INTRODUCTION

I. Metabolic Syndrome

Metabolic syndrome (MetS) is a constellation of physiological and biochemical abnormalities characterized by diabetes or high fasting glucose, central obesity, abnormal cholesterol and triglyceride (TG) levels, and hypertension.[1,2] This clustering of abnormalities is frequently seen and attributed to people's dietary habits. One in approximately every 4 or 5 adults has developed MetS depending on the environmental conditions and daily lifestyle habits of the country where he or she resides. The prevalence of MetS is rapidly increasing worldwide not only in industrialized countries but also in developing countries associated with an increase in high-calorie food intake.[3] It affects about one in four people in the Middle East, and prevalence increases with age.[4] MetS affects 27% of the population in India, nearly 30% in Europe,[2] and more than 40% in the US.[5] MetS has been accepted worldwide as a clinical marker for earlier detection of cardiovascular disease and type 2 diabetes.[6,7] People with MetS are estimated to have twice the risk of developing cardiovascular disease compared to healthy individuals and a five-fold increased risk of type 2 diabetes.[1,7] However, the underlying pathophysiological processes leading to its development are unclear and there is confusion over its conceptual definitions and criteria, allowing the medical controversy over MetS to continue. An increase in total body fatness and preferential upper body accumulation of fat is independently related to insulin resistance (IR). Obese women with a greater proportion of upper body fat tend to be more insulin-resistant, hyperinsulinemic, glucose-intolerant, and dyslipidemic than obese women with a greater proportion of lower body fat. Therefore, the distribution of body fat is an important correlate of MetS. The term "metabolic" refers to the biochemical processes involved in the body's normal functioning. Risk factors are behaviors or conditions that increase a disease.[8]

Risk factors

The following factors increase chances of developing MetS:

Age:

The risk of MetS increases with increasing age, affecting less than 10% of people in their 20s and 40% of people in their 60s. However, some research shows that about one in eight school children has three or more components of MetS. Other research has also identified an association between childhood MetS and adult cardiovascular disease decades later.[9]

Obesity:

A body mass index (BMI), a measure of percentage of body fat based on height and weight, greater than 25 increases the risk of MetS. Excess fat in the abdominal area is a greater risk factor for heart disease than excess fat in other parts of the body, such as on the hips. Therefore, so does abdominal obesity, i.e., having an apple shape rather than a pear shape.[10]

History of diabetes:

There is a greater likelihood of MetS if a family history of type 2 diabetes or a history of diabetes during pregnancy (gestational diabetes) is present.[11]

Other diseases:

A diagnosis of fatty liver, gallstones, breathing problems during sleep, cardiovascular disease, or polycystic ovary syndrome (such metabolic problems affect a woman's hormones and reproductive system) also increases the risk of MetS.[8]

Diagnosis of MetS

The risk factors seen in MetS include: IR, obesity (especially abdominal obesity), high blood pressure, high fasting glucose or hyperglycemia, and lipid abnormalities. There must be at least three of the following five metabolic risk factors for an individual to be diagnosed with MetS:

a) A higher triglycerides (TG) level (≥ 150 mg/dL than normal). TGs are a kind of fat and hang out in fat cells but they also circulate in the blood.

b) A lower high-density lipoprotein (HDL) cholesterol level (< 50 mg/dL for women and < 40 mg/dL for men than normal). HDL is sometimes called "good" cholesterol because it helps in removing cholesterol from arteries. It cleans out the low-density lipoproteins (LDLs) or "bad" cholesterol from blood. When there are not enough HDLs, the LDLs can run rampantly, causing plaque to build up in artery walls and put a strain on the heart and circulatory system. A low HDL cholesterol level increases the risk of coronary heart disease.

c) Higher blood pressure (≥ 130/85 mmHg than normal). Blood pressure pushes the blood against the arterial walls as the heart pumps out blood. If this pressure rises and remains high, it can damage the heart and lead to plaque buildup.

d) Higher fasting blood glucose level (more than 100 mg/dL). Fasting blood glucose between 100 and 110 mg/dL is a sign of MetS. A mildly high blood sugar (between 100 and 125 mg/dL) may be an early predictor of diabetes. About 85% of people who have type 2 diabetes, the most common type of diabetes, also have MetS. These people have a much higher risk of heart disease than the 15% of people who have type 2 diabetes but not MetS.

e) Large waist circumference (≥ 88 cm for women and ≥ 102 cm for men). Having a large waist circumference or apple-shaped figure means that there is excess weight around the waist (abdominal obesity), representing an increased risk of heart disease and other health problems.[12]

Throughout the years, several definitions of MetS have been proposed, emphasizing IR or abdominal/visceral obesity. However, the 4 main definitions are from the World Health Organization (WHO) definition (1998)[13], the Adult Treatment Panel III (ATPIII) Report (2001)[14], the European Group for the Study of IR (EGIR) (1999)[15], and the International Diabetes Federation (IDF) consensus[16] on MetS **(Table 1).** The definition of MetS according to the National Cholesterol Education Program (NCEP) was slightly updated by the American Heart Association (AHA) and National Heart, Lung, and Blood Institute (NHLBI) in (2005)[17] and the same year IDF proposed a new definition[16] based

on clinical criteria. The two are very similar and should presumably identify many of the same individuals as having MetS. This modification of the IDF and ATP III definitions increased the emphasis on abdominal obesity as the core feature of MetS. The two differences are that the IDF definition excludes any subject lacking an increased waist circumference while the NCEP definition diagnoses MetS based on other criteria. Secondly, the IDF definition uses physical feature-specific cut-off points for waist circumference, while the NCEP definition uses only one set of cutoff points for waist circumference regardless of physical features. Abdominal obesity measured by waist circumference is an essential requirement for the diagnosis, while other variables featured in the ATP III definition have changed slightly.[18] **(Table 1)**.

WHO criteria:

According to WHO criteria (1998), the presence of MetS requires the presence of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or IR, and at least two of the above factors.[13]

EGIR criteria:

The EGIR definition (1999) requires IR in the top 25% of the fasting insulin values among non-diabetic individuals and two or more risk factors.[15]

NCEP-ATP III criteria:

The NCEP-ATP III definition (2001) requires at least three of the risk factors.[14]

IDF:

According to the International Diabetes Federation (IDF) definition: a person is defined as having the metabolic syndrome if he has central obesity (waist circumference≥94cm for European men and ≥80cm for European women) with ethnicity specific values for other groups plus any two of the following four additional factors:

a) Raised triglycerides (TG) level: ≥150mg/dL (1.7mmol/L); or specific treatment for this lipid abnormality.

b) Reduced high-density lipoprotein (HDL) cholesterol <40mg/dL (1.03mmol/L) in males and <50mg/dL (1.29mmol/L) in females; or specific treatment for this lipid abnormality.

c) Raised blood pressure: systolic blood pressure ≥130mmHg or diastolic blood pressure ≥85mmHg; or treatment of previously diagnosed hypertension.

d) Raised fasting plasma glucose ≥100mg/dL (5.6mmol/L); or previously diagnosed type 2 diabetes. If above 5.6mmol/L or 100mg/dL, oral glucose tolerance test is strongly recommended to identify impaired glucose tolerance (IGT) or undiagnosed diabetes, but is not necessary to define the presence of the MetS.[16]

Table 1. Diagnostic criteria for metabolic syndrome (8)

S. No.	Criteria for metabolic syndrome	Obesity		Dyslipidemia		Blood pressure (Systolic and Diastolic)	Glucose	Insulin resistance	Other
		Male	Female	Male	Female				
1.	WHO (5th or 6th + ≥ 2 criteria), 1999	WHR > 0.90 and/or BMI > 30 kg/m^2	WHR > 0.85 and/or BMI > 30 kg/m^2	TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 35 mg/dL (< 0.9 mM)	TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 39 mg/dL (<1.0 mM)	≥ 140/90 mmHg	T2DM, impaired glucose tolerance, impaired fasting glucose	IR measured under hyperinsulinemiceu glycemic conditions	Urinary albumin excretion rate ≥ 20 µg/min or albumin: creatinine ratio ≥ 30 mg/g
2.	EGIR (5th + ≥ 2 criteria), 1999	WC ≥ 94 cm	WC ≥ 80 cm	TG ≥ 177 mg/dL (≥ 2.0 mM); HDL-C < 39 mg/dL (<1.0 mM)		≥ 140/90 mmHg or on medication	Fasting glucose > 110 mg/dL (≥ 6.1 mM)	IR	–
3.	NCEP-ATP III (≥ 3 criteria), 2001	Abdominal obesity WC ≥ 102 cm or 40 inches	Abdominal obesity WC > 88 cm or 36 inches	TG ≥ 150 mg/dL; HDL-C < 40 mg/dL or on therapy	TG ≥ 150 mg/dL; HDL-C < 50 mg/dL or on therapy	≥ 130/85 mmHg or on therapy	Fasting glucose > 110 mg/dL (≥ 6.1 mM)	–	–
4.	AHA/NHLBI or Updated NCEP criteria, 2005	WC ≥ 102 cm (Asian ≥ 90) or 40 inches	WC > 88 cm (Asian ≥ 80) or 36 inches	TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 40 mg/dL (1.0 mM) or on therapy	TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 50 mg/dL (1.0 mM) or on therapy	≥ 130/85 mmHg or on medication	Fasting glucose > 100 mg/dL (5.6 mM)	–	–
5.	IDF (1st + ≥ 2 other criteria), 2005	Ethnicity-specific WC (≥ 90 cm for men and ≥ 80 cm for women) for Indian subjects (If BMI is > 30 kg/m^2, central obesity can be assumed and WC does not need to be measured)		TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 40 mg/dL (1.03 mM) or on therapy	TG ≥ 150 mg/dL (≥ 1.7 mM); HDL-C < 50 mg/dL (1.3 mM) or on therapy	≥ 130/85 mmHg or on therapy	Fasting glucose > 100 mg/dL (5.6 mM) or DM	–	–

Abbreviation: Waist circumference (WC).

