increases the plasma cholesterol and LDL-cholesterol while decreasing HDL cholesterol, thus increasing the cardiovascular risk.

Klevay theorized that a metabolic imbalance between zinc and copper, but more a copper deficiency than zinc excess, is a major factor in the genesis of coronary heart disease. Other investigators found that copper complexes also can minimize damage to the aorta and heart muscle following myocardial infarction. 47

Severe copper deficiency results in heart abnormalities and damage (cardiomyopathy) in some animals.

A multimember placebo-controlled study found copper supplementation with 3 or 6 mg/day increased the resistance of red blood cells to damaging oxidation indicating that relatively high intakes of copper do not increase the susceptibility of LDL or red blood cells to oxidation. Copper supplementation in humans does not affect the susceptibility of oxidation. 74

Rats on a copper deficient diet had a decrease in aortic integrity that produces eventually aneurysm.
Free Fatty Acids

Free fatty acid (FFA) elevation is known to be associated with diabetes type 2 the metabolically active form of FFA’s are cytosolic long chain acyl-coA esters (LCACOA) and are responsible for cytosolic neutral triglyceride deposition in adipose and non-adipose tissues.

Central obesity is associated with increased cytosolic neutral fat triglyceride stores in adipose and non-adipose tissues such as muscle (skeletal and cardiac), the liver, pancreatic beta cells and possibly, endothelial cells.\(^\text{18}\)

Intra my cell lipid was found to be more highly correlated with insulin resistance than any other commonly measured indicates such as body mass index, waist-to-hip ratio or total body fat. Low insulin sensitivity was accompanied by a marked increase in intramyocellular lipid. The chronic low grade production of ROS produced by respiring mitochondrial is enhanced by excessive cytosolic triglyceride stores and LCACOA esters in non-adipose tissue.\(^\text{19}\)

LCACOA esters exert an inhibitor effect on the adenosine nucleotide translocation with a resultant decrease in ADP available. This decrease in ADP slows the flow of electrons along the electron transfer chain and increases the possibility of having single impaired electrons to eat the superoxide anion (\(\overline{O}_2\)) increasing oxidative mitochondrial stress, thus resulting in a dysfunctional cell. Moreover they suggested that these phenomena not only accelerate the atherosclerotic process but also induce endothelial dysfunction and microabluminurea prior to the development of T2DM and possible beta cell dysfunction and failure.\(^\text{72}\)

It is difficult to completely separate FFA toxicity from the sections which follow on lipoprotein toxicity and triglycerides toxicity as there is a
dynamic relationship between these three in a FLIGHT toxicities. In FFAs are transported by the protein fraction albumin, and lipases are constantly removing the long chain fatty acids from the glycerol backbone of triglycerides at the interface of the capillary endothelial cells creating free fatty acids, which can freely move into cells throughout the body. Intracellularly the FFAs are the added to the glycerol backbone in order to form cytosolic triglycerides stores as neutral fat, or are oxidized for fuel and energy generating ATP. If mitochondrial beta oxidation is over utilized or dysfunctional, the excess may then undergo the toxic non beta non mitochondrial pathway generating toxic FFAs. 75
BIOCHEMISTRY OF LIPID REGULATION

Triglycerides synthesized in the liver are incorporated into the core of VLDL and secreted into the blood stream. This synthesis is enhanced in the postprandial state or when the diet contains excess carbohydrates. In such situations excess fatty acids are generated and incorporated by the liver into triglycerides. Cholesteryl esters are also packed into the VLDL core constituting about one-tenth of triglycerides. When VLDL particles reach the capillaries they are hydrolyzed by the lipoprotein lipase that catabolizes chylomicrons. Removal of VLDL triglycerides from the core results in the formation of smaller VLDL remnant particles called IDLs. The LDL particle is similar to the chylomicron remnant but has a different metabolic fate. Only zero portions of the IDL particles is
catabolised by the liver after the IDLS interact with specific receptors or hepatocyte membranes called LDL receptors. These receptors bind either to apo-B 100 or to apo-E; more likely to the later in the case of IDL. The other portion of IDL particles remains in the plasma, where most of the IDL triglycerides are removed until an LDL particle is formed with a core containing cholesteryl ester and a surface containing B 100 as the only protein component.  

One function of the LDL is to supply cholesterol to cells for membrane synthesis and steroid hormone synthesis. These cells contain protein molecules called LDL receptors on their surface membrane. The number of LDL receptors is thought to be a major determinant of the concentration of LDL in the blood. Exposure of LDL to endothelial cells results in the peroxidation of LDL, which also causes the lipoprotein to become a substrate for the scavenger receptor. Normal LDL does not appear to go through to be a major determinant of the concentration of LDL in the blood. Exposure of LDL to endothelial cells results in the peroxidation of LDL, which also causes the lipoprotein to become a substrate for the scavenger receptor. Normal LDL does not appear to go through the scavenger pathway. Oxidized or modified LDL could thus lead to cholesterol and cholesteryl esters accumulation in macrophages and smooth muscle cells, leading to the development of an atherosclerotic plaque.  