Insulin resistance – A key aspect of MetS

A key aspect of MetS is IR. In the body's attempt to counterbalance IR, extra insulin is produced, leading to increased insulin levels. The increased insulin levels can directly or indirectly lead to the characteristic metabolic abnormalities seen in patients. Frequently, the IR will progress to overt type 2 diabetes, further increasing the risk of cardiovascular complications.[19]

Risk of developing CVD in individuals with MetS

The relative risk of developing Cardiovascular disease (CVD) associated with MetS as defined by NCEP-ATP III or by other organizations has increased 2- to 5-fold in both men and women and in various populations.[20,21,22] Data from a Quebec cardiovascular study of individuals with several risk factors associated with MetS were characterized by a tremendous increase in the relative risk of CVD compared to individuals who had only one or none of the risk factors. For example, non-diabetic men who had hyperapobetalipoproteinemia (hyperapo B), small dense LDL, and hyperinsulinemia simultaneously had a 20-fold increase in the risk of CVD over 5 years compared to men who had none of these metabolic perturbations.[23]

Another study showed that the risk associated with hypertriglyceridemia was modulated, to a significant extent, by the presence or absence of other components of MetS. For example, men with marginally increased plasma TG levels (above 1.6 mmol/L or 141 mg/dL) but with no other features of MetS had a 3-fold increase in the risk of CVD compared to men with normal plasma TG levels.[24] The risk of CVD increased 13-fold for subjects with moderate hypertriglyceridemia who also had hyperapo B, reduced HDL-C levels, and increased insulin concentrations.[24] These data clearly indicate that MetS, irrespective of its definition, may be associated with a significantly increased risk of CVD. Therefore, components of MetS may significantly contribute to this increased risk of CVD.[25]

Etiology

The cause of MetS is unknown. Its pathophysiology is extremely complex and has been only partially elucidated. Most patients are older, obese, sedentary, and have a degree of IR. The most important factors are, in order: i) aging, ii) genetic makeup, and iii) daily lifestyle and habits (e.g. low physical activity and excess caloric intake).

There is debate regarding whether obesity or IR is the cause of MetS or if obesity and IR are consequences of more far-reaching metabolic dysfunction. A number of markers of systemic inflammation, including C-reactive protein (CRP), often increase, as do interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), resistin, leptin, and adiponectin.[26]

Pathophysiology

Obesity and metabolic abnormalities were known to be associated with poor cerebrovascular outcomes when the concept of MetS first appeared. In 1995, Dr. G Reaven noticed that those outcomes were found in people who had hyperinsulinemia, high TG, low HDL cholesterol, and hypertension, all of which were considered factors for the

development of CVD. Many earlier studies measured only serum total cholesterol regardless of LDL-cholesterol levels, although most total cholesterol consists of LDLs. Thus, the robust relationship between total cholesterol and coronary heart disease found in epidemiological studies strongly implies that an elevated LDL is a highly prevalent and powerful risk factor. Epidemiological investigations of human populations point to high levels of LDL cholesterol as being atherogenic lipoprotein.[27]

Previous research demonstrated that adipose tissue plays an important role in energy regulation via endocrine, paracrine, and autocrine signals[28] and various factors known as 'adipokines' released by adipose cells cause IR. These adipokines are defined as insulin antagonists (TNF-α, IL-6, and resistin) and insulin sensitizers (leptin and adiponectin). These adipokines markedly affect peripheral functions and influence the pathogenesis of obesity-related disease, particularly diabetes and cardiovascular disorders.[29]

Visceral fat accumulation is found to be specifically associated with metabolic alteration of obesity in both men and women. Increasing accumulation of visceral fat leads to the overproduction of some adipokines such as IL-6, TNF-α and resistin, decreasing insulin action in muscles and/or the liver, while some adipokines like leptin and adiponectin have a beneficial effect on energy balance, insulin action, and vasculature. Leptin regulates energy balance and has an insulin-sensitizing effect. These beneficial effects are reduced in obesity due to leptin resistance. Adiponectin increases insulin action in muscles and the liver and has an anti-atherogenic effect. Conversely, excessive production of other adipokines is deleterious. The increased levels of circulating adipokines associated with visceral obesity may be attributed to production by ectopic adipose tissue. Adiponectin is the only known adipokine with circulating levels that decrease as a result of visceral obesity while levels of other adipokines increase. This dysregulation of adipokine production may promote obesity-linked metabolic disorders and CVD.[30]

The accumulation of visceral fat hastens the release of non-esterified fatty acids (NEFAs), resulting in greater lipolytic activity in obese individuals and increasing NEFA levels in systemic circulation. This increased release of NEFAs into the portal circulation stimulates hepatic glucose production and reduces hepatic insulin clearance, ultimately resulting in insulin resistance, hyperinsulinemia, and hyperglycemia.[31] When obesity develops, abnormal production of these adipokines by more visceral fat contributes to a proinflammatory state. This state of inflammation is likely to contribute to the health problems associated with obesity such as dyslipidemia, insulin resistance, and atherosclerosis. In contrast to these adipokines, levels of, the insulin sensitizing and anti-inflammatory adipokine, adiponectin are reduced in visceral obesity,[32] and this may further exacerbate the state of low-grade systemic inflammation. In the liver, adiponectin increases insulin sensitivity by lowering NEFA uptake, increasing fatty acid oxidation, and reducing hepatic gluconeogenesis and very low density lipoprotein (VLDL) production. In muscle, adiponectin stimulates glucose uptake and fatty acid oxidation.[33] Therefore, the altered expression of adipokines associated with visceral obesity induces a state of low-level systemic inflammation and dyslipidemia, eventually leading to atherosclerosis. In visceral obesity, dysregulated production of specific adipokines may contribute to hypertension and CVD. Hypertension is a common manifestation of the metabolic disturbances associated with insulin resistance and hyperinsulinemia, a key factor for MetS. Studies using the hyperinsulinemic-euglycemic clamp technique have demonstrated

that hyperinsulinemia occurs in hypertension as a compensatory response to reduced insulin-stimulated glucose uptake by skeletal muscle.[34,35] Adipocytes synthesize and release several factors that have been linked to blood pressure control, including adiponectin, leptin, and resistin. Increasing evidence suggests that aberrant production and release of such adipokines as adiponectin, leptin, and resistin by adipocytes may contribute to the high prevalence of hypertension in visceral obesity. Therefore, adipokines are potential causes of insulin resistance, endothelial dysfunction, and hypertension and reflect the role visceral obesity plays as a causal factor in metabolic and vascular disease.[36]

Treatments and drugs for MetS

Tackling one of the risk factors for MetS is tough – taking them all on might seem overwhelming. That says, healthy or aggressive lifestyle changes and, in some cases, medication can improve every component of MetS. Lifestyle changes include losing weight, getting more regular physical activity, following a heart healthy diet, quitting smoking, reducing one's blood pressure, and improving one's cholesterol and blood sugar levels. The main focus of treating MetS is managing the risk factors that are under control, such as being overweight or obese, having an inactive lifestyle, or consuming an unhealthy diet. These changes are the key factors in reducing metabolic risk.[37]

Exercise:

More activity means more of a benefit. The four main types of physical activity are aerobic, muscle strengthening, bone strengthening, and stretching. Physical activity can be light, moderate, or vigorous. Doctors recommend 30 to 60 min of moderate-intensity exercise, such as brisk walking, every day.[38,39]

Losing weight:

In general, people who have MetS and are overweight or obese and who then lose as little as 7-10% of their body weight can reduce their insulin levels and blood pressure and decrease their risk of diabetes.[40,41]

Lipid abnormalities:

While the lipid abnormalities seen with MetS (low HDL, high LDL, and high TGs) respond nicely to weight loss and exercise, drug therapy is often required. Treatment should be aimed primarily at reducing LDL levels according to specific recommendations. Once reduced LDL targets are reached, efforts should be made to reduce TG levels and raise HDL levels.[42]

Clotting disorders:

People with MetS can have several coagulation disorders that facilitate the forming of blood clots within blood vessels. These blood clots are often a precipitating factor for a heart attack. Excessive blood clotting is a condition that often occurs with MetS.[43]

Eating healthy:

A heart healthy diet is an important part of a healthy lifestyle. The Dietary Approaches to Stop Hypertension (DASH) diet and the Mediterranean Diet, like many healthy-eating plans, limit unhealthy fats and emphasize fruits, vegetables, fish, and whole grains. Both of these dietary approaches have been found to offer important health benefits –in addition to weight loss– for people who have components of MetS.[44,45,46]

Stopping smoking:

Smoking can increase the risk of heart disease and heart attacks and worsen other heart disease risk factors.[47] Smoking cigarettes increases IR and worsens the health consequences of MetS.[48] A doctor should be consulted for help in quitting cigarettes. The doctor can help the individual to monitor weight and blood glucose, cholesterol, and blood pressure levels in order to ensure that lifestyle modifications are working.[49,50]

Medication:

If goals cannot be achieved with lifestyle changes, a doctor may also prescribe medications to lower blood pressure with diuretics or angiotensin-converting enzyme (ACE) inhibitors, reduce unhealthy cholesterol levels with statins, fibrates, or nicotinic acid, or provide help in losing weight. High blood sugar is treated with oral medicines such as metformin, insulin injections, or both. Low-dose aspirin can help reduce the risk of blood clots, especially for people at high risk of heart disease.[51]

II. Statins

Statins (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors or HMG-CoA reductase inhibitors) are a class of drug used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases (CVD), and statins are therefore used in the prevention of these diseases. Some types of statins are naturally occurring, and can be found in such foods as oyster, mushrooms and red yeast rice.[52]

Randomized controlled trials have shown that they are most effective in those already suffering from cardiovascular disease (secondary prevention), but they are also advocated and used extensively in those without previous CVD but with elevated cholesterol levels and other risk factors (such as diabetes and high blood pressure) that increase a person's risk. A number of statins are on the market: atorvastatin (Lipitor and Torvast), fluvastatin (Lescol), lovastatin (Mevacor, Altocor, Altoprev), pitavastatin (Livalo, Pitava), pravastatin (Pravachol, Selektine, Lipostat), rosuvastatin (Crestor) and simvastatin (Zocor, Lipex).[53]

Comparative effectiveness

An independent analysis has been done to compare atorvastatin, pravastatin and simvastatin, based on their effectiveness against placebos. It found that, at commonly prescribed doses, there are no statistically significant differences amongst statins in reducing cardiovascular morbidity and mortality. The CURVES study (A Comparison of LDL-C Lowering Among Statins), which compared the efficacy of different doses of atorvastatin, simvastatin, pravastatin, lovastatin, and fluvastatin for reducing LDL and total cholesterol in patients with hypercholesterolemia, found that atorvastatin was more effective without increasing adverse events.[52,54]

Statins differ in their ability to reduce cholesterol levels. Doses should be individualized according to patient characteristics such as goal of therapy and response. After initiation and/or dose changes, lipid levels should be analyzed within 1–3 months and dosage adjusted accordingly, then every 6–12 months afterwards. A link between cholesterol and cardiovascular disease, known as the lipid hypothesis, had already been suggested.[55]

Effects of Statins

Statins exhibit action beyond lipid-lowering activity in the prevention of atherosclerosis. The ASTEROID trial (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden) showed direct ultrasound evidence of atheroma regression during statin therapy. Researchers hypothesize that statins prevent cardiovascular disease via four proposed mechanisms:

1. Improve endothelial function.
2. Modulate inflammatory responses.
3. Maintain plaque stability.
4. Prevent thrombus formation.

Cholesterol is the main constituent of atheroma, the fatty lumps in the wall of arteries that occur in atherosclerosis and, when ruptured, cause the vast majority of heart attacks. Treatment consisted mainly of dietary measures such as a low-fat diet, and poorly tolerated medicines such as clofibrate, cholestyramine and nicotinic acid. Cholesterol researcher Daniel Steinberg wrote that while the Coronary Primary Prevention Trial of 1984 demonstrated that cholesterol lowering could significantly reduce the risk of heart attacks and angina, physicians, including cardiologists, remained largely unconvinced.(56)

Mechanism of action

Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway **(Figure 1)**. HMG-CoA reductase inhibitors are a group of prescription drugs used to lower cholesterol, a white waxy substance that can stick to the inside of blood vessels, resulting in clogged arteries, heart disease, and strokes. These medicines work by slowing down the body's ability to make cholesterol. Because statins are similar to HMG-CoA on a molecular level they take the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate, the next molecule in the cascade that eventually produces cholesterol, as well as a number of other compounds. This ultimately reduces cholesterol via several mechanisms.(57)

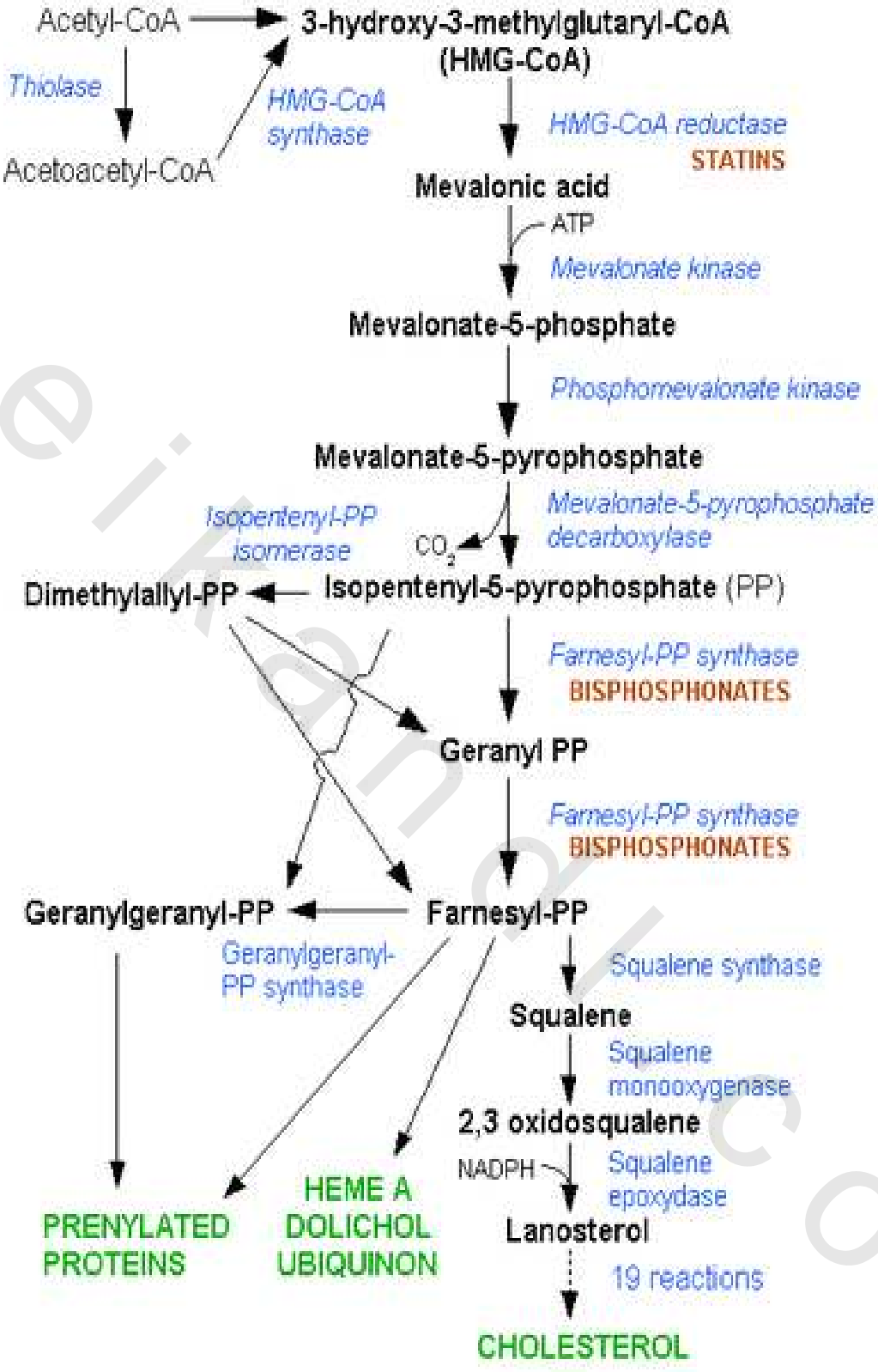


Figure (1): The HMG-CoA reductase pathway, which is blocked by statins via inhibiting the rate-limiting enzyme HMG-CoA reductase.[57]

a) Inhibiting cholesterol synthesis:

By inhibiting HMG-CoA reductase, statins block the pathway for synthesizing cholesterol in the liver **(Figure 2)**. This is significant because most circulating cholesterol comes from internal manufacture rather than the diet. When the liver can no longer produce cholesterol, levels of cholesterol in the blood will fall. Cholesterol synthesis appears to occur mostly at night, so statins with short half-lives are usually taken at night to maximize their effect. Studies have shown greater LDL and total cholesterol reductions in the short-acting simvastatin taken at night rather than the morning, but have shown no difference in the long-acting atorvastatin.[57]

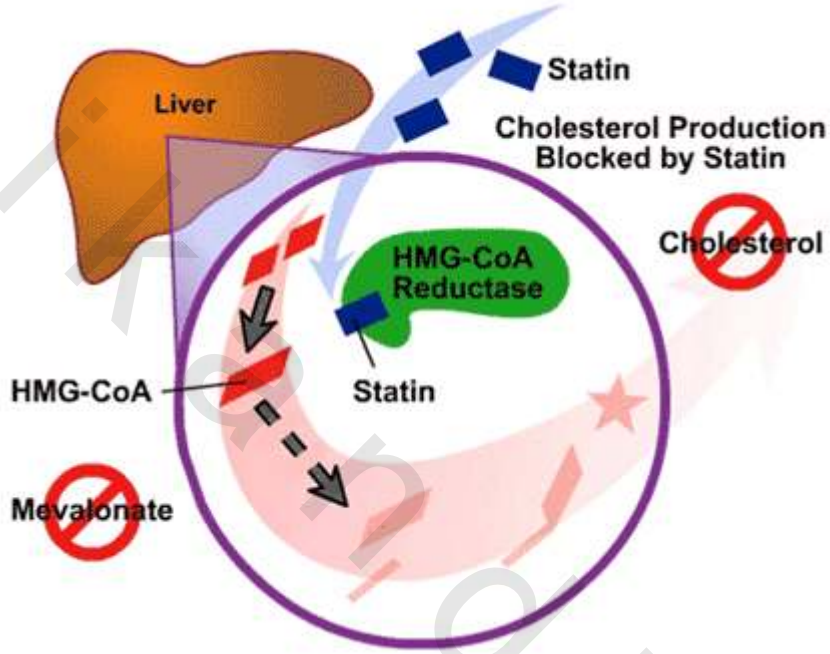


Figure (2): Statins block the pathway for synthesizing cholesterol in the liver.[57]

b) Increasing LDL uptake:

Liver cells sense the reduced levels of liver cholesterol and seek to compensate by synthesizing LDL receptors to draw cholesterol out of the circulation. This is accomplished via protease enzymes that cleave a protein called "membrane-bound sterol regulatory element binding protein", which migrates to the nucleus and causes increased production of various other proteins and enzymes, including the LDL receptor. The LDL receptor then relocates to the liver cell membrane and binds to passing LDL and VLDL particles (the "bad cholesterol" linked to disease). LDL and VLDL are drawn out of circulation into the liver where the cholesterol is reprocessed into bile salts. These are excreted, and subsequently recycled mostly by an internal bile salt circulation.[57]

Atorvastatin

Atorvastatin is a cholesterol-lowering medication that blocks the production of cholesterol (a type of lipid) in the body. Atorvastatin reduces low-density lipoprotein (LDL) cholesterol and total cholesterol in the blood. Lowering cholesterol levels can help to prevent heart disease and hardening of the arteries, conditions that can lead to heart attack, stroke, and vascular disease. Atorvastatin is used to treat high cholesterol. Atorvastatin is also used to lower the risk of stroke, heart attack, or other heart complications in people with coronary heart disease or type 2 diabetes.[58]

Atorvastatin is used to treat dyslipidemias, which are disorders characterized by abnormal levels of lipids in the blood. Specifically, atorvastatin is used along with dietary therapy to decrease elevated serum: total cholesterol, low-density lipoprotein cholesterol (LDL-C; so-called "bad" cholesterol), apolipoprotein B (apo B), and triglyceride concentrations. It is also used to increase concentrations of high-density lipoprotein cholesterol (HDL-C; the so-called "good" cholesterol).[59,60]

Familial hypercholesterolemia is an inherited condition characterized by high cholesterol levels. Atorvastatin is used to lower cholesterol in individuals as young as ten years who have familial hypercholesterolemia (LDL-C levels higher than 190 mg/dl), or higher than 160 mg/dl and who have a family history of coronary heart disease (CHD).[61]

The lipid-lowering effect of atorvastatin reduces the risk of CHD. Therefore, atorvastatin is used as primary prevention of heart attack, stroke, or angina in people who have multiple risk factors for CHD: age, smoking, high blood pressure, low HDL-C, or a family history of early CHD. Primary prevention refers to interventions that prevent the first occurrence of a disease or condition. Primary prevention of CHD is done for people who have no clinical evidence of cardiovascular disease but who are at risk. Atorvastatin is also used in primary prevention of cardiovascular events (e.g., heart attack, stroke) in people with type 2 diabetes.[60,62]

Atorvastatin is also used as secondary prevention. Secondary prevention refers to interventions that protect against recurrence of a disease or condition. Secondary prevention with atorvastatin is done in people who have CHD. In these people, atorvastatin is used to reduce the risk of heart attack, stroke, or hospitalization for congestive heart failure (CHF). Atorvastatin has also been shown to slow the progression of coronary atherosclerosis in patients with CHD.[63]

Atorvastatin is taken as tablets by mouth once a day, with or without food. Tablets containing 10 mg, 20 mg, 40 mg, or 80 mg are available. Low doses may be given initially, with gradual escalation depending on changes in blood lipid concentrations and the presence or absence of side effects.[63,64]

III. Oxygen Free Radicals

Oxygen, while indisputably essential for life, can also participate in the destruction of tissue and/or impair its ability to function normally.[65,66] Oxygen-free radicals (OFR), or more generally, reactive oxygen species (ROS) are products of normal cellular metabolism. It has been estimated that the average person has around 10,000–20,000 free radicals attacking each body cell each day.

In some cases, ROS are produced specifically to serve essential biological functions, whereas in other cases, they represent byproducts of metabolic processes.[67] Despite the cell's antioxidant defence system to counteract oxidative damage from OFR, radical-related damage of DNA and proteins have been proposed to play a key role in the development of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders and others.[68,69] All ROS have the potential to interact with cellular components including DNA bases or the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks.[70] Oxygen radicals can also oxidise lipids or proteins thus generating intermediates that react with DNA by forming adducts.[71] Some oxidative DNA lesions are pro-mutagenic and oxidative damage is proposed to play a role in the development of certain cancers.[72]

Electronic structure of oxygen radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals.[73] This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems.[74] Molecular oxygen (dioxygen) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical ($O_2^{\bullet -}$).[75]

Sources and properties of oxygen radicals

Superoxide radical

With the exception of unusual circumstances such as ionizing radiation, ultraviolet light, and other forms of high energy exposure, free radicals are produced in cells generally by electron transfer reactions, which can be enzymatically mediated or non-enzymatically mediated. Cellular sites of superoxide generation and its compartmentalization are shown in **Figure 3**. The major source of free radicals under normal circumstances is the electron leakage that occurs from electron transport chains, such as those in the mitochondria and endoplasmic reticulum, to molecular oxygen, which generates superoxide.[76]

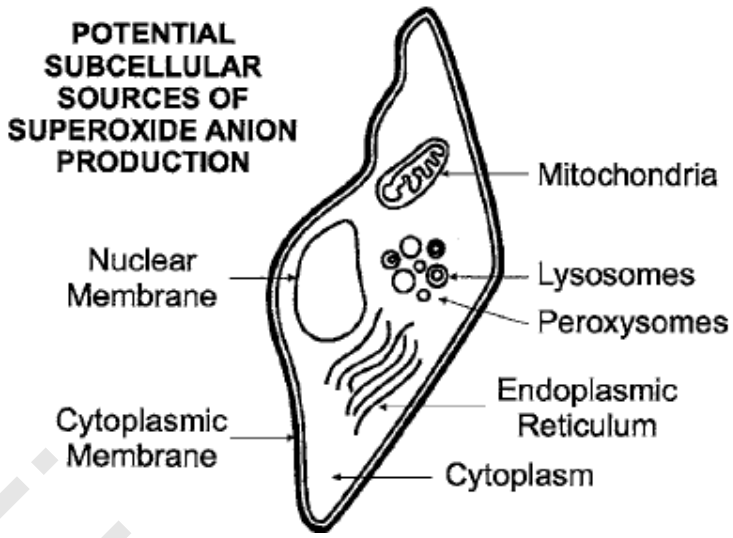


Figure (3): Cellular sites of superoxide generation and its compartmentalization.[77]

Intracellular sources of superoxide/hydrogen peroxide

Mitochondria:

The production of superoxide, the most common radical in biological systems, occurs mostly within the mitochondria of a cell. The mitochondrion is a small intracelullar organelle which is responsible for energy production and cellular respiration. Mitochondria accomplish this task through a mechanism called the "electron transport chain (ETC)." In this mechanism, electrons are passed between different molecules, with each pass producing useful chemical energy. Oxygen occupies the final position in the electron transport chain. Even under ideal conditions, some electrons "leak" from the electron transport chain.[78,79] These leaking electrons interact with oxygen to produce superoxide radicals, so that under physiological conditions, about 1–3% of the oxygen molecules in the mitochondria are converted into superoxide.[80-83]

The primary site of radical oxygen damage from superoxide so produced is mitochondrial DNA (mtDNA). The cell repairs much of the damage done to nuclear DNA, but mtDNA cannot be readily fixed. Therefore, extensive mtDNA damage accumulates over time and shuts down mitochondria, causing cells to die and the organism to age.[77]

Cytochrome P-450:

The phase I cytochrome P-450 is the terminal component of the mono-oxygenase system found within the endoplasmic reticulum of most mammalian cells.[84] The main role of cytochrome P-450 is that of detoxification of foreign compounds into less toxic products. In order to perform this detoxification function, this enzyme uses oxygen to oxidize the foreign compounds. This enzyme is also involved in hydroxylation reactions, which also remove/inactivate toxic compounds in the body and are heavily involved in steroidogenesis. During these oxidation and hydroxylation reactions electrons may be 'leaked' onto oxygen molecules, forming superoxide radicals ($O_2^{\bullet -}$).[84]

Cytoplasmic oxidases:

Cytochrome oxidase is found at the end of the electron transport chain in the mitochondrion.[85] The electron transport chain uses oxygen to oxidise nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) during aerobic respiration to generate energy. Cytochrome oxidase adds four electrons onto a molecule of dioxygen in a series of reduction reactions **(Figure 4)**. Each of these reduction reactions may potentially have superoxide radicals as a byproduct, which are potentially damaging.

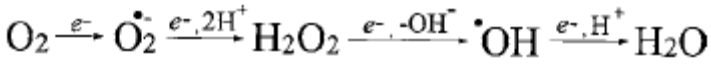


Figure (4): The active oxygen system: four single-electron reduction steps from molecular oxygen to water.[77]

Xanthine oxidase:

Xanthine oxidase (XO) is a highly versatile enzyme that is widely distributed among species (from bacteria to man) and within the various tissues of mammals.[86] XO is an important source of OFR. It is a member of a group of enzymes known as molybdenum iron–sulfur flavin hydroxylases and catalyses the hydroxylation of purines.[87,88] In particular, XO catalyses the reaction of hypoxanthine to xanthine and xanthine to uric acid **(Figure 5)**. In both steps, molecular oxygen is reduced, forming the superoxide anion in the first instance and hydrogen peroxide in the second.