HDL cholesterol, the good cholesterol shows a strong negative association with coronary artery disease and is transported by high density lipoproteins (HDLs). HDL consists of two major subclasses, HDL₂ and HDL₃. High HDL cholesterol which is caused by high levels of HDL₂, provides protection from coronary artery disease. HDL₂ and HDL₃ are not secreted into the secretion are as mature lipoproteins but are assembled in the blood from components derived from the intestine, liver cell membrane and triglycerides rich lipoproteins during lipolysis.
Cholesterol and phospholipids originating from membranes during cellular renewal and death became associated with HDL addition sources of cholesterol and lecithin are the surface components transferred to HDL from Chylomicrons and VLDL during their lipolysis. The cholesterol transferred in this way from HDL to chylomicrons can exit the body via the chylomicrons remnant to the liver, where it is secreted into the bile as cholesterol or bile acids. Cholesterol transferred from HDL to VLDL is the redistributed to other cells through the VLDL - IDL - LDL cascade via the LDL receptors. This process in which HDL returns cholesterol from peripheral tissue to the liver for excretion or for redistribution to other cells has been termed reverse cholesterol transport.

The triglycerides transferred from chylomicrons and VLDL to HDL is hydrolyzed by a triglycerides hydrolase’s called hepatic lipase which is located in the endothelial cells of the liver. Hydrolysis of these triglycerides in the care of HDL leaves only cholesteryl esters in the core and reduces the size of the HDL particle. In this way, the larger HDL$_2$ is converted to the smaller HDL$_3$ particles.

This mechanism is the basis for the well established clinical observation that individuals with permanent or temporary hypertriglyceridemia.
LIPOPROTEIN DISORDER IN DIABETES AND MECHANISM TO CARDIOVASCULAR DISEASE.

For patients with type II diabetes lipid abnormalities are related not only to hyperglycemia but also to the interplay of the insulin resistant state. Patients with type 2 diabetes may have normal LDL levels but elevated level of the very low density triglycerides moiety and reduced HDL levels. The expected elevation in VLDL triglyceride is usually not more than 100 percent. 73

LOW DENSITY LIPOPROTEIN:

Although LDL level in patients with controlled type 2 diabetes are normal the atherogenic properties of LDL are increased. There is glycosylation of both apoprotein B and the phospholipid component of LDL which changes LDL clearance and susceptibility to oxidative modifications. Glycosylation of apoprotein B occurs mainly in the LDL receptor binding area and is directly related to glucose levels. As a result there is impairment in the LDL Receptor mediated uptake and therefore clearance of LDL. Glycosylation also makes LDL more susceptible to oxidative modification. The product generated by the combined glycosylation and oxidation of LDL is more atherogenic than is either glycosylated or oxidized LDL alone. Such LDL molecules are taken up more easily by the aortic intimal cells and macrophages, resulting in the formation of foam cells.

Type 2 diabetic patients with insulin resistance have LDL particles that are small and rich with triglycerides but have little cholesterol in them. These LDL particles increase the risk of CAD independent of the total LDL level, probably because of their increased susceptibility to oxidative modification. High levels of small dense LDL may contribute the increase risk of CAD. 77
VERY LOW DENSITY LIPOPROTEIN:

Diabetes patients have elevated levels of VLDL as a result of increased free fatty acid mobilization and high glucose levels. There is an increase in triglycerides product by the liver, which results in large. Triglyceride rich VLDL particles. The size of these VLDL particles, which is dependent primarily on the amount of triglycerides available, is an important factor in determining their eventual fate. The conversion of large VLDL particles to LDL is not efficient. Therefore, they are cleared from circulation by other pathways. Since the removal of VLDL by lipoprotein lipase also is affected the level of VLDL triglycerides rises. Furthermore, the abundance of large triglyceride rich VLDL is associated with an increase in small, dense, atherogenic LDL particles. Elevated triglyceride level is associated with increased risk for CAD in diabetic patients.  

HIGH DENSITY LIPOPROTEIN:

Low HDL level is a strong risk factor for the development of CAD in both diabetic and nondiabetic patients. There is decreased production and increased catabolism of HDL in diabetes. The decreased HDL (LPL) activity. The failure of LDL to efficiently catabolize VLDL results in reduced availability of surface components for HDL results form the hyper triglyceridemia of diabetes, producing triglyceride rich HDL₂ that is prone to catabolism by liver enzymes.