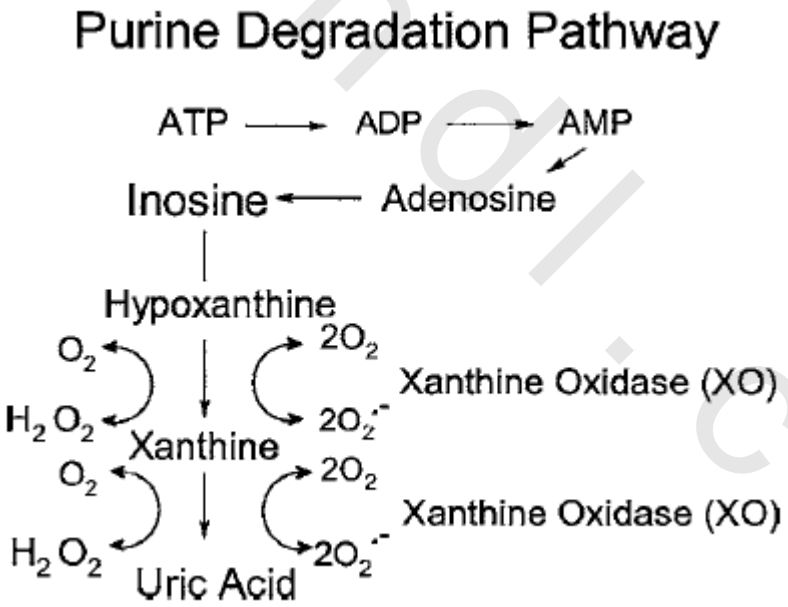


Figure (5): Superoxide generation within enzymatic oxidations as, e.g. in the biological degradation of purines.[77]

Microsomes and peroxisomes:

Microsomes are responsible for 80% of the Hydrogen peroxide (H_2O_2) produced in vivo at 100% hyperoxia sites.[89] Peroxisomes are known to produce H_2O_2, but not $O_2^{\bullet -}$, under physiological conditions.[90] Although the liver is the primary organ where peroxisomal contribution to the overall H_2O_2 production is significant, other organs that contain peroxisomes are also exposed to these H_2O_2-generating mechanisms. Peroxisomal oxidation of fatty acids has recently been recognized as a potentially important source of H_2O_2 production with prolonged starvation.

Extracellular sources of superoxide

Membrane NADPH oxidase(s):

The NADPH oxidases are a group of plasma membrane-associated enzymes found in a variety of cells of mesodermal origin. The most thoroughly studied of these is the leukocyte NADPH oxidase, which is found in phagocytes and B-lymphocytes. If a phagocytic cell such as the neutrophil is exposed to a stimulus, it has the ability of recognizing the foreign particle and undergoing a series of reactions called the respiratory burst.[91] The respiratory burst enables the cell to provide oxidising agents for the destruction of the target cells. When NAD(P)H oxidase is activated, it takes nicotinamide adenine dinucleotide phosphate (NAD(P)H) from the cytoplasm and passes electrons to O_2, producing superoxide within the plasma membrane or on its outer surface[92,93]

$$2O_2 + NAD(P)H \rightarrow 2\ O_2^{\bullet -} + NADP^+ + H^+ \qquad (1)$$

In chronic granulomatous disease, there is a hereditary defect of NAD(P)H oxidase enzyme resulting in decreased production of superoxide and the patient's leukocytes cannot kill the pathogens.[94]

As summarized in **Figure 6**, granulocytes and other phagocytic cells possess a membrane NADPH oxidase, which takes reducing equivalents from the hexose monophosphate shunt and transfers these to molecular oxygen to produce superoxide and other active oxygen species. A further myeloperoxidase converts peroxide produced in this system to microbiocidal products, probably including hypochlorite.[95] Production of activated products by this system probably plays a key role in cell-mediated immunity and microbiocidal activity. There is evidence for similar systems in T-lymphocytes, platelets, and mucus. An NADPH oxidase of non-inflammatory cells may have a role in mediating cyclic nucleotide metabolism.[95]

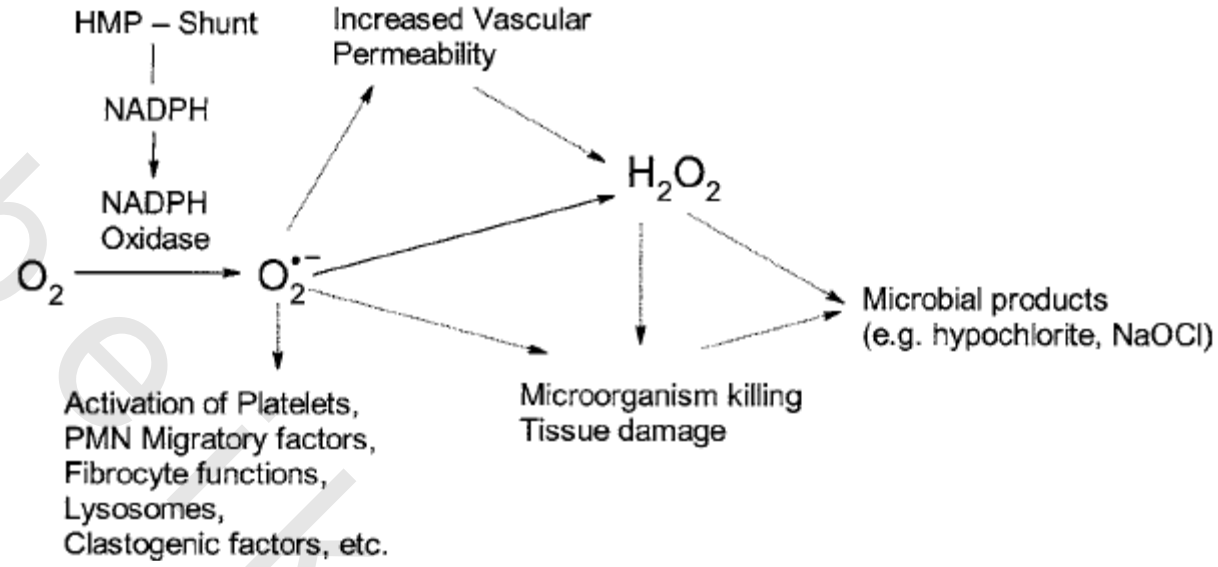


Figure (6): Role of active oxygen species in inflammation.[77]

Properties of superoxide

Despite the moderate in vitro chemical reactivity of superoxide in aqueous solution, it has been proven to be able to do a considerable degree of in vivo damage. However, superoxide can undergo a dismutation reaction[96]

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (2)$$

This reaction is accelerated in biological systems by the superoxide dismutase (SOD) enzymes by about 4 orders of magnitude. It should be noted that SOD enzymes work in conjunction with H_2O_2-removing enzymes, such as catalases and glutathione (GSH) peroxidases.[97]

a. Superoxide as a reducing agent:

One route to generate hydroxyl radicals in biological systems is mediated by $O_2^{\bullet -}$ through a metal-catalyzed Haber–Weiss reaction;[98-101]

$$O_2^{\bullet -} + H_2O_2 \xrightarrow{Fe2+} O_2 + OH^{\bullet} + OH^- \quad (3)$$

which is an overall reaction and consists of two steps:

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (4)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (5)$$

It is proposed that the above mechanism in vivo proceeds through catalytic activity of metal ions bound to biomolecules (Biol) in the close proximity of the target molecule (DNA)

$$\text{Biol-M}^{n+1} + O_2^{\bullet -} \rightarrow \text{Biol-M}^{n+} + O_2 \quad (6)$$

$$\text{Biol-M}^{n+} + H_2O_2 \rightarrow \text{Biol-M}^{n+1} + {}^{\bullet}OH + OH^- \quad (7)$$

$$DNA + {}^{\bullet}OH \rightarrow \text{damaged-DNA} \quad (8)$$

b. Superoxide as an oxidant:

In 1976, Klug-Roth and Rabani[102] published the kinetics of copper(II)-catalyzed decomposition of superoxide to dioxygen, with the following proposed mechanism:

$$Cu^{2+} + O_2^{\bullet -} \rightarrow Cu^{+} + O_2 \quad (9)$$

$$Cu^{+} + O_2^{\bullet -} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2 \quad (10)$$

$$Cu^{2+} + O_2^{\bullet -} + 2H^{+} \rightarrow Cu^{3+} + H_2O_2 \quad (11)$$

$$Cu^{3+} + O_2^{\bullet -} \rightarrow Cu^{2+} + O_2 \quad (12)$$

A similar mechanism applies to iron(III) involving Fe^{3+}/Fe^{2+} and Fe^{3+}/Fe^{4+} couples.[103] The above reactions imply that the superoxide anion is a precursor of Cu^{3+} or Fe^{4+} formation. These cations are strong oxidants and therefore may be involved in oxidative damage to biologically important molecules, as follows:

$$\text{Biol-M}^{n+} + O_2^{\bullet -} + 2H^{+} \rightarrow \text{Biol-M}^{n+1} + H_2O_2 \quad (13)$$

$$\text{Biol-M}^{n+1} \rightarrow \text{(damaged-Biol)-M}^{n+} \quad (14)$$

Hydroxyl radical

Hydroxyl radical (${}^{\bullet}OH$) is highly reactive with a half-life in aqueous solution less than 1ns. Thus when produced in vivo it reacts close to its site of formation. It can be generated through a variety of mechanisms. Ionizing radiation causes decomposition of H_2O, resulting in formation of ${}^{\bullet}OH$ and hydrogen atoms. ${}^{\bullet}OH$ is also generated by photolytic decomposition of alkyl hydroperoxides (ROOH). Production of ${}^{\bullet}OH$ close to DNA could lead to this radical reacting with DNA bases or deoxyribosyl backbone of DNA to produce damaged bases or strand breaks. It has been proposed that the extent of DNA strand breaking by ${}^{\bullet}OH$ is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone.[104]

The majority of the hydroxyl radicals generated in vivo comes from the metal catalyzed breakdown of hydrogen peroxide, according to the reaction[105-108]

$$M^{n+}(= Cu^{+}, Fe^{2+}, Ti^{3+}, Co^{2+}) + H_2O_2 \rightarrow M^{(n+1)+} (= Cu^{2+}, Fe^{3+}, Ti^{4+}, Co^{3+}) + {}^{\bullet}OH + OH^- \quad (15)$$

where M^{n+} is a transition metal ion. The most realistic in vivo production of hydroxyl radical according to reaction (15) occurs when M^{n+} is iron or copper.[109-111] The Fe^{2+}-dependent decomposition of hydrogen peroxide is called the Fenton reaction.[108,112-114] In addition to reaction (15), the following reactions may occur:

$${}^{\bullet}OH + H_2O_2 \rightarrow H_2O + H^{+} + {}^{\bullet}O_2^{-} \quad (16)$$

$${}^{\bullet}O_2^{-} + Fe^{3+} \rightarrow Fe^{2+} + O_2 \quad (17)$$

$${}^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (18)$$

Generally, the hydroxyl radical may react by (i) hydrogen abstraction, (ii) electron transfer and (iii) addition reactions. The reaction of hydroxyl radical with a biomolecule will produce another radical, usually of lower reactivity. As a result of the high reactivity of •OH, it often abstracts carbon-bound hydrogen atoms more or less non-selectively, e.g. from glucose. Production of •OH close to an enzyme molecule present in excess in the cell, such as lactate dehydrogenase, might have no biological consequences. However, attack by •OH on a membrane lipid can cause a series of radical reactions that can severely damage the membranes.[114,115] Hydroxyl radical also causes addition to DNA bases leading to generation of a variety of oxidative products. The interaction of •OH with guanine leads to the generation of 8-oxo-7,8-dihydro-2`-deoxyguanosine (8-oxo-dG) and 2,6-diamino-5-formamido-4-hydroxypyrimidine (FAPy-G) **(Figure 7)**.[116,117] Adenine reacts with •OH in a similar manner to guanine, although oxidative adenine lesions are less prevalent in DNA damage.[118] It has been demonstrated that in the presence of Fe(III) or Fe(III)–EDTA complex, endogenous reductants such as ascorbate, GSH, and the reduced form of NADH, caused DNA damage at every type of nucleotide with a slight dominance by guanine.[119] Specifically, NADH in the presence of Fe(III)–EDTA and H_2O_2 generated •OH, lead to formation of 8-oxo-dG.[120] The DNA damage was inhibited by typical •OH scavengers and by catalase,[121-123] suggesting that these reductants cause DNA damage via the Fenton reaction.

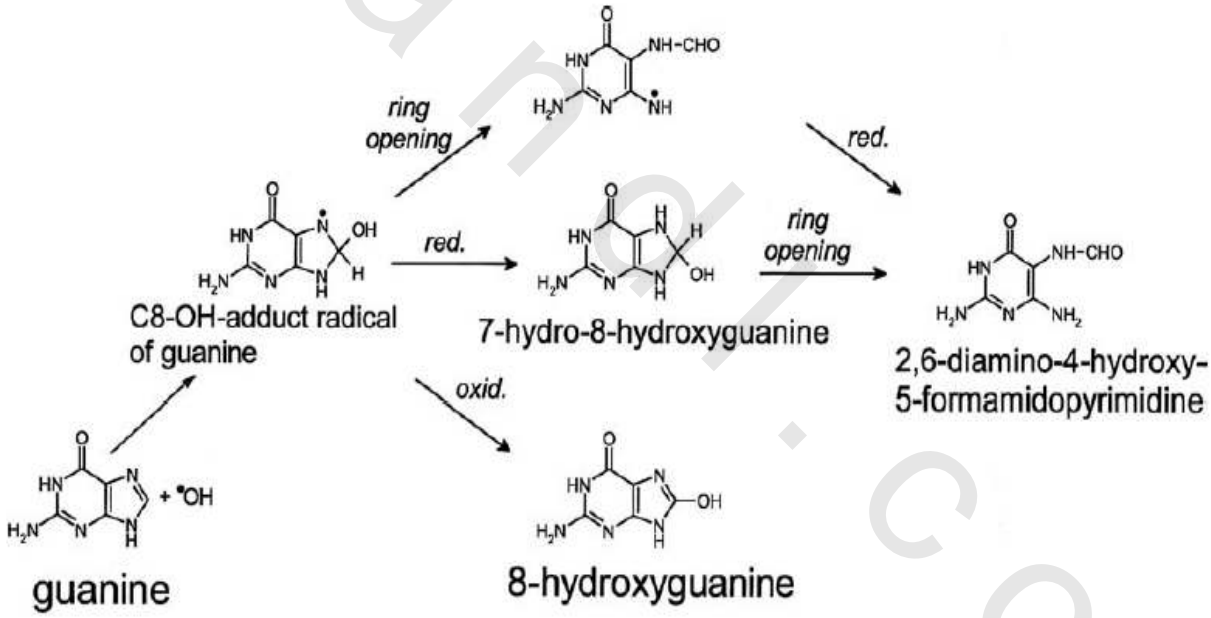


Figure (7): Structures of various products formed from the C8-OH–adduct radical of guanine, which itself is formed by attack of •OH on the C8-position of guanine.[77]

Peroxyl radicals

The reactions of peroxyl radicals (ROO•) are prevalent in all aspects of life, ranging from interactions with DNA[124] to "knocking" in the internal combustion engines of automobiles.[125] They are high-energy species, with a reduction potential ranging from +0.77 to +1.44 V, depending on the R group. Several methods, ranging from chemical and physical to enzymatic techniques, may be used to generate peroxyl radicals.[126]

The simplest peroxyl radical is dioxyl radical $HOO^{\bullet}$, the conjugate acid of superoxide, $O_2^{\bullet -}$. There are many, more complex peroxyl radicals, including cholesterol derivatives, fatty acids, etc.[127] The chemistry of this type of molecule is variable due to the identity of the R group, the local environment, and the concentration of oxygen and other reactants.

Perhaps the most interesting feature of peroxyl radicals is the diversity of biological reactions in which they participate. The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in human disease and toxicology. Peroxyl radicals are involved in DNA cleavage[128] and protein backbone modification.[129] Peroxyl radicals synergistically enhance the induction of DNA damage by superoxide.[130]

The pathway of reactions of peroxyl radicals is as follows.[131] Chain initiation refers to the attack of any species that has sufficient reactivity to abstract a hydrogen atom from a methylene group. Hydroxyl radical is sufficiently reactive to do this, although superoxide is not.

$$-CH_2- + {}^{\bullet}OH \rightarrow -{}^{\bullet}CH- + H_2O \quad \text{(Ri = Rate of initiation)} \qquad (19)$$

Under aerobic conditions the radical $-{}^{\bullet}CH-$ produced in (19) reacts with dioxygen to yield a peroxyl radical:

$$-{}^{\bullet}CH- + O_2 \rightarrow -CHOO^{\bullet} \qquad \text{(Rp1 = rate of propagation)} \qquad (20)$$

It should be noted that a very low oxygen pressure might favor the self-reaction of carbon-centered radicals, terminating the process.

$$2CHOO^{\bullet} \rightarrow \{\text{Non-radical products, i.e. termination}\} \qquad (21)$$

$$\text{or } 2CH^{\bullet} \rightarrow \{\text{Non-radical products, i.e. termination}\}$$

Peroxyl radicals produced in (20) are capable of abstracting hydrogen from another adjacent lipid molecule to propagate the process.

$$-CHOO^{\bullet} + -CH_2 \rightarrow \quad -CHOOH + -CH^{\bullet} \quad x \text{ (Rp2 = rate of propagation)} \qquad (22)$$

IV. Antioxidant Systems

A number of sophisticated antioxidant systems exist in aerobic organisms, and they function to balance the cellular production of ROS that has been described before. Endogenous antioxidant defenses include a network of compartmentalized antioxidant enzymes that are usually distributed within the cytoplasm and among various organelles in cells. A variety of small non-enzymatic molecules present in the internal milieu are also capable of scavenging ROS. In eukaryotic organisms, several ubiquitous primary antioxidant enzymes, such as SOD, catalase, and different forms of peroxidases work in a complex series of integrated reactions to convert ROS to more stable molecules, such as water and O_2. Besides the primary antioxidant enzymes, a large number of secondary enzymes act in concert with small molecular-weight antioxidants to form redox cycles that provide necessary cofactors for primary antioxidant enzyme functions. Small molecular-weight antioxidants (e.g., GSH, NADPH, thioredoxin, vitamins E and C, and trace metals, such as selenium) can also function as direct scavengers of ROS. These enzymatic and non-enzymatic antioxidant systems are necessary for sustaining life by their ability to both maintain a delicate intracellular redox balance and reduce or prevent cellular damage caused by ROS.[132]

Superoxide Dismutases

Superoxide dismutases (SOD) form a group of enzymes important for removing biologically generated superoxide anion radical ($O_2^{\bullet -}$). These enzymes function by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and oxygen, and their action helps to protect cells from oxidation of lipids, proteins and DNA. These enzymes are crucial in maintaining a proper balance of superoxide within specific cellular compartments that is essential for normal cellular signaling and stress responses.[133]

Three forms of SOD exist in humans, depending on the type of metal ion: copper and zinc containing SOD (Cu/Zn-SOD or SOD1), manganese containing SOD (Mn-SOD or SOD2) and extracellular SOD (EC)-SOD (or SOD3). Each of the three SOD proteins plays a unique physiological role based, in part, on its tissue distribution and subcellular localization.[134]

The first SOD discovered contains both copper and zinc; hence, its name copper and zinc containing superoxide dismutase (Cu/Zn-SOD). In humans, the liver has a relatively high amount and activity of SOD1.[135] This enzyme is predominantly located in the cytoplasm, but has also been found in the nucleus and lysosomes under certain conditions.[136] Its localization allows it to protect proteins, lipids and DNA from oxidation and it is an important regulator of intracellular superoxide signaling.[137]

The second SOD is the manganese containing SOD (Mn-SOD). SOD2 is generally located in the mitochondrial matrix[135] and reduces oxidative stress in the mitochondria by catalyzing the rapid dismutation of superoxide formed by the electron transport chain during respiration. This reaction proceeds with an impressively rapid rate constant.[138] Overall, the action of Mn-SOD protects the respiratory machinery and mitochondrial DNA, thus ensuring maintenance of mitochondrial integrity.[139] In humans, the activity of SOD2 in the renal cortex is higher than in other tissues.[135]

Finally, extracellular superoxide dismutase (EC)-SOD is the most recently discovered form of SOD. Unlike the previously described SODs, (EC)-SOD is found in extracellular fluids such as human plasma and lymph. Its expression is greatest in endothelial cells of blood vessels where its key role appears to be regulating superoxide interactions with nitric oxide. Superoxide and nitric oxide react to form peroxynitrite, a toxic species.[140] This reaction decreases the bioavailability of nitric oxide, so it can no longer function to maintain vascular tone and health. The balance of superoxide and nitric oxide is, therefore, vital for proper vasculature function.[141]

In disease processes, dysregulation of SODs results in a pathogenic imbalance of superoxide, leading to profound effects on cells and tissues. For example, aberrant structure or activity of Cu/Zn-SOD has been linked to the neurological disease,[142] amyotrophic lateral sclerosis (ALS)[143] and developmental abnormalities seen in Down's syndrome.[144] Similarly, decreases in the function of Mn-SOD have been shown to play a role in carcinogenesis.[145,146] Finally, cardiovascular disease is associated with poor vascular tone following lowered nitric oxide bioavailability. Decreased (EC)-SOD activity results in increased peroxynitrite formation that causes damage to the vasculature, thus accelerating cardiovascular disease.[147]

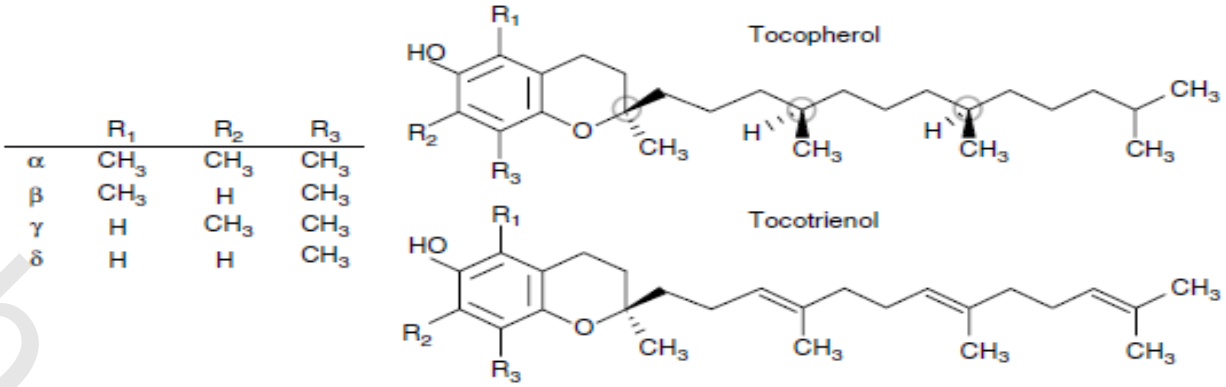
In summary, SODs are an essential group of enzymes that must be present at appropriate levels, structures and locations to maintain a healthy balance of superoxide, necessary for development and disease prevention.[133]

Vitamin E

Vitamin E a highly potent antioxidant

Vitamin E is an effective lipophilic vitamin, whose major role is to act as a highly potent antioxidant against the damaging ROS and peroxyl free radicals, which are produced as a result of cellular oxidative stress. The main dietary sources of vitamin E include vegetable seeds and their oils, nuts, almonds, fish, some cereals and some of the green leafy vegetables. The term vitamin E refers to eight essential naturally occurring isomeric forms that possess a similar chemical structure comprising a chromanol ring with a 16-carbon side chain **(Figure 8)**. The isomeric forms with saturated side chain are called "tocopherols", while isomeric forms with unsaturated side chain are termed as "tocotrienols".[148] The significance of vitamin E has been subsequently proven as an essentially required cellular component that can protect the integrity of cellular membranes and play an important role in various biological processes.

The most abundant and biologically active isoform of vitamin E in the human body is alpha-tocopherol.[149] Alpha-tocopherol has the maximum bioavailability among all the forms and is selectively recognized and transported inside the cells with the help of a cytosolic liver protein, termed as α-tocopherol transfer protein.[150] The most well-known function of vitamin E is to terminate a radical-propagated chain of lipid peroxidation reaction. Vitamin E protects the polyunsaturated fatty acids (PUFAs) of the cellular membranes and low-density lipoproteins (LDL) from oxidative damage by free radicals. In this manner, vitamin E serves to protect and stabilize the cellular membranes through its role as a powerful antioxidant.[151]



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Figure 8: Vitamin E structures are shown. The methyl groups on the chromanol head determine whether the molecule is α-, β- or γ-, or δ-, while the tail determines whether the molecule is a tocopherol or a tocotrienol.[152]

Non-antioxidant functions of vitamin E

Molecules in biological systems often perform more than one function. In particular, many molecules have the ability to chemically scavenge free radicals, but they may also act as hormones, ligands for transcription factors, modulators of enzymatic activities or as structural components. During the last two decades, the potential impact of vitamin E on health and various life processes has been intensely studied and debated. More recently, vitamin E has been found to possess functions that are independent of its well-defined cellular membrane stabilizing and freeradical scavenging ability.[153]

Vitamin E has been suggested to regulate enzyme activities, signal transduction and gene expression.[154] It has been reported that the activity of the enzymes such as protein kinase C, tyrosine kinase, 5-lipoxygenase, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and phospholipase A_2 is significantly reduced, while that of diacylglycerol kinase and protein phosphatase 2A is increased in the presence of α-tocopherol.[155] Vitamin E has been shown to modulate the activity of both inflammatory cytokines and immune cells.[156] Vitamin E has also been reported to inhibit smooth muscle cell proliferation.[157] Epidemiological and experimental studies have suggested that antioxidants like vitamin E may play an important role in the prevention of certain chronic diseases. Large scale clinical trials have been carried out to find out the effect of vitamin E supplementation in the prevention of ischemic heart disease, cancers and neurodegenerative diseases.[158-160] However, the results of these clinical studies have been inconclusive, and the expected benefits of vitamin E supplementation in the prevention of some of the chronic diseases need to be clearly shown.[161,162]

Absorption of vitamin E

Vitamin E is absorbed in the intestine and enters the circulation via the lymphatic system. It is absorbed together with lipids, packed into chylomicrons, and transported to the liver with the chylomicrons and the remnants derived thereof.[163] This process is similar for all forms of vitamin E tested. Only after passage through the liver does α-tocopherol preferentially appear in the plasma.[163] Most of the ingested β-, γ-, and δ-tocopherol is secreted into bile or not taken up and excreted in the feces.[164] The reason for the plasma preference for α-tocopherol is its specific selection by the hepatic α-tocopherol transfer protein (α-TTP).[165]

V. Oxidative Stress

In health, balance between production of ROS and antioxidant defenses lies slightly in favour of ROS production. Oxidative stress can be defined as an excessive amount of ROS, which is the net result of an imbalance between production and destruction of ROS (the latter is regulated by antioxidant defences).[166] In principle, oxidative stress can result from increased production of ROS, excessive activation of phagocytic cells in chronic inflammatory diseases, diminished antioxidants, e.g. mutations affecting antioxidant defense systems and depletions of dietary antioxidants and micronutrients.[167]

Consequences of oxidative stress are either adaptation or cell injury, i.e. damage to DNA, proteins, and lipids; disruption in cellular homeostasis and accumulation of damaged molecules.[167]

The tissue level of antioxidants critically influences the susceptibility of various tissues to oxidative stress. Enhanced oxidative stress and oxidative damage to tissues are general features of most chronic diseases as Alzheimer's disease, cancer, atherosclerosis and rheumatoid arthritis.[168]

Biomarkers of oxidative stress

Biomarkers are compounds whose variation can indicate an alteration in physiological processes and can be used as an early warning against ensuring pathology. The harmfulness of free radicals is their extreme reactivity due to their unpaired electron; this reactivity also determines the very short half-life of these species. It has been calculated that the OH˙ radical can travel a maximum of only 15A from its site of generation before it reacts with another molecule. This fact illustrates the difficulty in the measurement of free radicals directly, but the products of radical damage in the cell, mainly DNA, lipids and proteins are also good markers of oxidative stress. Moreover, cells (and organisms) developed several different mechanisms to control these harmful substances; therefore oxidative stress could also be monitored by evaluating levels of those protective species.[169,170]

DNA:

Oxidative changes to DNA can occur by many routes including oxidative modifications of the nucleotide bases or sugars, or forming crosslinks. It is well known that such modifications can lead to mutations, cellular aging and death.[171,172] In vivo, damaged DNA is repaired by endonucleases and glycosylases liberating deoxynucleotides and bases respectively that are excreted in urine.[173] ROS can cause DNA oxidation by different mechanisms, such as via hydroxyl radical (derived from the reduction of H_2O_2). Most interest to date has been focused on measuring 8-hydroxy-2`-deoxyguanosine (8-OHdG) and its free base 8-hydroxyguanine (8-OHG) in blood or urine.[174,175]

Proteins:

The modifications of proteins may result in crosslinking, peptide fragmentation and a conversion of one amino acid to another, or to a modified residue by oxidation of the amino acid side chain. Such modifications can result in the alteration of secondary and tertiary structures of a protein, and these conformational changes may expose previously shielded regions to further oxidation or to other types of spontaneous modifications such as deaminations.(176)

A good marker of oxidative damage to protein and of oxidative stress is nitrotyrosine. When superoxide and nitric oxide exist in close proximity, they can spontaneously form peroxynitrite ($ONOO^-$), a powerful oxidant. Nitration in the 3-position (ortho) of tyrosine is the major product of the peroxynitrite attack on proteins.(177) Protein tyrosine nitration by peroxynitrite interferes with phosphorylation/dephosphorylation signaling pathways and alters protein function.(178)

Lipids:

Lipid peroxidation is probably the most extensively investigated process induced by free radicals. These compounds are abundant at the membrane level, where most of the reactive radicals, especially reactive oxygen species are formed. Moreover, lipid peroxidation may result in a chain reaction that auto-propagates once started, leading to the formation of many lipid peroxide radicals and amplifying the ROS effect.(179)

Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. The most studied are isoprostanes (such as 8-isoprostane), a prostaglandin-like compound generated in vivo by the free radical-catalyzed peroxidation of arachidonic acid, independently of the cyclooxygenase.(180)

Other important compounds are bioactive aldehydes generated by extensive oxidation of polyunsaturated fatty acids (PUFA). This process generates compounds such as alkanals (hexanal), 4-hydroxy alkenals (mainly 4-HNE), malondialdehyde (MDA) and acrolein.(181)

Lipid peroxidation changes molecule characteristics, making it more hydrophilic; this can alter structure and function of the membrane.(182) Moreover, some molecules also posses an independent biological activity. The isoprostanes, for example, have vasoconstrictor and mitogenic properties towards vascular smooth muscle cells in renal and pulmonary artery walls. Some compounds may also be toxic, leading to cell death (necrosis or apoptosis).(183)

VI. Component Pathologies of the Metabolic Syndrome and Oxidative Stress

Oxidative Stress is a Hallmark of the Metabolic Syndrome

Patients with metabolic syndrome often develop advanced atherosclerosis. Oxidative stress plays a central role in the initiation and progression of atherosclerosis. NAD(P)H oxidases are the primary source of ROS in the vasculature. Increased expression and activity of the phagocytic NAD(P)H oxidases with a parallel increase of oxidized LDL (oxLDL) and nitrotyrosine levels accompanied by thickened intima to media ratio in the carotid arteries, indicative of early subclinical atherosclerosis, have been demonstrated in metabolic syndrome patients.[184] It has also been found that subfractions of small HDL cholesterol particles, which are normally protective, posses lower antioxidant capacity in the metabolic syndrome.[185] Increased oxidative stress associated with increased production of ROS is augmented by decreased expression of antioxidant enzymes. Studies in a diet-induced rat model of metabolic syndrome found increased oxidative stress and endothelial dysfunction. This study further demonstrated increased ROS production capacity by the NAD(P)H oxidase along with down-regulation of key superoxide dismutase (SOD) isoforms indicating a disrupted antioxidant defense system in metabolic syndrome.[186] Reports from the Third National Health and Nutrition Examination Survey indicate diminished concentrations of the antioxidant vitamins C and E and several carotenoids, even after adjusting for lower fruit and vegetable consumption in participants with metabolic syndrome.[187] Thus, it is clear that the human metabolic syndrome is characterized by oxidative stress precipitated by excess production of ROS and diminished antioxidant defenses.

Oxidative Stress and Obesity

Recently, there have been some attempts to define the contribution of the individual components of the metabolic syndrome to oxidative stress evident in the metabolic syndrome patients.[7] Obesity is a core component in the development of metabolic syndrome and plays a central role in amplified oxidative stress. Obese patients have shown oxidative stress-induced decreased vasodilatory response to acetylcholine, which was inversely related to body mass index, waste to hip ratio, fasting insulin, and insulin resistance.[188] Obesity in children, without any other metabolic syndrome components, has been repeatedly correlated with increased oxidative stress and endothelial dysfunction.[189] Weight loss (10% of body weight) by moderate diet restriction and moderate-intensity aerobic exercise in metabolic syndrome patients has been shown to improve markers of oxidative stress.[190] On the other hand, data from an intensive 21-day residential diet and exercise program in overweight or obese patients revealed a decrease in oxidative stress and improvement in other markers of cardiovascular risk associated with metabolic syndrome even before significant weight loss.[191] This effect could have been mediated by a reduction in oxidative stress through exercise-mediated improvement in endothelial function and nitric oxide (NO) production or up-regulation of antioxidant defenses. In animal models, ROS production in adipose tissue of obese mice was reduced by treatment with, the NAD(P)H oxidase inhibitor, apocynin resulting in improvement in glucose and lipid metabolism independent of body weight.[192] Long-term studies are needed to see if these short-term effects translate to long-term cardiovascular outcomes.

Oxidative Stress and Insulin Resistance

The isolated contribution of insulin resistance to oxidative stress is difficult to ascertain. Studies which address the question of oxidative stress in type II diabetes typically do not distinguish between the study participants on the basis of obesity or their lipid profile. Since both obesity and dyslipidemia, independently, significantly contribute to oxidative stress, and obesity is the primary risk factor for the development of insulin resistance, with dyslipidemia now emerging as a possible contributing factor, this presents a significant obstacle with respect to determining whether insulin resistance alone elevates oxidative stress in humans. Likewise, the animal models of insulin resistance are obese, and the insulin resistance develops secondary to obesity. Increased ROS have also been shown to have a causal role insulin resistance.[193] Both tumor necrosis factor-α (TNF-α) and dexamethasone decreased Protein Kinase B (Akt) phosphorylation and consequently glucose uptake into cultured muscle cells, which was reversed by antioxidant treatment (N-acetyl cysteine (NAC), SOD, catalase, manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP)). The same study furthermore showed that glucose uptake was compromised in obese (db/db) mice in vivo resulting in increased blood glucose and antioxidants lowered blood glucose.[193]

Oxidative Stress and Hyperglycemia

While hyperglycemia per se is not a defining parameter of the metabolic syndrome, hyperglycemia, which results from primary β-cell destruction in the absence of any other components of the metabolic syndrome, has been shown to correlate with elevated oxidative stress (decreased glutathione, GSH/GSSG ratio) in type I diabetes.[194] However, this may or may not be relevant to the metabolic syndrome, where hyperglycemia develops secondary to the development of insulin resistance.

Oxidative Stress and Dyslipidemia

Dyslipidemia, characterized by elevated LDL and triglycerides and decreased HDL, is also a frequent component of the metabolic syndrome phenotype. A positive correlation between elevated LDL and triglycerides and low HDL and oxidative stress in animal models is well established. LDL receptor-deficient mice fed a cholesterol-enriched diet developed elevated LDL levels and consequently oxidative stress.[195] These observations extend to human studies. High plasma oxidative stress markers positively correlated with elevated plasma triglycerides and inversely correlated with HDL[196] in a group of metabolic syndrome patients with end-stage renal disease, after all other factors (presence of obesity, hypertension, and/or type II diabetes) were adjusted for. Lipid peroxidation, as an index of oxidative stress, inversely correlated with HDL levels, irrespective of age, gender, and presence of the other metabolic syndrome components.[197] It is also now accepted that the numerous positive effects of some statins in the cardiovascular system are independent of their lipid-lowering effect and a consequence of a direct decrease in oxidative stress. For example, short-term pravastatin treatment reduced myocardial infarct (MI) size in hypercholesterolemic rabbits through reduction in peroxynitrate and nitrotyrosine formation.[198] Similar results, with regards to the atherogenic index, were achieved with rosuvastatin, which lowered: oxidative stress by elevating the expression of antioxidant enzymes (SOD, catalase, glutathione, glutathione peroxidase), LDL, triglycerides, and C-reactive protein (CRP), and elevated HDL.[199]

Oxidative Stress and Hypertension

Hypertension is another component of the metabolic syndrome which is independently associated with increased cardiovascular risk. While animal models of hypertension have also been rather consistently associated with elevated oxidative stress, whether hypertension alone increases oxidative stress in humans is somewhat controversial. One study found no difference in markers of oxidative stress when comparing hypertensive and normotensive patients,[200] while several studies found increased oxidative stress in hypertensive patients.[201,202] A study in metabolic syndrome patients showed that other metabolic syndrome components (low HDL, triglycerides, abdominal obesity, and fasting glucose) had minimal contribution to increased oxidative stress, whereas hypertension alone was responsible for elevated oxidative stress in these patients.[203] This study, however, used the International Diabetes Federation (IDF) definition for metabolic syndrome, but used body mass index (BMI) as an indicator of central obesity and has been criticized for underdiagnosing metabolic syndrome patients.[204] The metabolic syndrome definition, which uses waist circumference as a measurement of central obesity, has been shown to be a better predictor of mortality than others.[205] It is not clear how the effects of hypertension were separated from the effects of the other risk factors in the established pathology of the metabolic syndrome in this study. In another study showing a positive link between hypertension and oxidative stress, seemingly essential hypertension was found to, in fact, be secondary to insulin resistance.[206] Another study determined that CRP, an inflammatory marker, known to be elevated in metabolic syndrome, is a better predictor of oxidative stress in "essential" hypertension, than high blood pressure.[207] These studies illustrate that attempts to identify the etiology of oxidative stress in human metabolic syndrome where hypertension is a component are complicated by the propensity of additional metabolic syndrome components to complicate the interpretation of the study results as confounding factors so that the isolated contribution of hypertension to oxidative stress becomes difficult to determine.

Furthermore, unlike the other component pathologies of the metabolic syndrome, hypertension is itself a multifactorial disease with a variety of possible etiologies. Oxidative stress has been shown to increase with deoxycorticosterone acetate (DOCA)-salt-induced hypertension,[208] angiotensin II (AngII)-infusion[209] and 2-kidney-1-clip-induced hypertension[210] as well as in genetic animal models of spontaneous hypertension (SHR). However, norepinephrine-induced hypertension does not increase oxidative stress in a rat model.[211] These studies may indicate that whether or not human hypertension is associated with oxidative stress depends on the predominant etiology of the disease in the individual patient. This may explain the seeming discrepancy among the studies outlined above.